UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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MOM Enterprises, LLC,
Petitioner,

v.

ELAINE AND REINHOLD W. VIETH,
Patent Owner,

_____

Case IPR 2023-00726
Patent 9,066,958

_____

DECLARATION OF PAUL HOROWITZ

I, Paul Horowitz, declare and state as follows under penalty of perjury:

1. This declaration is based on personal knowledge unless otherwise indicated. All statements made in this declaration are true and accurate. I believe that any statements made on information and belief are true and accurate. I make these statements knowing that willful false statements are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001.
2. I have been retained by the Patent Owners as an expert witness in the MOM vs. Vieth IPR Proceeding, Case IPR2023-00726, and have been asked to provide this testimony to the US Patent Office
3. This testimony will respond to the two questions I have been asked to address: First, does US Patent No. 9066958 ("the '958 patent") solve a long-felt but unmet need and, second, whether medications/supplements can effectively and consistently be administered via the tip of a spoon to an infant as described by the "Harder reference".
4. Expert Qualifications
    a. I am knowledgeable in, and an expert in clinical pediatrics. I am a Board Certified Pediatrician. I have nearly 30 years of experience in children's health and wellness. My experiences include, but are not limited to, clinical practice which includes the care of infants and children, participation on corporate advisory boards of infant

consumer products companies, international lectures to other pediatricians, and clinical research on infants. A copy of my curriculum vitae is attached as Exhibit A.

b. I earned a Bachelor's degree from UC Berkeley in 1988 in Anatomy and Physiology, then my MD from Tel Aviv University's Sackler School of Medicine in 1992. My clinical training was at Children's Hospital Los Angeles, and I was selected Chief Resident, so I stayed on to teach in that role for another academic year from 1995 to 1996. Since then, I have been in clinical practice and held various leadership roles within those practices and medical groups. I am currently a co-founder of a private pediatric practice where I have been working since 2008.

c. In my clinical practice, I care for children ranging from birth and their first moments of life until their 21st birthday. I help families with many aspects of health, wellness, nutrition, and prevention and have cared for thousands of children over the years.

d. I am being compensated for my services in this matter at a rate of $600 per hour, and if called to testify, it is at a rate of $900 per hour. No part of my compensation is contingent on the outcome of this litigation, nor on the opinions provided by my report(s).

e. My opinions are based on my education, training, decades of experience, and review of referenced materials. Documents I have reviewed or considered in the preparation of this report are identified and listed in Exhibit B.

f. All other Exhibits referenced in this report are done so by citation to documents in the record, or are otherwise attached as Exhibits.

5. Reason for Invention

a. Bone disease and the consequences of reduced mineralization have been characterized for over 350 years. In the late 1960's, the active form of Vitamin D was identified and since then, scientists have gained further understanding into how it prevents and cures vitamin D sensitive rickets. Rickets is the result of poor bone mineralization causing disfigurement such as scoliosis, dental abnormalities, fractures, respiratory problems, seizures, and even difficulties in childbirth due to pelvic deformities in some young women.[1]

b. Vitamin D is a fat-soluble vitamin and, unlike water soluble vitamins which get excreted through the urine if given in excess, it can reach toxic levels causing hypercalcemia, vomiting, muscle weakness, pain,

[1] Exhibit C.

kidney problems, and nephrolithiasis (kidney stones).[2] Indeed, the FDA has even warned the industry about the risk of overdose.[3]

c. Since 2008 the American Academy of Pediatrics has recommended: "Supplementation with 400 IU of vitamin D should be initiated within days of birth for all breastfed infants, and for non-breastfed infants and children who do not ingest at least 1L of vitamin D-fortified milk daily."[4]

d. Specific amounts of vitamin D supplementation have been recommended by experts and organizations around the world such as the World Health Organization[5], the US Institute of Medicine[6], and the American Academy of Pediatrics[7]. However, anyone who has tried to feed a baby knows that they do not always cooperate with the adult trying to get them to take it. Indeed, in my nearly 30 years of

[2] Exhibit D.

[3] Exhibit E.

[4] Exhibit F.

[5] Exhibit G.

[6] Exhibit H.

[7] IPR Exhibit 1012 ("Gartner").

experience caring for infants and children, administering a medication, nutrient, or supplement via the oral route has been a source of frustration for most caregivers at one time or another.

6. Long Felt but Unmet Need --

    a. Counsel has advised that in determining whether a claimed invention is obvious, the USPTO should consider any objective evidence (sometimes called "secondary considerations") that may shed light on whether or not the claimed invention would have been obvious to one of ordinary skill in the art. The objective evidence that I considered and discuss herein is whether the claimed invention satisfied a long-felt but unmet need. At the time of the invention, there were other ways for infants to either make vitamin D (via adequate sun exposure), or get it exogenously (via injection, or as a dietary supplement). There were problems with each of the then-available methods of delivery. As will be shown below, the solution provided by the inventors and the '958 patent holders solved a problem that was hitherto unmet and that had existed for a long time.

    b. Although vitamin D can be made in the skin with enough UV exposure, the risk of skin damage is felt to be greater than the benefits of UV exposure. According to The American Academy of

Dermatology, there is not a safe level of UV exposure from the sun or indoor tanning devices that allows for maximum vitamin D synthesis without increasing skin cancer risk.[8]

c. Furthermore, UV exposure adequate to meet vitamin D needs in infants and children is impossible to achieve year-round in many parts of the world and in certain seasons in other parts of the world.

d. Giving vitamin D via injection delivers the compound in a reliable way, but it is not a practical method of universal administration for parents to inject their baby daily for many reasons, including but not limited to the pain of injection, needle management, cost of syringes, access to alcohol swabs, and risk of infection.

e. Oral ingestion of 5ml of cod liver oil had been used for generations to prevent rickets, but it was an inexact endeavor in the sense that the person administering the vitamin D supplement could not be certain they were giving enough and could not be certain they were not administering a dangerous, toxic, amount. The amount of vitamin D administered is measured in units which can be calculated by multiplying the concentration times the volume administered. But

[8] Exhibit I.

O'Keefe noted in 1986 that the concentration of vitamin D in cod liver oil varies depending on the source of the oil[9]. Therefore, the exact amount in 5ml administered is impossible to determine. Furthermore, in my opinion, 5ml is too much fluid to reliably administer every day to an infant and cod liver oil carries with it the risk or underdosing or overdosing for the reasons above.

f. It is my understanding that Harder is a midwife who co-authored parts of a book in German in 2003 about postpartum care with the intended audience of other midwives[10]. In one of the chapters she wrote, she included a brief mention about administering vitamin D as an oil called Vigantol® oil as one option in a section about administering vitamin D to infants. As a pediatrician, skilled in the art, I have conferred directly with midwives but I have never relied upon information from a book by a midwife as if it were an academic medical reference because midwife education and training is generally not as rigorous or comprehensive as that of physicians or PhD's whom I consider experts. In her book, Harder advises that one option for

[9] Exhibit J.

[10] IPR Exhibit 1006, ("Harder").

administering a vitamin D supplement called Vigantol® oil is via the tip of a spoon. Specifically, based upon a translation from Rebecca Tinworth in 2017 (MOM 1021, page 5), the reference states:

> Vigantol® oil is a prescription-only medicine, as there is a risk of an overdose if it is not used properly. Before asking their pediatrician for a prescription, parents must be given full details of the benefits and drawbacks of the oil, and also of its use; if this is not done, their pediatrician will refuse to prescribe the oil for fear of incorrect dosage.
>
> Use of the oil is simple: 1 drop of Vigantol® oil is given to the baby from the tip of a spoon once a day before a breastfeed or a meal. Under no circumstances should it be put directly in the baby's mouth! Should 2 drops fall onto the spoon, the spoon should be washed and the process started again. In my experience, skeptical parents are more likely to be accepting of administering drops on a daily basis than a daily dose of tablets. I have also now been able to convince some pediatricians of the advantages of the oil.

g. Modifications have been made to Tinworth's 2017 translation by her then-supervisor Sherman, MOM 1022, page 2, and again by

Benyunes, MOM 1023, pages 7-8. The changes add the word "lick[ed]" to the translated passage about how an infant might transfer the substance from the spoon to the mouth.

h. Vigantol® oil is a prescription-only product, available in limited countries and it is not available in the United States per online search on the sites of our clinic's medical suppliers Henry Schein, McKesson, and Medline on January 16, 2024. As with all vitamin D supplements, Vigantol® oil can be dangerous if given in excess. The translated reference, MOM 1021, page 5, emphasizes the danger of administering too much vitamin D and even points out the need for training by stating "parents must be given full details of the benefits and drawbacks of the oil, and also of its use…." Furthermore, Harder's advice to administer from the "tip of a spoon" is ineffective; although administering medication to school-aged children is routinely given by spoon, this would be an ineffective method of delivery to infants. Infants don't consistently lick as their primary method of ingestion the way a dog or cat does. Infants might lick the lips as they root to find mother's nipple, but this is not consistent enough to be relied upon as a method to administer a medication or supplement consistently and efficaciously. The natural suckle action is

latching onto the nipple, compressing the front of the tongue with cyclic movements of the mandible and then then peristaltic wave-like movements of the back of the tongue initiate swallowing.[11] Infants naturally thrust their tongue when hard objects like the tip of a spoon or other feeding utensils are inserted into their mouths until 4 to 6 months of age when this protective reflex typically fades. Since the recommended time to start administering vitamin D is in the first few days of life, using a hard object like a spoon in the way Harder advises, makes it impossible to meet the need.

i. There are physical properties of the substance that Harder suggests using (Vigantol®) oil that are unknown such as the viscosity and adhesion (see below). Because they remain unknown, and are not identified as concerns, Harder et al. does not suggest a solution to the long-felt but unmet need. Without a firm understanding of a supplement's viscosity and adhesion to the spoon, and appropriate testing to demonstrate that an adequate, but not toxic amount of vitamin D will be consistently and efficaciously taken by the infant, Harder's instructions to give an infant Vigantol Oil® by a spoon

[11] Exhibit K.

introduced the unacceptable risk that well-meaning caregivers will administer too little or toxic amounts of vitamin D.

j. Appropriate adhesion (stickiness of the solution to a delivery object) is an important characteristic of the invention described in the '958 patent. The supplement must stick to the surface of the nipple, pacifier, or knuckle (the object) to which it is initially applied without running off, yet leave less than or equal to 10% of the provided supplement behind. This ensures the delivery of the intended amount of the supplement to the child within acceptable limits. Documentation in the inventor's lab notebook[12] shows the invention meets this requirement. The invention, as tested by the inventor, was shown to deliver an appropriate dose of Vitamin D in an efficacious way.

k. The first days and weeks of an infant's life are intensely emotional for most parents and other caregivers and I have found it is helpful to help them feel successful. Successfully administering vitamin D in a form that infants, who are totally dependent on their caregivers, will find acceptable, and which can be administered by the non-medical

[12] RVIETH000-0000394-000414.

caregiver, and which reliably delivers just the right amount of vitamin D was needed because of the shortcomings of then-available options such as cod liver oil, Vigantol® oil, and injectable vitamin D. The need was met with the product and method of delivery specified in the '958 patent.

l. The invention, with its formulation and method of delivery, solved the unmet need to efficaciously administer just the right amount of a supplement of vitamin D in a form that is acceptable to infants and caregivers. The invention solved the problem of getting infants to ingest a recommended amount of vitamin D as specified by healthcare experts in nutrition like those at the American Academy of Pediatrics. Since advocating for the use of a single drop to administer vitamin D, I have not heard of any conflicts around its administration from families with infants under my care.

m. Prior to the invention described by the '958 patent, vitamin D was available in several forms, including but not limited to those described above. As discussed, each of these forms had shortcomings regarding administration to infants.

n. Blass[13] suggests that dipping a pacifier into an unquantifiable sweet solution "provide[s] … potent and easily implemented treatment against local pain in newborns…" But this has nothing to do with administering a pharmacological dose of medication.

o. The German-language book for midwives, with its section by Harder, describes using the tip of a spoon to administer a drop of liquid with some concentration of vitamin D notwithstanding the shortcomings outlined above. This differs tremendously from the invention of the '958 patent claims. Directly pouring liquid vitamin D into the mouth of infants such as from a spoon or dropper puts them at risk of gagging or aspirating it. The claimed invention of the '958 patent, thanks to its physical properties and method of administration, is superior to any of the prior art. In contrast with trying to get an appropriate amount of an oily substance into an infant from the bowl of a spoon as described by Harder, the inventor came up with something new that met all the needs of a vitamin D supplement, and a new method of its administration to infants.

[13] IPR Exhibit 1011, ("Blass").

p. The above references, alone or in combination, don't lead to the development of the inventor's vitamin D formulation and method of administration as described in the '958 patent. The inventor undertook experimentation with various formulations to get to the final product which is adherent but not too adherent to the object on which it was placed. Indeed, if it did not adhere enough to the pacifier or caregiver's finger, it would run off. If it were too adherent, not enough would be transferred to the infant. Further experimentation was undertaken by the inventor who tested taste, mouth-feel, and stability when stored[14]. The spark that led to the innovation described by the '958 patent is analogous to the spark that led to the identification of vitamin C in 1928. The discovery of vitamin C was not obvious even though it is the mysterious ingredient in lemons, limes, and other citrus known for more than 200 years to prevent and treat scurvy[15]. Awareness that willow bark can treat fever and minor pain had been

[14] RVIETH000-0000394-000414.

[15] Exhibit L.

known for more than 3000 years, yet that didn't make the discovery and synthesis of aspirin, willow bark's active ingredient, obvious.[16]

q. The '958 patented product and method of delivery solved an unmet need which was not apparent to a person having ordinary skill in the art. The prior art does not lead to the invention.

7. Using a Spoon to Deliver –

a. The natural suck/swallow of infants is described above. Harder, with her description of administering vitamin D via the tip of a spoon, completely disregards the natural method by which newborns feed from mother's nipple. The reader is left to to guess what side of the tip she means for the supplement to be placed. Notwithstanding, if she meant the interior surface (bowl) near the tip or the exterior surface of the spoon near the tip, we have no reason to think this is an efficacious way to administer vitamin D to an infant. My years of experience tell me that this is a suboptimal method of delivery to infants because she does not demonstrate that an appropriate amount was transferred to the infant (with a minimal amount of residual remaining on the spoon). She does not address the issue that the

[16] Exhibit M.

infant's tongue would be beneath the bowl of the spoon so there's no way to "lick" the drop out of the spoon's bowl, and she does not address the fact that the oil would run (due to gravity) into the bottom of the bowl of the spoon making it unavailable to the infant.

8. Importance of the invention –

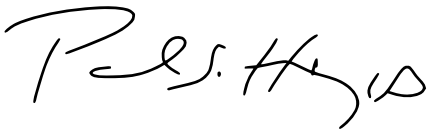
    a. The invention enables the caregiving adult to administer the recommended amount of vitamin D efficiently and consistently. As a result, the invention helps prevent rickets and prevents the complications of vitamin D deficiency safely and efficaciously. It provides vitamin D in a form and method of administration that is natural for infants to take from the exterior surface of an object (via suckling from the surface of a pacifier or nipple).

    b. Although caregivers receive information about childcare from multiple sources, they generally trust their pediatrician's recommendations about health, prevention of disease, and treatment. When a pediatrician advises a caregiver to administer vitamin D supplements, in my experience, they are receptive, responsive, and act by following that advice. This invention gives the ideal tool for caregivers to use to meet our collective objective of consistently and conveniently administering the recommended amount of vitamin D

consistently and efficaciously. As a pediatrician, I am confident that when I advise parents and caregivers to administer vitamin D as described in the '958 patent claims, the infant will receive the intended amount of vitamin D, without leading to a battle between the child and caregiver.

I, Paul Horowitz, declare and state that all statements made herein on my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: February 5, 2024



Paul Horowitz

Exhibit A

Paul S. Horowitz, M.D., F.A.A.P.

Curriculum Vitae

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Suite 150
Valencia, CA 91355
V: (661) 259-8999
F: (661) 705-0110
PHorowitz@DiscoveryPeds.com

POST-TRAINING PRACTICE EXPERIENCE

2008-Present CO-FOUNDER DISCOVERY PEDIATRICS, INC. VALENCIA, CA
A unique private practice.

Hospital privileges at:
Henry Mayo Newhall Memorial Hospital Newhall, CA

Other Capacities:
2008 to present: Monthly presenter: "BABY BASICS" class to expectant couples

Teaching and Academic Activities:
October 2023 AAP NCE Exhibit Hall Educational Speaker for Johnson's Baby (Kenvue) on benefits of emollients early in life as well as the power of colloidal oatmeal.
December 2022 Oat Roundtable about the benefits of colloidal oatmeal in prevention of atopic dermatitis in infants (Virtual)
August 2020 Advisory Board Member (Virtual) Acne Profiles — Beyond the Lesions
January 2020 Lecture and Panelist at PEDCON 2020 Indor, India on the Continuum of Pediatric Skincare
January 2020 Lecture at ITC Hotel in Hyderabad, India with transmission throughout India on Pediatric Skincare Spectrum in Clinical Practice
August 2018 PR Event to Re-Launch an international baby brand of skin products
January 2018 Consultant on Tolerance Study looking at infant skin tolerance of a new line of baby products.
October 2017 AAP NCE Product Theater Speaker about Atopic Dermatitis

October 2016 AAP NCE Product Theater Speaker about the unique needs of infant skin and eczema
September 2015 Co-Chair Johnson's Baby North American Advisory Board meeting Toronto, Canada
July 2014 Clinical Study on Tolerance of a Skin Product Regimen for Newborns (unpublished)
May 2014 Skin Summit Participant Scottsdale, AZ
October 2013 AAP NCE Product Theater Speaker on Scientific Evidence to Guide Clinicians on Skin Care Products for Children
October 2012 AAP NCE Product Theater speaker on Newborn Skin Care Guidelines and Pediatric Practices
April 2012 Guest Editor for Dermatology Research and Practice special edition on "Skin Barrier Protection"
March 2012 Presenter in Exhibit Hall of American Academy of Dermatology annual meeting about Minimal Criteria for Infant Skin Products, San Diego, CA
April 2011 to present Primary investigator: Effect of Oral Antibiotics on Children's Skin Flora – research in progress.
Feb 2011 presentation on UV Protection in children at scientific symposium Global Clinical Advisory Board (corporate sponsor) Orleans, LA
August 2010 Chair of luncheon symposium on Baby Wellness at International Pediatric Association meeting Johannesburg, SA
Video of above symposium re-purposed as CME program through Pediatric News as a webcast
May 2010 Moderator of study panel on Evidence-based infant skin cleansing practices at Global Scientific Summit
Poster presentation: Penetrance of Zinc Oxide and Titanium Dioxide Through Infant Skin
April 2010 Faculty member of Council for Baby Wellness RE: Soothing babies with cold, flu, and allergy symptoms. Peer-reviewed article submitted for publication
January 2010 Satellite Media Tour for SkinWear USA hand sanitizer 31 total interviews

Media Activities:

January 18, 2020 Quoted extensively in Times of India article "Expert shares 3 things parents get wrong about infant care"
April 2019 Dr. Paul was the special guest on the wildly successful Nick Jr. show called "Ryan's Mystery Playdate"
August 2011 Quoted in WebMD article on New Fathers

Spokesman for the Clorox Company
Quoted in lay-press magazines and websites (e.g. Real Simple, Parents, Lifescript)
Back-to-school emphasis on hygiene taped for "The View" Aug. 2008
Featured presenter at AMA Back-to School health forum in NYC 2005
Board of Advisors for DIC Entertainment
Board of Advisors for Major Consumer Health Company
Board of Advisors for MediaScope
Consultant to Jumbo Pictures/Disney
Consultant to Fox Family Channel
Advisor to National Educational Television Committee/Mediascope project on E/I Guidelines
Member Acad. Of Television Arts and Sciences Children's Peer Group
Interviewed on multiple news shows including KOIN TV Portland
Interviewed Los Angeles Times "Healthy Traveler" section
Promotion for National Pharmaceutical Company and Satellite Media Tour
Member of National Advisory Committee to "The All New Captain Kangaroo"
AAP Production: "Straight Talk for New Dads" (video only).
Special guest on "The All New Captain Kangaroo"; PSA development and guest.
Advisor and consultant for the award-winning Caillou animated pre-school show.
Panelist on Public Broadcasting KCET Southern California
Special guest on "1…2…3…Grow"
Panelist on KTLA channel 5 "Pacesetters"
Discovery Channel: "Kids Healthworks" (4 episodes)
Respondent to kids' questions at PBS Kids website: "It's My Life"
Interviewed on Northwest Television News regarding Co-sleeping
Interviewed on Northwest Television News regarding Children and sleeping pills

2005-2007 MEDICAL DIRECTOR AND OFFICE-BASED PEDIATRICIAN LEGACY EMANUEL CHILDREN'S HOSPITAL PEDIATRIC AND ADOLESCENT CLINICS — PORTLAND, OR

Hospital privileges at:

Legacy Portland Hospitals — PORTLAND, OR

Other Capacities:

Champion for bringing physicians into Electronic Health Record
Champion for Voice Recognition pilot and promotion
Member of Quality Strategy and Leadership Committee
Member of task force evaluating on-line resources

Member of e-chart Clinical Applications Committee
Computerized Physician Order Entry templates task-force

1996-2005 OFFICE-BASED PEDIATRICIAN SANTA CLARITA, CA
FACEY MEDICAL GROUP

2002 PHYSICIAN OF THE YEAR
FACEY MEDICAL GROUP

2000-2005 Regional Medical Director, Santa Clarita Valley
FACEY MEDICAL GROUP

Hospital privileges at:

Henry Mayo Newhall Memorial Hospital	Newhall, CA
Providence Holy Cross Medical Center	Mission Hills, CA
Northridge Hospital Medical Center	Northridge, CA

Other Capacities:

Chair: Technology Implementation Committee Facey Medical Group
Physician Clinical Advisory Group Allscripts Healthcare Solutions
Education Committee member in Facey Medical Group
Clinical Research Liaison for Facey Medical Group Pediatrics and AAP
Instructor of Preparation for the Newborn Classes
Periodic Ward Attending at Children's Hospital Los Angeles Until 2002
Representative of Facey Medical Group at Santa Clarita Valley Business Expo2004 Keynote address to Food Service Workers representing 80 local schools on "OBESITY"

American Academy of Pediatrics Highlights:

2008-Present AAP Media Spokesman

2005-Present Founding member: Council on Communications

2004 Recognized Contributor to "The Complete and Authoritative Guide Caring for your Baby and Young Child Birth to Age 5" by the American Academy of Pediatrics 4th edition

Oct. 2001 Presenter at AAP National Conference and Exhibition; San Francisco

1999-2005 Chair: Media Resource Team

1999-2005 Liaison to AAP Committee on Public Education

Post-Doctoral Training

1995-1996 CHIEF PEDIATRIC RESIDENT LOS ANGELES, CA
CHILDREN'S HOSPITAL LOS ANGELES

Responsibilities:
Education and management of 75 pediatric residents.
Emergency Room and General Pediatrics Service clinic preceptor for medical students of University of Southern California.

Other Capacities/Memberships:
Counselor for Los Angeles Pediatric Society Gene Black High School Summer Program.
House-staff Representative at American Academy of Pediatrics Meeting in San Francisco, CA in October, 1995.
Member of Hospital Ancillary Services Committee.
Member of Hospital CPR Committee.
Representative for Children's Hospital Los Angeles at Medical Information Systems Physician Association Meeting in Colorado.

1992-1995 CHILDREN'S HOSPITAL LOS ANGELES LOS ANGELES, CA
PEDIATRIC INTERNSHIP AND RESIDENCY

Assistant Clinical Professor University of Southern California Keck School of Medicine.
Member of Pediatrics Clinic Waiting Room Enhancement Committee.
Member of Emergency Room Education Enhancement Task Force.
Presented at Grand Rounds: 1994
House-staff Representative to Information Services Division/TDS Software Update and Review Taskforce.
Volunteer Physician to Camp for Blind Children.

Volunteer at numerous Health Fairs as a representative Physician for Children's Hospital Los Angeles.

Education

1988-1992	SACKLER SCHOOL OF MEDICINE NY STATE/AMERICAN PROGRAM	TEL AVIV, ISRAEL

M.D., May 1992.
Student Council Class Representative: 1988-1992.
Pediatric Journal Club: 1990-1992.

1984-1988	UNIVERSITY OF CALIFORNIA	BERKELEY, CA

B.S. in Physiology/Anatomy; 1988.
Dormitory President; 1986-1986.
Alcohol and Drug Peer Educator; 1986-1987.
Physiology Undergraduate Research Club; 1986-1987.

Research/Work Experience

1995-1996	CHILDREN'S HOSPITAL LOS ANGELES EMPLOYEE HEALTH	LOS ANGELES, CA
1995	ALHAMBRA HIGH SCHOOL	ALHAMBRA, CA
1992-1995	GENERAL PEDIATRICS SERVICE OUTPATIENT CLINIC (CHLA)	LOS ANGELES, CA
1985-1987	U.C.L.A. BONE RESEARCH LABORATORY	LOS ANGELES, CA

Professional Affiliations/Memberships

1996-Present FELLOW, AMERICAN ACADEMY OF PEDIATRICS
1992-Present MEMBER OF AMERICAN MEDICAL ASSOCIATION

Awards

PHYSICIAN OF THE YEAR FACEY MEDICAL GROUP 2002
NOMINATED FOR FRANCIS NUNNALLY WINZER HUMANISM AWARD
("FOR DEMONSTRATION OF CONSISTENT AND OUTSTANDING KINDNESS AND THOUGHTFULLNESS IN DEALING WITH OTHERS")

Certification

BOARD CERTIFIED IN PEDIATRICS
AMERICAN BOARD OF PEDIATRICS CERTIFYING EXAM--OCTOBER, 1995—PASSED
RECERTIFIED 2002
NATIONAL BOARD OF MEDICAL EXAMINERS--PART I--JUNE, 1990--PASSED
NATIONAL BOARD OF MEDICAL EXAMINERS--PART II--SEPTEMBER, 1991--PASSED
CALIFORNIA MEDICAL BOARD ORAL EXAMINATION MARCH, 1994--PASSED
CALIFORNIA MEDICAL BOARD LICENSED JUNE, 1994 TO PRESENT (Lic. A053073)
U.S. DRUG ENFORCEMENT AGENCY LICENSED 1994 TO PRESENT (Lic. BH4234631)
OREGON STATE MEDICAL BOARD CERTIFIED JANUARY, 2005

Languages

English, essential Hebrew, and Spanish.

Personal Interests/Hobbies

Physician leadership, General Pediatrics, and Parenthood. I am married to Daphne Horowitz MD FAAP, also a pediatrician. We have 3 daughters; Ariel, Danielle, and Emma. I enjoy being with my family as well as many activities. I build and fly radio-controlled airplanes, play golf, and enjoy computers. In addition, I am an award-winning magician; a member of the International Brotherhood of Magicians, the Academy of Magical Arts, Inc. and the Magic Castle in Hollywood.

References

Available upon request

Exhibit B

Exhibit B

Material Reviewed in the Preparation of this Report in Addition to Footnotes

1. FDA Letter to Industry Concerning Liquid Vitamin D Dietary Supplements (June 2010).
2. Carol L. Wagner, Frank R. Greer, and the Section on Breastfeeding and Committee on Nutrition; Prevention of Rickets and Vitamin D Deficiency in Infants, Children, and Adolescents. Pediatrics November 2008; 122 (5): 1142–1152.
3. World Health Organization/Food and Agriculture Organization (WHO/FAO). Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation. Bangkok, Thailand; 2004, 21-30 September, 1998.
4. Institute of Medicine (U.S.), Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Dietary Reference Intakes: calcium and vitamin D. Washington, D.C.: National Academies Press; 2011.
5. "Vitamin D." aad.org, 2019, www.aad.org/media/stats-vitamin-d.
6. O'Keefe Proc. N.S. Inst. SCI (1986) volume 37, pp. 1-7.
7. Elad, D., et al. "Biomechanics of Milk Extraction during Breast-Feeding." Proceedings of the National Academy of Sciences, vol. 111, no. 14, 24 Mar. 2014, pp. 5230–5235; edited by Sheldon Weinbaum, The City College of New York, City University of New York, New York, NY, and approved February 25, 2014 (received for review October 24, 2013) located at: (www.pnas.org/content/111/14/5230, https://doi.org/10.1073/pnas.1319798111).
8. Colley J.R.T. Sucking and Swallowing in Infants. British Medical Journal. 1958 Aug 16.
9. "The British 'Limeys' Were Right: A Short History of Scurvy" Health.mil, 10 Jan. 2022, located at: (https://www.health.mil/News/Articles/2022/01/10/The-British-Limeys-Were-Right-A-Short-History-of-Scurvy#:~:text=Scurvy%2C%20a%20disease%20caused%20by,16th%20and%2018th%20centuries%20alone).
10. Aspirin Foundation. "The Story of Aspirin | the International Aspirin Foundation" located at: (www.aspirin-Foundation.mtcdevserver4.com, 2020, www.aspirin-foundation.com/history/the-aspirin-story/).
11. UpToDate Overview of Rickets: Topic 5856 Version 30.0
12. Vitamin D Deficiency in Children and Its Management: Review of Current Knowledge and Recommendations, Madhusmita Misra, MD, MPHa, Danie`le Pacaud, MDb, Anna Petryk, MDc, Paulo Ferrez Collett-

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    b. Paper 6 Patent Owner Preliminary Response filed 06/29/2023;
    c. Paper 7 Order: Conduct of the Proceeding 37 C.F.R. sec. 42.5 filed 08/04/2023;
    d. Paper 8 Petitioners Reply to Patent Owners Preliminary Response filed 08/11/2023;
    e. Paper 9 Patent Owners' Pre-institution Sur-reply filed 08/25/2023;
    f. Paper 10 Granting Institution of Inter Partes Review 35 U.S.C. § 314 filed 09/15/2023;
    g. Paper 11 Scheduling Order filed 09/15/2023;
    h. Paper 21 Updated PO Ex List 20231024 filed 10/24/2023;
    i. Paper 23 Conduct of the Proceeding 37 C.F.R. § 42.5 filed 10/30/2023;
    j. Paper 25 PO Updated Exhibit List filed 11/08/2023;
    k. Paper 26 Petitioners Motion to Submit Supplemental Information filed 11/09/2023;
    l. Paper 27 Petitioners Motion to Compel Routine Discovery filed 11/09/2023;
    m. Paper 28 Patent Owner Motion for Additional Discovery filed 11/09/2023;
    n. Paper 34 ORDER Conduct of the Proceeding Sua Sponte Expunging Exhibits 2016 and 2017 37 C.F.R. § 42.5 filed 11/17/2023;
    o. Paper 35 ORDER Granting Petitioner's Unopposed Motion to Submit Supplemental Information 37 C.F.R. § 42.123(a) filed 11/17/2023;
    p. Paper 36 Patent Owners Opposition to Motion to Compel filed 11/20/2023;

q. Paper 37 Petitioners Opposition to Motion for Additional Discovery filed 11/20/2023;
r. Paper 38 Petitioners Updated Exhibit List filed 11/20/2023;
s. Paper 43 Notice of Stipulation to Change Due dates 1, 2, and 3 filed 12/05/2023;
t. Paper 44 Denying Petitioner's Motion to Compel Routine Discovery, or in the Alternative, for Additional Discovery 37 C.F.R. § 42.51 filed 12/06/2023;
u. Paper 45 Granting Patent Owner's Motion for Additional Discovery 37 C.F.R. § 42.51(b)(2)(i) filed 12/06/2023;
v. Paper 46 Order: Conduct of the Proceeding filed 12/21/2023;
w. Paper 47 Joint Motion for Entry of Stipulated Protective Order filed 12/28/2023;
x. Paper 49 Patent Owners Motion for Additional Discovery - Depositions filed 12/28/2023;
y. Paper 51: Redacted Petitioners Opposition to Motion for Additional Discovery filed 01/04/2024;
z. Paper 52: Confidential Petitioners Opposition to Motion for Additional Discovery filed 01/04/2024;
aa. Paper 53: Order Granting-in-Part Patent Owner's Motion for Additional Discovery 37 C.F.R. § 42.51(b)(2) filed 01/11/2024;
bb. Transcript of Markman Hearing June 1, 2023, filed 06/29/2023
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dd. PTAB Transcript for Teleconference Hearing Before John G. New, Sheridan K. Snedden, And Cynthia M. Hardman, Administrative Patent Judges Dated October 27, 2023
ee. Transcript for the Deposition of Robert O. Williams, III, and Exhibits dated December 7, 2023.
ff. Transcript for the Deposition of David J. Sherman, and Exhibits dated December 5, 2023.
gg. Transcript for the Deposition of Stephen Benyunes, and Exhibits dated December 5, 2023.

16. <u>PTAB IPR Filed Exhibits:</u>
a. Exhibit 1001 U.S. Patent No. 9,066,958 File History filed 03/16/2023; Exhibit 1003 Complaint_1:22-cv-00332-GBW filed 03/16/2023; Exhibit 1004 Declaration of Dr. Robert Williams filed 03/16/2023; Exhibit 1005 Declaration of June Munford and Exhibits filed 03/16/2023; Exhibit 1006 Harder filed 03/16/2023; Exhibit 1007 Translation of Harder filed 03/16/2023; Exhibit 1008

Wolf filed 03/16/2023; Exhibit 1009 Translation of Wolf filed 03/16/2023; Exhibit 1010 European Pharmacopoeia Supplement 4.3 filed 03/16/2023; Exhibit 1011 Blass filed 03/16/2023; Exhibit 1012 Gartner filed 03/16/2023; Exhibit 1013 Wjst filed 03/16/2023; Exhibit 1014 Vieth filed 03/16/2023; Exhibit 1016 Bauer filed 03/16/2023; Exhibit 1017 Canadian Patent Application No. 2,558,202 filed 03/16/2023; Exhibit 1021 Declaration of Amy Tinworth filed 11/20/2023; Exhibit 1022 Supplemental Declaration of David Sherman filed 11/20/2023; Exhibit 1023 Declaration of Dr. Stephen Benyunes filed 11/20/2023; Exhibit 1024 Supplemental Declaration of Simon Liu filed 11/20/2023; Exhibit 1025 Declaration of June Munford filed 11/20/2023; Exhibit 2001 Docket Sheet: 1:22-cv-00332-GBW Ddrops Company et al v. MOM Enterprises, LLC, filed 06/29/2023; Exhibit 2004 Declaration of Chris Temovsky filed 06/29/2023; Exhibit 2005 Claim Construction Order filed 06/29/2023; Exhibit 2006 MARC Standards filed 06/29/2023; Exhibit 2007 USBLS Occupational Outlook Handbook-Interpreters and Translators filed 06/29/2023; Exhibit 2008 Declaration of Reinhold Vieth filed 06/29/2023; Exhibit 2009 Declaration of Warren D. Woessner filed 06/29/2023; Exhibit 2010; Exhibit 2001 Docket Sheet: 1:22-cv-00332-GBW Ddrops Company et al v. MOM Enterprises, LLC, filed 06/29/2023; Exhibit 2010 Transcript of Markman Hearing June 1, 2023, filed 06/29/2023; Exhibit 2011 Oxford English Reference Dictionary, 2nd Edition, the Oxford University Press, 1996 (Definitions of adhere and viscosity) filed 06/29/2023; Exhibit 2012 Anderson Declaration filed 10/24/2023; Exhibit 2013 Florence Declaration filed 10/24/2023; Exhibit 2014 Palen Declaration filed 10/24/2023; Exhibit 2015 Teleconference Hearing Transcript dated 11/08/2023; Exhibit 2017 Anderson Declaration in Support of Motion for Additional Discovery dated 11/09/2023; Exhibit 2018 Stipulated Proposed Protective Order filed 12/28/2023; Exhibit 2019 Redline of Stipulated Proposed Protective Order filed 12/28/2023; Exhibit 3001 filed 08/04/2023; Exhibit 3002 filed 10/30/2023; Exhibit 3003 filed 10/30/2023; Exhibit 3004 filed 10/30/2023; Exhibit 3005 filed 10/30/2023; Exhibit 3006 filed 12/21/2023.

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a. Declaration of Robert O. (Bill) Williams III, Ph.D. in Support of Defendants' Opposition to Plaintiffs' Renewed Motion for Preliminary Injunction dated April 28, 2017 (Doc. 71).
b. Declaration of David B. Conrad in Support of Defendants' Opposition to Plaintiffs' Renewed Motion for Preliminary Injunction and Exhibits dated April 28, 2017 (Doc. 67).
c. Second Declaration of Warren Woessner and Exhibits dated May 26, 2017 (Doc. 75);
d. 26-17 First Declaration of Reinhold Vieth and Exhibits dated May 26, 2017 (Doc. 76);

18. Ddrops Co. et al., v. MOM Ent., Civil Case No. Case 1:22-cv-00332 Documents:
    a. Complaint Ddrops Co. et al., v. MOM Ent., with Exhibits A-C, dated March 16, 2022;
    b. Defendant MOM Enterprises Answers and Affirmative Defenses to Complaint, dated April 28, 2022;
    c. Plaintiffs' Ddrops Answer to Counterclaims dated May 19, 2022;
    d. Protective Order (Doc. 25) dated August 25, 2022;
    e. Transcript for the Rule 30(b)(6) Deposition of Innovative Labs (Chantel Wood), and Exhibits Nos. 1-23, dated September 6, 2023;
    f. Plaintiffs' Identification of The Accused Products, Asserted Patent, and Damages Model dated September 15, 2023.
    g. Claim Construction Order (Doc. 72) dated June 2, 2023.
    h. Plaintiffs' Initial Disclosures dated August 19, 2022.
    i. Plaintiffs' Rule 3 ESI Disclosures dated September 6, 2022;
    j. Plaintiffs' Responses to 1st Set of Requests for Production dated March 17, 2023
    k. Plaintiffs' Responses to 1st Set of Interrogatories dated December 13, 2022
    l. Plaintiffs' Responses to 2nd Set of Requests for Production dated April 4, 2023
    m. Plaintiffs' Answers to 2nd Set of Interrogatories dated May 30, 2023
    n. Plaintiffs' Responses to 3rd Set of Requests for Production dated August 17, 2023
    o. Plaintiffs' Answers to 3rd Set of Interrogatories dated August 17, 2023
    p. Plaintiffs' Answers to Defendant's Fourth Set of Interrogatories dated September 15, 2023

q. Plaintiffs' Responses to Defendant's First Set of Requests for Admission dated September 15, 2023
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s. Plaintiffs' Amended Answers to Defendant's 1st Set of Interrogatories dated June 22, 2023
t. Defendant's Supplemental Disclosures and 1st Supplemental Responses to 1st Interrogatories dated 2023-09-18
u. Defendant's Objection and Responses to 1st Set of Discovery Interrogatories dated 2023- 01-26
v. Defendant's Responses to 4th Set Requests for Production (Nos. 79-90) dated 2023-08-18
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aa. Defendant's Responses to 2nd Requests for Production (Nos. 53-66) dated 2023-05-04
bb. Defendant's Responses to 3rd Set of Requests for Production; Nos. 67-78 dated 2023-07-17
cc. Defendant's Responses to 4th Set of Requests for Production; Nos. 79-90 dated 2023-08-17
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19. Ddrops' & Vieth Bates No. Documents:
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Exhibit C

REVIEW

100 YEARS OF VITAMIN D

Historical aspects of vitamin D

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This paper forms part of a special series on 100 Years of Vitamin D. The guest editors for this section were Josef Köhrle, Susan Lanham-New and Martina Rauner.

Abstract

Vitamin D has many physiological functions including upregulation of intestinal calcium and phosphate absorption, mobilization of bone resorption, renal reabsorption of calcium as well as actions on a variety of pleiotropic functions. It is believed that many of the hormonal effects of vitamin D involve a 1,25-dihydroxyvitamin D_3-vitamin D receptor-mediated transcriptional mechanism involving binding to the cellular chromatin and regulating hundreds of genes in many tissues. This comprehensive historical review provides a unique perspective of the many steps of the discovery of vitamin D and its deficiency disease, rickets, stretching from 1650 until the present. The overview is divided into four distinct historical phases which cover the major developments in the field and in the process highlighting the: (a) first recognition of rickets or vitamin D deficiency; (b) discovery of the nutritional factor, vitamin D and its chemical structure; (c) elucidation of vitamin D metabolites including the hormonal form, 1,25-dihydroxyvitamin D_3; (d) delineation of the vitamin D cellular machinery, functions and vitamin D-related diseases which focused on understanding the mechanism of action of vitamin D in its many target cells.

Key Words

- vitamin D
- vitamin D metabolism
- rickets and osteomalacia
- calcium and phosphate homeostasis
- vitamin D analogs
- vitamin D function
- 7-dehydrocholesterol
- UV light

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Introduction

The history of vitamin D is a rich and storied subject and is now over 350 years old. It began in the early 1600s with the first descriptions of the human deficiency disease: rickets in children and osteomalacia in adults. Of course, there were no precise medical details that distinguished it from other bone diseases, but treatises describing the symptoms and lithographs from that time showing bone deformities resembling rickets leave little doubt that it was vitamin D deficiency. It took another 250 years to define the cause of vitamin D deficiency in the 1900–1920 period when physicians and biochemists elucidated the role of sunlight and identified the chemical structure of the two main forms of the vitamin D molecule, vitamin D_2 and vitamin D_3.

Another 50 years elapsed before the metabolism of vitamin D was first described in 1967 and the active form of vitamin D, namely 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D$), was discovered. The period of time since has witnessed the exciting realization that vitamin D has its own set of dedicated specialized machinery consisting of transport proteins, metabolic enzymes and vitamin D receptor (VDR) to mediate the actions of vitamin D, not only in bone but also in many other tissues around the body where it has a myriad of different physiological effects.

Before we get into the history of vitamin D, let us first remind the reader of the general aspects of its nomenclature, origins and principal functions. Vitamin D is a steroidal substance required by all vertebrates including humans to maintain blood calcium and phosphate within a narrow normal range and thereby support a healthy skeleton, muscle contraction, immune function and optimal cellular functions in many locations around the body (1). The name vitamin D is a term coined by nutritionists, and

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is not a chemical term, which is defined as '*a substance with anti-rachitic properties that will cure rickets*'. In human biology, vitamin D usually refers to two substances: vitamin D_3 (usually known as cholecalciferol) of animal origin and vitamin D_2 (referred to as ergocalciferol) of plant or fungal origin. These two forms have roughly equal potencies, similar metabolic patterns and identical effects in the body.

Because of the four phases of vitamin D history, this review is divided into four sections each summarizing one particular time period:

1. 1650–1890: history of vitamin D deficiency (rickets)
2. 1890–1930: history of the discovery of vitamin D and its structural elucidation
3. 1930–1975: history of the discovery of vitamin D metabolites including $1,25\text{-}(OH)_2D_3$
4. 1975–present: history of the discovery of the vitamin D cellular machinery, functions and vitamin D-related human diseases.

Since the different facets of the history of vitamin D represent interesting topics and span many centuries, they have been reviewed by many previous historians, including the current author, and interested readers are invited to further access these because they focus on different aspects of the overall story (2, 3, 4, 5, 6, 7, 8).

1650–1890: history of vitamin D deficiency (rickets)

There is no doubt that rickets was prevalent in Europe long before it was recognized as a specific disease in the 15th century, but the earliest documentation of the word 'rickets' was in a domestic receipt book of an English family in 1632 and the earliest printed record of rickets as a disease causing death in the London Bill of Mortality in 1634 (reviewed by (2, 3, 4)). The term *rickets* is thought to have its origins in the verb in the Dorset dialect to '*rucket*,' which means to breathe with difficulty. However, some claim the term rickets is derived from the Anglo-Saxon word '*wrikken*,' meaning to twist. Rickets and osteomalacia were first clearly described by Daniel Whistler in the Netherlands (1645) as a condition in which the skeleton was poorly mineralized and deformed (9). Francis Glisson (1650) provided the first documented records with his book entitled *De Rachitide* first published in Latin in 1650 and then translated into English in 1671 (10). It features a lithograph of children with bowing of the legs and skeletal deformities which are the hallmarks



Figure 1
Lithograph from Glisson's *De Rachitide* (1671) (10) also depicted as the frontispiece of Hess AF's book (11) *Rickets Including Osteomalacia and Tetany*. Reproduced from the US National Library digital collection. Credit: Rickets, including osteomalacia and tetany / by Alfred F Hess.

of vitamin D deficiency. One of those Glisson lithographs was reproduced as a frontispiece in a landmark treatise on *Rickets Including Osteomalacia and Tetany* by AF Hess in 1929 (11). It is reproduced here as Fig. 1.

A more recent definition of vitamin D deficiency has grown to include defective chondrocyte differentiation and lack of mineralization of the growth plate, but the common feature of vitamin D deficiency is insufficiently mineralized or calcified bone matrix (1, 12, 13). Rickets is characterized by a deformed and misshaped skeleton, particularly bending and bowing of the long bones and enlargement of the epiphyses of the joints of the rib cage,

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arms, legs and neck. Victims have painful movements of the rib cage and difficulty breathing. In China, medical texts refer to deformities of the rib cage in severe rickets as 'chicken breast' (5). Severe rickets is often accompanied by pneumonia. The loss of the important role of vitamin D in strengthening the immune system compounds this problem. Though rarely is rickets life-threatening, it certainly lowers the quality of life for the afflicted individual and leads to secondary problems. One of these secondary effects of rickets occurs in young women who had vitamin D deficiency in childhood causing deformities of the pelvis which result in difficulties in childbirth (14). Shorter (14) speculates that rickets in early life must have resulted in numerous deaths of women during their first delivery.

Vitamin D deficiency is partly the result of inadequate skin synthesis of vitamin D_3 from 7-dehydrocholesterol compounded by a low dietary intake of vitamin D_2 from plant or fungal sources or vitamin D_3 from animal products. The advent of the Industrial Revolution in Western Europe heralded in massive air pollution in the form of smoke from mills and burning of fossil fuels. This dramatically reduced the amount of UV light reaching the ground. Since the workers needed for these new industrial jobs were required to move from their rural locations into dingy, poorly-lit cities, their exposure to UV light diminished and skin synthesis of vitamin D was reduced. Rickets resulted and was associated with lack of exposure to sufficient sunlight. Thus, the 18th and 19th centuries saw a higher increase in rickets in the industrialized cities of northern Europe. The Dickensian character Tiny Tim, of the novel *A Christmas Carol*, clearly represents a child with a deformed skeleton who must have been a common sight in the dark cities of the late 19th century (7). Rickets was particularly prevalent in the industrialized Britain of the 16th–20th centuries, and thus, it is no surprise that it was referred to in old texts as 'the English disease' (7, 15).

Despite the fact that rickets seemed to be associated with lack of exposure to sunlight, by the late 1700s, some, including Percival (16) in the UK, were advocating the use of cod liver oil for the treatment of rickets suggesting a nutritional aspect to vitamin D. In contrast, in the early 1800s, Sniadecki (17) in Poland was documenting the differential incidence in city-dwellers and rural-dwellers suggesting some environmental factor was involved. He speculated that sunlight or fresh air might be involved in the etiology of the disease. By the end of the 19th century, a rigorous debate roared on whether rickets was caused by the lack of some dietary substance or an environmental factor and how could these two points of view be reconciled.

1890–1930: history of the discovery of vitamin D and its structural elucidation

By the 1890s, some researchers such as Owen (18) and Palm (19), who clearly supported the environmental theory, produced evidence that there were big geographical differences in the incidence of rickets in different parts of the UK and northern and southern China. Palm, a medical missionary, went on to suggest that exposure of children to sunlight would cure rickets (19). Subsequently, researchers in Europe and the United States namely Buchholtz (1904), Raczynski (1913), Huldshinsky (1919), and later Chick (1922) and Hess & Weinstock (1924) performed experiments in which laboratory animals and children with rickets could be cured with sunlight or light from mercury arc lamps (7, 20, 21, 22, 23, 24). This clearly demonstrated that lack of exposure to UV light was one cause of rickets.

But the proponents of the theory that a dietary factor could also be involved continued with their experiments too. The early 20th century was a momentous period in nutritional research in which nutritionists showed that a diet of highly purified carbohydrates, protein, fat and salt is unable to fully support growth and life of experimental animals (25). By adding various 'trace factors', researchers were able to restore growth and a full range of physiological actions. The first of these trace factors was thiamin discovered by Funk (26) which cured neuritis in what Funk termed the '*vital amine or vitamin theory*.' Thiamin was later renamed vitamin B_1, but it was one of a number of vitamin substances that are defined as '*trace compounds which are derived from the diet and are required in small amounts per day and perform an essential role critical to life*.' Vitamin D was identified as one of these substances playing a critical role in skeletal growth and calcium and phosphate homeostasis. However, strictly speaking, vitamin D has been misnamed since it can also be derived from exposure to UV light and is not required to be in the diet. In practise and for a variety of social and religious reasons, many populations around the world do not receive adequate UV light, especially during the winter months, so that a dietary intake is essential.

The discovery of the nutritional factor, later termed vitamin D by McCollum (27), came largely as the result of the work of a number of researchers: Mellanby, McCollum, Steenbock and Hart working independently. Sir Edward Mellanby (28) in the UK reasoned that rickets might be due to a dietary deficiency and managed to produce beagle dogs with severe rickets by feeding them oatmeal and then cured their rickets with cod liver oil. Since cod liver oil is a mixture of lipids and a rich source of vitamin A, it was not clear what the active ingredient might be. McCollum (29),

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working first at the U Wisconsin and then Johns-Hopkins, heated and bubbled oxygen through the cod liver oil to destroy the vitamin A and found that the product still cured rickets. Building on the new vitamin nomenclature, he termed the new substance vitamin D. But how was the field to reconcile the apparently unconnected findings that UV light and a nutritional substance termed vitamin D could both cure rickets? Harry Steenbock also working at the U Wisconsin-Madison performed the definitive experiment. Steenbock & Black experimented with the diets of goats and found that sunlight or UV irradiation of the animals or their diets resulted in rickets being cured in the goats (30). Steenbock traced the bioactive substance in irradiated food to the non-saponifiable fraction of lipids in the diet and showed that it cured rickets (31). Dietary vitamin D was born.

Subsequently, Steenbock was able to show that irradiated yeast contained significant amounts of vitamin D, later shown to be vitamin D_2 and that the yeast could be irradiated and added to milk which formed the basis of the first food fortification with vitamin D (5). Though Steenbock and the University of Wisconsin filed a patent for milk fortification with vitamin D, the proceeds from this discovery were used to establish the Wisconsin Alumni Research Foundation (WARF) which was one of the prototypical organizations intended to allow universities to plough the benefits of their research into future research. WARF funded the research of a number of scientists inside and outside of the vitamin D field, included several Nobel laureates, with the proceeds of Steenbock's patent. Furthermore, vitamin D fortification of a variety of foodstuffs (including milk, margarine, bread and even beer) has become a major nutritional tool in the fight to prevent rickets and osteomalacia around the world (5).

In the late 1920s, Windaus and colleagues (32) isolated the key anti-rachitic substance from a mixture of irradiated plant sterols and named it vitamin D_1, although they did not identify its structure. Later, vitamin D_1 was shown to be a mixture of vitamin D_2 and tachysterol. A British group headed by Askew (33) successfully identified and determined the structure of the anti-rachitic, plant-derived sterol as vitamin D_2 or ergocalciferol. Windaus's group confirmed the structure of vitamin D_2 (34) and also isolated and identified the animal-derived, anti-rachitic vitamin D_3 or cholecalciferol and its skin precursor, 7-dehydrocholesterol (35). For his discovery of the structures of vitamin D_3, 7-dehydrocholesterol and several other sterols, Adolf Windaus was awarded the 1928 Nobel Prize for Chemistry (Fig. 2).

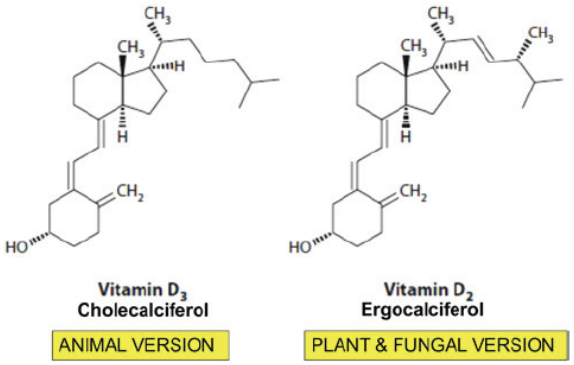


Figure 2
Structures of vitamin D_2 and D_3. The two versions of vitamin D differ only in their side chains vitamin D_2 possessing an additional C-22-23 double bond and a C-24 methyl group. The modifications make little significant difference in their metabolism or biological actions.

1930–1975: history of the discovery of vitamin D metabolites including 1,25-$(OH)_2D_3$

Chemically synthesized vitamin D_2 and vitamin D_3 have been available since the 1930s and paved the way for the study of their biological functions and metabolism. The physiological roles of vitamin D are primarily its roles in calcium and phosphate homeostasis (1) and include:

(1) stimulation of intestinal calcium and phosphate absorption;
(2) mobilization of calcium from bone;
(3) renal reabsorption of calcium.

All three of these functions serve to raise blood calcium and phosphate and ensure that these ions are available to ensure health and prevent rickets. Elucidating the details of these physiological functions became the main foci during the 1930–1960 time period, and research revealed that vitamin D was intimately connected to the roles of other calcium and phosphate-related hormones including parathyroid hormone (PTH) and calcitonin. Details of these connections are beyond the scope of this chapter and are described in reviews (1) and in other articles in this special series.

In the 1960s, there was considerable debate over whether the functions of vitamin D were carried out by vitamin D itself or its possible metabolites. Consequently, intense effort was put into studying the metabolism of vitamin D by using chemically synthesized radioactive versions of vitamin D_2 and vitamin D_3. The pioneer in this area was Egon Kodicek at the Dunn Nutritional Laboratories, U Cambridge UK. After 10 years of work, Kodicek (36) concluded that vitamin D was active without

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Table 1 History of the discovery of the major metabolites of vitamins D_2 and D_3.

Metabolite	Tissue source	Biosynthetic enzyme	Biological role	Discovery
Vitamin D_3 metabolites				
25-OH-D_3	Liver	25-Hydroxylase (CYP2R1)	Main circulating metabolite	Blunt *et al.* 1968 (38)
1,25-$(OH)_2D_3$	Kidney (major) Extra-renal sites	1α-Hydroxylase (CYP27B1)	Active hormonal form	Lawson *et al.* 1969 (39) Myrtle *et al.* 1970 (40) Holick *et al.* 1971 (41)
24,25-$(OH)_2D_3$	Kidney (major) Extra-renal sites	24-Hydroxylase (CYP24A1)	Principal catabolite	Suda *et al.* 1970a (48) Holick *et al.* 1972 (49)
25,26-$(OH)_2D_3$	Unknown	26-Hydroxylase (?)	Catabolite	Suda *et al.* 1970b (50)
25-OH-D_3-26,23-lactone	Kidney (major) Extra-renal sites	24-Hydroxylase (CYP24A1)	Presumed catabolite	Wichmann *et al.* 1979 (51)
1,24,25-$(OH)_3D_3$	Kidney (major) Extra-renal sites	24-Hydroxylase (CYP24A1)	Unknown possible catabolite	Holick *et al.* 1974 (52)
Calcitroic acid	Kidney (major) Extra-renal sites	24-Hydroxylase (CYP24A1)	Excretory form	Esvelt *et al.* 1981 (53)
Calcioic acid	Kidney (major)	24-Hydroxylase (CYP24A1)	Excretory form	Kaufmann *et al.* 2019 (76)
4α,25-$(OH)_2D_3$ 4β,25-$(OH)_2D_3$	Liver	General cytochrome P450 (CYP3A4)	Excretory form	Wang *et al.* 2013 (77)
Vitamin D_2 metabolites				
25-OH-D_2	Liver	25-Hydroxylase (CYP2R1)	Main circulating metabolite	Suda *et al.* 1969 (45)
1,25-$(OH)_2D_2$	Kidney (major)	1α-Hydroxylase (CYP27B1)	Active hormonal form	Jones *et al.* 1975 (46)
24,25-$(OH)_2D_2$	Kidney (major)	24-Hydroxylase (CYP24A1)	Principal catabolite	Jones *et al.* 1980 (47)
1,24,25-$(OH)_3D_2$	Kidney (major)	24-Hydroxylase (CYP24A1)	Presumed catabolite	Reddy *et al.* 1986 (78)

being metabolized. In retrospect, the radioactive vitamin D that his group were using was insufficiently labeled to detect its metabolites. However, Hector DeLuca, again at the U Wisconsin-Madison, and the final graduate student of Harry Steenbock, synthesized radioactive vitamin D_3 with much higher specific activity (37) and was able to demonstrate metabolism to more polar metabolites, the principal one being 25-hydroxyvitamin D_3 (25-OH-D_3) (38) made in the liver and the first identified natural vitamin D metabolite.

25-OH-D_3 proved to be more potent biologically than vitamin D_3 and was present in the bloodstream at a higher concentration (38). We now identify 25-OH-D_3 as the principal circulating form of vitamin D. But that is not the extent of vitamin D metabolism. Several other groups then entered or re-entered the picture, including Dr Kodicek's, as well as that of one of Dr DeLuca's former graduate students Dr Anthony Norman. Among the other polar products of vitamin D_3 was a metabolite even more potent than 25-OH-D_3, namely 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) which is now universally accepted as the hormonal form of vitamin D_3. Several groups including Dr Kodicek's, (39) Dr Norman's (40) and Dr DeLuca's (41) were credited with playing a role in the discovery and/or

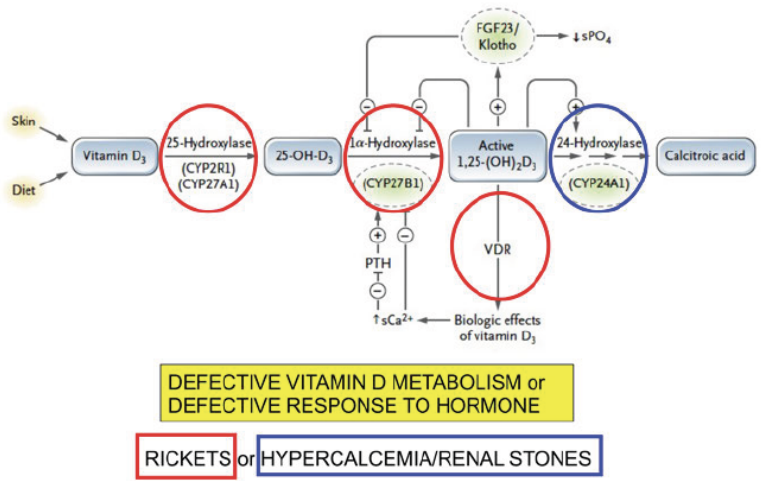


Figure 3
Metabolism and mechanism of action of vitamin D_3. Skin-synthesized or dietary vitamin D_3 is converted via a two-step hydroxylation process into the active hormonal form 1,25-$(OH)_2D_3$. The hormone binds to the vitamin D receptor (VDR) and regulates serum calcium (sCa^{2+}) and serum phosphate (sPO_4) levels ensuring sufficient minerals for normal cellular activity around the body including bone. Insufficient vitamin D results in insufficient 1,25-$(OH)_2D_3$ and vitamin deficiency rickets. Circled in red are the proteins in the vitamin D-specific machinery that when mutated also result in some type of rickets. Circled in blue is the enzyme CYP24A1 that when mutated results in elevated 1,25-$(OH)_2D_3$ and hypercalcemia and/or kidney stones.

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Table 2 History of the main protein components of the specific* vitamin D signal transduction machinery.

Protein	Abbreviation	Tissue location or source	Biological function	Discovery	Gene cloning
Vitamin D-binding globulin	DBP	Liver	Transport of vitamin D and its metabolites	Daiger *et al.* 1975 (64)	Cooke *et al.* 1991 (79)
Vitamin D receptor	VDR	Most tissues except liver	Regulation of vitamin D-dependent genes	Haussler 1969 (80) Brumbaugh *et al.* 1975 (55)	McDonnell *et al.* 1987 (56)
25-Hydroxylase	CYP2R1	Liver	25-hydroxylation of vitamins D_2 and D_3	Cheng *et al.* 2003 (81)	Cheng *et al.* 2004 (75)
1α-Hydroxylase	CYP27B1	Kidney (major) Extra-renal sites	1α-hydroxylation of 25-OH-D_2 & 25-OH-D_3	Fraser *et al.* 1970 (42)	St-Arnaud *et al.* 1997 (70) Takeyama *et al.* 1997 (71)
24-Hydroxylase	CYP24A1	Kidney (major) Extra-renal sites	24-hydroxylation of (& 23- & 26-hydroxylation) 25-OH-D_2 & 25-OH-D_3 Complete catabolism of vitamin D	Knutson *et al.* 1972 (66)	Ohyama & Okuda 1991 (72)

Other cellular proteins play a general role in vitamin D metabolism and action, for example, CYP3A4 but this degrades many other molecules and drugs.
*The specific vitamin D signal transduction machinery is specialized to transport, activate, mediate the biological effects of and catabolize vitamin D.

in the structural identification of 1,25-$(OH)_2D_3$. Kodicek's group administered a mixture of radioactive [4-^{14}C] and [1-^{3}H]vitamin D_3 preparations and showed that one polar metabolite lost its tritium atom during metabolism that aided in its identification as a 1-hydroxylated compound (39). Furthermore, the Cambridge group also showed that the hormone was biologically generated in the kidney (39, 42). Dr Norman's group showed that the new metabolite was associated with the chromatin of intestinal mucosal cells and had greater biological activity than even 25-OH-D_3 (40). Holick *et al.* (41) showed that the additional 1-hydroxyl group was in the 1α orientation and supported their identification of the metabolite as 1α,25-$(OH)_2D_3$ with mass spectrometry. Chemically synthesized 1,25-$(OH)_2D_3$ was first produced by Semmler *et al.* (43) and made commercially by a group headed by Dr Milan Uskokovic at Hoffmann-La Roche in the early 1970s and is known clinically by the name calcitriol (44).

The identification of the principal metabolites, 25-OH-D_3 and 1,25-$(OH)_2D_3$, spawned a frenzy of research activity in the vitamin D area and the discovery of a number of other vitamin D metabolites (1). Among these are the principal metabolites of vitamin D_2 including 25-OH-D_2 (45), 1,25-$(OH)_2D_2$ (46) and 24,25-$(OH)_2D_2$ (47). Also identified in that mixture of metabolites arising from radioactive vitamin D_3 were several compounds that are presumed to be inactive catabolites including, 24,25-$(OH)_2D_3$, 25,26-$(OH)_2D_3$, 25-OH-D_3-26,23-lactone, 1,24,25-$(OH)_3D_3$ and calcitroic acid (48, 49, 50, 51, 52, 53). A summary of the main metabolites of both vitamin D_3 and vitamin D_2 along with their tissue source, biosynthetic enzyme, details of first reporting and biological role is presented in Table 1 and depicted in a metabolic pathway diagram (Fig. 3).

1975–present: history of the discovery of the vitamin D cellular machinery, functions and vitamin D-related human diseases

The discovery of the active forms of vitamin D heralded in a search for

(a) the signal transduction mechanisms to explain how 1,25-$(OH)_2D_3$ was able to produce its various biological effects;
(b) identification of the enzymes responsible for the synthesis and catabolism of 1,25-$(OH)_2D_3$;
(c) a clear understanding of the regulation of the vitamin D endocrine system.

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Table 3 History of the main vitamin D-related genetic and acquired human diseases and animal models generated to study them.

Disease	Cause	Initial report	Animal model equivalent	Generated by
Vitamin D deficiency rickets	Lack of dietary vitamin D Lack of skin synthesis of D	F Glisson 1671 (10)	Beagle dog on oatmeal diet Lactating goat model	Mellanby 1919 (28) Steenbock & Black 1924 (30)
Vitamin D dependency rickets type 1A	Genetic defect in CYP27B1	Fraser *et al.* 1972 (82)	CYP27B1 null mouse	Kato 1999 (83) Panda *et al.* 2001 (84) St-Arnaud *et al.* 2003 (85)
Vitamin D dependency rickets type 1B	Genetic defect in CYP2R1	Cheng *et al.* 2004 (75)	CYP2R1 null mouse	Zhu *et al.* 2013 (86)
Vitamin D dependency rickets type 2	Genetic defect in VDR	Rosen *et al.* 1979 (87) Eil *et al.* 1981 (88)	VDR null mouse	Yoshizawa *et al.* 1997 (89) Li *et al.* 1998 (90)
Idiopathic infantile hypercalcemia	Genetic defect in CYP24A1	Lightwood 1953 (91) Schlingmann *et al.* 2011 (92)	CYP24A1 null mouse	St-Arnaud *et al.* 2000 (93)
Chronic kidney disease	Loss of Kidney CYP27B1 enzyme activity	DeLuca & Avioli 1970 (94) Brickman *et al.* 1974 (95)	Dog nephrectomy models	Rutherford *et al.* 1977 (96)

These studies began almost as soon as metabolism was recognized in the late 1960s when Mark Haussler, in AW Norman's laboratory, demonstrated that vitamin D metabolites were associated with the chromatin (54). Clear evidence of the protein that is now termed the vitamin D receptor (VDR) was produced by Haussler's lab (55). The VDR protein from various species was later purified and its gene was cloned by Haussler's group (56, 57). Study of the pure protein has led to a determination of its crystal structure (58). Parallel to these investigations of the VDR have come other studies on how it works both at the whole-body level in calcium and phosphate homeostasis and other pleiotropic functions (1, 8, 59) and at the cellular level in a classic steroid hormone super-family like process through a transcriptional mechanism (60). Over the past 30 years, Mark Haussler, Wes Pike and colleagues (61) have demonstrated that 1,25-$(OH)_2D_3$ works through a VDR-mediated mechanism that involves many coactivators and repressors to directly interact with and regulate hundreds of genes around the body. Other researchers, most notably Anthony Norman (62), have proposed that some of the actions of vitamin D occur through rapid non-genomic signaling pathways, possibly involving a plasma membrane VDR but this protein has never been fully characterized at the molecular level. Nevertheless, there remains some uncertainty that all vitamin D ligands and analogs produce their effects through a genomic VDR mechanism (63).

The history of two other components of the vitamin D machinery deserves some mention.

These are vitamin D-binding globulin (64, 65) and the cytochromes P450-containing enzymes that metabolize vitamin D into its many metabolites (66). Being a fat-soluble vitamin, vitamin D requires a protein to transport it around the body and the vitamin D-binding globulin (usually abbreviated as DBP) performs this function. DBP was first identified as Gc (group-specific component) in the 1970s, and its properties have been reviewed extensively by the father figure of the field Roger Bouillon, U Leuven, Belgium (65). DBP has a high affinity for most of the main metabolites of vitamin D, most notably 25-OH-D, and because of this, 25-OH-D is the main circulating form in the blood.

The cytochrome P450-containing enzymes (CYPs) responsible for vitamin D metabolism were first studied in the early 1970s in tissue extracts of liver and kidney (67, 68, 69) and then in tissue culture and given names based upon their hydroxylation activity: 25-hydroxylase, 1α-hydroxylase and 24-hydroxylase. In the early 1990–2005 period, all three enzymes were purified, cloned and expressed in cell culture systems, principally by Canadian group of St-Arnaud (70) as well as the Japanese groups of Kato S (71), Okuda (72) and Sakaki (73, 74) as well as Russell's group at the U Texas (75). The three enzymes are now known as CYP2R1, CYP27B1 and CYP24A1. A review of the CYP field and how these enzymes operate and how they are regulated is provided (66). A summary of the history of the signal transduction protein machinery for vitamin D including VDR, DBP and the various CYPs is provided in Table 2.

No review of the recent history of vitamin D would be complete without an overview of how defects in vitamin D metabolism result in human disease. It is now evident that vitamin D deficiency and rickets are caused by several genetic and acquired errors in vitamin D metabolism which involve any of the major protein components of the

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Table 4 History of the commercially approved vitamin D drugs (vitamin D analogs) used to treat rickets and related diseases.

Vitamin D analog	Drug name	Marketed by	Field of use*	Initial report	Comments
25-OH-D_3	Calderol	Organon	Vitamin deficiency	Blunt & DeLuca 1969 (97)	First vitamin D metabolite
	Rayaldee	OPKO Renal	Chronic kidney disease		Licensed by Upjohn, Kalamazoo
1,25-$(OH)_2D_3$	Calcijex	Roche	Vitamin D dependency type 1A	Semmler *et al.* 1972 (43)	First vitamin D active analog
	Generic		Chronic kidney disease		
1α-OH-D_3	One-alpha	Leo Pharma	Vitamin D deficiency	Holick *et al.* 1973 (98)	1-hydroxylated prodrug not requiring activation by kidney
	Alfacalcidiol		Chronic kiidney disease	Barton *et al.* 1973 (99)	
1α-OH-D_2	Hectorol	Genzyme/Sanofi	Chronic kidney disease	Lam *et al.* 1974 (100)	1-hydroxylated prodrug not requiring activation by kidney
	Doxercalciferol	Sandoz			
19-nor-1,25-$(OH)_2D_2$	Paricalcitol	Abbott	Chronic kidney disease	Takahashi F *et al.* 1997 (101)	Active 'low-calcemic' vitamin D analog
Calcipotriol	Daivonex	Leo Pharma	Psoriasis	Calverley 1987 (102)	Topical rapidly metabolized side-chain modified vitamin D analog

*Many of the vitamin D drugs used in chronic kdney disease stages 3–4 and beyond are used to suppress secondary hyperparathyroidism, as well as having a moderate serum calcium-raising activity.

vitamin D machinery described above. These are compiled into Table 3 where we document the disease name, the component of the vitamin D machinery affected, as well as the publication first describing it. Besides diseases involving too little 1,25-$(OH)_2D_3$ and resulting in rickets, diseases involving too much 1,25-$(OH)_2D_3$ which cause hypercalcemia are also included in Table 3. Most of these diseases involving a shortage of 1,25-$(OH)_2D_3$ are now treated with vitamin D analogs which were developed from knowledge of the metabolism and biological actions of vitamin D. Currently approved and marketed vitamin D analogs are listed in Table 4 along with their original publications.

Conclusions

The history of vitamin D is indeed a rich subject which has already stretched over 350 years and involved the four phases described in this review. While the chemical entity vitamin D remained unknown for all but 100 of those years, the significant medical consequences of vitamin D deficiency were evident for the whole of that time. Many physicians, nutritionists, biochemists, chemists and molecular biologists have worked to elucidate our current knowledge of the nature of vitamin D in addition to its metabolism, mechanism of action and biological activities. That knowledge has paid dividends by providing new therapies for the treatment of deficiency and excess vitamin D action. The field of vitamin D research is arguably one of the highlights of modern medicine.

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Exhibit D

National Institutes of Health
Office of Dietary Supplements

Vitamin D

Fact Sheet for Health Professionals

For information on vitamin D and COVID-19, see Dietary Supplements in the Time of COVID-19.

Introduction

Vitamin D (also referred to as calciferol) is a fat-soluble vitamin that is naturally present in a few foods, added to others, and available as a dietary supplement. It is also produced endogenously when ultraviolet (UV) rays from sunlight strike the skin and trigger vitamin D synthesis.

Vitamin D obtained from sun exposure, foods, and supplements is biologically inert and must undergo two hydroxylations in the body for activation. The first hydroxylation, which occurs in the liver, converts vitamin D to 25-hydroxyvitamin D [25(OH)D], also known as calcidiol. The second hydroxylation occurs primarily in the kidney and forms the physiologically active 1,25-dihydroxyvitamin D [1,25(OH)2D], also known as calcitriol [1].

Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal bone mineralization and to prevent hypocalcemic tetany (involuntary contraction of muscles, leading to cramps and spasms). It is also needed for bone growth and bone remodeling by osteoblasts and osteoclasts [1-3]. Without sufficient vitamin D, bones can become thin, brittle, or misshapen. Vitamin D sufficiency prevents rickets in children and osteomalacia in adults. Together with calcium, vitamin D also helps protect older adults from osteoporosis.

Vitamin D has other roles in the body, including reduction of inflammation as well as modulation of such processes as cell growth, neuromuscular and immune function, and glucose metabolism [1-3]. Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated in part by vitamin D. Many tissues have vitamin D receptors, and some convert 25(OH)D to 1,25(OH)2D.

In foods and dietary supplements, vitamin D has two main forms, D_2 (ergocalciferol) and D_3 (cholecalciferol), that differ chemically only in their side-chain structures. Both forms are well absorbed in the small intestine. Absorption occurs by simple passive diffusion and by a mechanism that involves intestinal membrane carrier proteins [4]. The concurrent presence of fat in the gut enhances vitamin D absorption, but some vitamin D is absorbed even without dietary fat. Neither aging nor obesity alters vitamin D absorption from the gut [4].

Serum concentration of 25(OH)D is currently the main indicator of vitamin D status. It reflects vitamin D produced endogenously and that obtained from foods and supplements [1]. In serum, 25(OH)D has a fairly long circulating half-life of 15 days [1]. Serum concentrations of 25(OH)D are reported in both

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nanomoles per liter (nmol/L) and nanograms per milliliter (ng/mL). One nmol/L is equal to 0.4 ng/mL, and 1 ng/mL is equal to 2.5 nmol/L.

Assessing vitamin D status by measuring serum 25(OH)D concentrations is complicated by the considerable variability of the available assays (the two most common ones involve antibodies or chromatography) used by laboratories that conduct the analyses [5,6]. As a result, a finding can be falsely low or falsely high, depending on the assay used and the laboratory. The international Vitamin D Standardization Program has developed procedures for standardizing the laboratory measurement of 25(OH)D to improve clinical and public health practice [5,7-10].

In contrast to 25(OH)D, circulating 1,25(OH)2D is generally not a good indicator of vitamin D status because it has a short half-life measured in hours, and serum levels are tightly regulated by parathyroid hormone, calcium, and phosphate [1]. Levels of 1,25(OH)2D do not typically decrease until vitamin D deficiency is severe [2].

Serum concentrations of 25(OH)D and health

Although 25(OH)D functions as a biomarker of exposure, the extent to which 25(OH)D levels also serve as a biomarker of effect on the body (i.e., relating to health status or outcomes) is not clear [1,3].

Researchers have not definitively identified serum concentrations of 25(OH)D associated with deficiency (e.g., rickets), adequacy for bone health, and overall health. After reviewing data on vitamin D needs, an expert committee of the Food and Nutrition Board (FNB) at the National Academies of Sciences, Engineering, and Medicine (NASEM) concluded that people are at risk of vitamin D deficiency at serum 25(OH)D concentrations less than 30 nmol/L (12 ng/mL; see Table 1 for definitions of deficiency and inadequacy) [1]. Some people are potentially at risk of inadequacy at 30 to 50 nmol/L (12–20 ng/mL). Levels of 50 nmol/L (20 ng/mL) or more are sufficient for most people. In contrast, the Endocrine Society stated that, for clinical practice, a serum 25(OH)D concentration of more than 75 nmol/L (30 ng/mL) is necessary to maximize the effect of vitamin D on calcium, bone, and muscle metabolism [11,12]. The FNB committee also noted that serum concentrations greater than 125 nmol/L (50 ng/mL) can be associated with adverse effects [1] (Table 1).

Table 1: Serum 25-Hydroxyvitamin D [25(OH)D] Concentrations and Health [1]

nmol/L*	ng/mL*	Health status
<30	<12	Associated with vitamin D deficiency, which can lead to rickets in infants and children and osteomalacia in adults
30 to <50	12 to <20	Generally considered inadequate for bone and overall health in healthy individuals
≥50	≥20	Generally considered adequate for bone and overall health in healthy individuals
>125	>50	Linked to potential adverse effects, particularly at >150 nmol/L (>60 ng/mL)

*Serum concentrations of 25(OH)D are reported in both nanomoles per liter (nmol/L) and nanograms per milliliter (ng/mL). One nmol/L = 0.4 ng/mL, and 1 ng/mL = 2.5 nmol/L.

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Optimal serum concentrations of 25(OH)D for bone and general health have not been established because they are likely to vary by stage of life, by race and ethnicity, and with each physiological measure used [1,13,14]. In addition, although 25(OH)D levels rise in response to increased vitamin D intake, the relationship is nonlinear [1]. The amount of increase varies, for example, by baseline serum levels and duration of supplementation.

Recommended Intakes

Intake recommendations for vitamin D and other nutrients are provided in the Dietary Reference Intakes (DRIs) developed by expert committees of NASEM [1]. DRI is the general term for a set of reference values used for planning and assessing nutrient intakes of healthy people. These values, which vary by age and sex, include the following:

- Recommended Dietary Allowance (RDA): Average daily level of intake sufficient to meet the nutrient requirements of nearly all (97%–98%) healthy individuals; often used to plan nutritionally adequate diets for individuals
- Adequate Intake (AI): Intake at this level is assumed to ensure nutritional adequacy; established when evidence is insufficient to develop an RDA
- Estimated Average Requirement (EAR): Average daily level of intake estimated to meet the requirements of 50% of healthy individuals; usually used to assess the nutrient intakes of groups of people and to plan nutritionally adequate diets for them; can also be used to assess the nutrient intakes of individuals
- Tolerable Upper Intake Level (UL): Maximum daily intake unlikely to cause adverse health effects

An FNB committee established RDAs for vitamin D to indicate daily intakes sufficient to maintain bone health and normal calcium metabolism in healthy people. RDAs for vitamin D are listed in both micrograms (mcg) and International Units (IU); 1 mcg vitamin D is equal to 40 IU (Table 2). Even though sunlight is a major source of vitamin D for some people, the FNB based the vitamin D RDAs on the assumption that people receive minimal sun exposure [1]. For infants, the FNB committee developed AIs based on the amount of vitamin D that maintains serum 25(OH)D levels above 20 ng/mL (50 nmol/L) and supports bone development.

Table 2: Recommended Dietary Allowances (RDAs) for Vitamin D [1]

Age	Male	Female	Pregnancy	Lactation
0-12 months*	10 mcg (400 IU)	10 mcg (400 IU)		
1–13 years	15 mcg (600 IU)	15 mcg (600 IU)		
14–18 years	15 mcg (600 IU)	15 mcg (600 IU)	15 mcg (600 IU)	15 mcg (600 IU)
19–50 years	15 mcg (600 IU)	15 mcg (600 IU)	15 mcg (600 IU)	15 mcg (600 IU)

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Age	Male	Female	Pregnancy	Lactation
51–70 years	15 mcg (600 IU)	15 mcg (600 IU)		
>70 years	20 mcg (800 IU)	20 mcg (800 IU)		

*Adequate Intake (AI)

Many other countries around the world and some professional societies have somewhat different guidelines for vitamin D intakes [15]. These differences are a result of an incomplete understanding of the biology and clinical implications of vitamin D, different purposes for the guidelines (e.g., for public health in a healthy population or for clinical practice), and/or the use in some guidelines of observational studies in addition to randomized clinical trials to establish recommendations [9,15]. The Endocrine Society states, for example, that to maintain serum 25(OH)D levels above 75 nmol/L (30 ng/mL), adults might need at least 37.5 to 50 mcg (1,500–2,000 IU)/day of supplemental vitamin D, and children and adolescents might need at least 25 mcg (1,000 IU)/day [11]. In contrast, the United Kingdom government recommends intakes of 10 mcg (400 IU)/day for its citizens age 4 years and older [16].

Sources of Vitamin D

Food

Few foods naturally contain vitamin D. The flesh of fatty fish (such as trout, salmon, tuna, and mackerel) and fish liver oils are among the best sources [17,1]. An animal's diet affects the amount of vitamin D in its tissues. Beef liver, egg yolks, and cheese have small amounts of vitamin D, primarily in the form of vitamin D_3 and its metabolite 25(OH)D_3. Mushrooms provide variable amounts of vitamin D_2 [17]. Some mushrooms available on the market have been treated with UV light to increase their levels of vitamin D_2. In addition, the Food and Drug Administration (FDA) has approved UV-treated mushroom powder as a food additive for use as a source of vitamin D_2 in food products [18]. Very limited evidence suggests no substantial differences in the bioavailability of vitamin D from various foods [19].

Animal-based foods typically provide some vitamin D in the form of 25(OH)D in addition to vitamin D_3. The impact of this form on vitamin D status is an emerging area of research. Studies show that 25(OH)D appears to be approximately five times more potent than the parent vitamin for raising serum 25(OH)D concentrations [17,20,21]. One study found that when the 25(OH)D content of beef, pork, chicken, turkey, and eggs is taken into account, the total amount of vitamin D in the food is 2 to 18 times higher than the amount in the parent vitamin alone, depending on the food [20].

Fortified foods provide most of the vitamin D in American diets [1,22]. For example, almost all of the U.S. milk supply is voluntarily fortified with about 3 mcg/cup (120 IU), usually in the form of vitamin D_3 [23]. In Canada, milk must be fortified with 0.88–1.0 mcg/100 mL (35–40 IU), and the required amount for margarine is at least 13.25 mcg/100 g (530 IU). Other dairy products made from milk, such as

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cheese and ice cream, are not usually fortified in the United States or Canada. Plant milk alternatives (such as beverages made from soy, almond, or oats) are often fortified with similar amounts of vitamin D to those in fortified cow's milk (about 3 mcg [120 IU]/cup); the Nutrition Facts label lists the actual amount [24]. Ready-to-eat breakfast cereals often contain added vitamin D, as do some brands of orange juice, yogurt, margarine, and other food products.

The United States mandates the fortification of infant formula with 1–2.5 mcg/100 kcal (40–100 IU) vitamin D; 1–2 mcg/100 kcal (40–80 IU) is the required amount in Canada [1].

A variety of foods and their vitamin D levels per serving are listed in Table 3.

Table 3: Vitamin D Content of Selected Foods [25]

Food	Micrograms (mcg) per serving	International Units (IU) per serving	Percent DV*
Cod liver oil, 1 tablespoon	34.0	1,360	170
Trout (rainbow), farmed, cooked, 3 ounces	16.2	645	81
Salmon (sockeye), cooked, 3 ounces	14.2	570	71
Mushrooms, white, raw, sliced, exposed to UV light, ½ cup	9.2	366	46
Milk, 2% milkfat, vitamin D fortified, 1 cup	2.9	120	15
Soy, almond, and oat milks, vitamin D fortified, various brands, 1 cup	2.5–3.6	100–144	13–18
Ready-to-eat cereal, fortified with 10% of the DV for vitamin D, 1 serving	2.0	80	10
Sardines (Atlantic), canned in oil, drained, 2 sardines	1.2	46	6
Egg, 1 large, scrambled**	1.1	44	6
Liver, beef, braised, 3 ounces	1.0	42	5
Tuna fish (light), canned in water, drained, 3 ounces	1.0	40	5
Cheese, cheddar, 1.5 ounce	0.4	17	2
Mushrooms, portabella, raw, diced, ½ cup	0.1	4	1
Chicken breast, roasted, 3 ounces	0.1	4	1
Beef, ground, 90% lean, broiled, 3 ounces	0	1.7	0
Broccoli, raw, chopped, ½ cup	0	0	0
Carrots, raw, chopped, ½ cup	0	0	0
Almonds, dry roasted, 1 ounce	0	0	0
Apple, large	0	0	0
Banana, large	0	0	0
Rice, brown, long-grain, cooked, 1 cup	0	0	0
Whole wheat bread, 1 slice	0	0	0
Lentils, boiled, ½ cup	0	0	0
Sunflower seeds, roasted, ½ cup	0	0	0

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Food	Micrograms (mcg) per serving	International Units (IU) per serving	Percent DV*
Edamame, shelled, cooked, ½ cup	0	0	0

* DV = Daily Value. The FDA developed DVs to help consumers compare the nutrient contents of foods and dietary supplements within the context of a total diet. The DV for vitamin D is 20 mcg (800 IU) for adults and children age 4 years and older [26]. The labels must list vitamin D content in mcg per serving and have the option of also listing the amount in IUs in parentheses. Foods providing 20% or more of the DV are considered to be high sources of a nutrient, but foods providing lower percentages of the DV also contribute to a healthful diet.
** Vitamin D is in the yolk.

The U.S. Department of Agriculture's (USDA's) FoodData Central (https://fdc.nal.usda.gov/) lists the nutrient content of many foods and provides a comprehensive list of foods containing vitamin D arranged by nutrient content (https://ods.od.nih.gov/pubs/usdandb/VitaminD-Content.pdf) and by food name (https://ods.od.nih.gov/pubs/usdandb/VitaminD-Food.pdf). However, FoodData Central does not include the amounts of 25(OH)D in foods.

Sun exposure

Most people in the world meet at least some of their vitamin D needs through exposure to sunlight [1]. Type B UV (UVB) radiation with a wavelength of approximately 290–320 nanometers penetrates uncovered skin and converts cutaneous 7-dehydrocholesterol to previtamin D_3, which in turn becomes vitamin D_3. Season, time of day, length of day, cloud cover, smog, skin melanin content, and sunscreen are among the factors that affect UV radiation exposure and vitamin D synthesis. Older people and people with dark skin are less able to produce vitamin D from sunlight [1]. UVB radiation does not penetrate glass, so exposure to sunshine indoors through a window does not produce vitamin D [27].

The factors that affect UV radiation exposure, individual responsiveness, and uncertainties about the amount of sun exposure needed to maintain adequate vitamin D levels make it difficult to provide guidelines on how much sun exposure is required for sufficient vitamin D synthesis [15,28]. Some expert bodies and vitamin D researchers suggest, for example, that approximately 5–30 minutes of sun exposure, particularly between 10 a.m. and 4 p.m., either daily or at least twice a week to the face, arms, hands, and legs without sunscreen usually leads to sufficient vitamin D synthesis [13,15,28]. Moderate use of commercial tanning beds that emit 2% to 6% UVB radiation is also effective [13,29].

However, despite the importance of the sun for vitamin D synthesis, limiting skin exposure to sunlight and UV radiation from tanning beds is prudent [28]. UV radiation is a carcinogen, and UV exposure is the most preventable cause of skin cancer. Federal agencies and national organizations advise taking photoprotective measures to reduce the risk of skin cancer, including using sunscreen with a sun protection factor (SPF) of 15 or higher, whenever people are exposed to the sun [28,30]. Sunscreens with an SPF of 8 or more appear to block vitamin D-producing UV rays. In practice, however, people usually do not apply sufficient amounts of sunscreen, cover all sun-exposed skin, or reapply sunscreen

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regularly. Their skin probably synthesizes some vitamin D, even with typically applied sunscreen amounts [1,28].

Dietary supplements

Dietary supplements can contain vitamins D_2 or D_3. Vitamin D_2 is manufactured using UV irradiation of ergosterol in yeast, and vitamin D_3 is typically produced with irradiation of 7-dehydrocholesterol from lanolin obtained from the wool of sheep [13,31]. An animal-free version of vitamin D3 sourced from lichen is also available [32]. People who avoid all animal-sourced products can contact dietary supplement manufacturers to ask about their sourcing and processing techniques.

Both vitamins D2 and D3 raise serum 25(OH)D levels, and they seem to have equivalent ability to cure rickets [4]. In addition, most steps in the metabolism and actions of vitamins D_2 and D_3 are identical. However, most evidence indicates that vitamin D_3 increases serum 25(OH)D levels to a greater extent and maintains these higher levels longer than vitamin D_2, even though both forms are well absorbed in the gut [33-36].

Some studies have used dietary supplements containing the 25(OH)D_3 form of vitamin D. Per equivalent microgram dose, 25(OH)D_3 is three to five times as potent as vitamin D_3 [37,38]. However, no 25(OH)D_3 dietary supplements appear to be available to consumers on the U.S. market at this time [32].

Vitamin D Intakes and Status

Most people in the United States consume less than recommended amounts of vitamin D. An analysis of data from the 2015–2016 National Health and Nutrition Examination Survey (NHANES) found that average daily vitamin D intakes from foods and beverages were 5.1 mcg (204 IU) in men, 4.2 mcg (168 IU) in women, and 4.9 mcg (196 IU) in children age 2–19 years [39]. In fact, 2013–2016 NHANES data showed that 92% of men, more than 97% of women, and 94% of people age 1 year and older ingested less than the EAR of 10 mcg (400 IU) of vitamin D from food and beverages [40].

The analysis of 2015–2016 data also showed that 28% of all individuals age 2 years and older in the United States took a dietary supplement containing vitamin D [39]. In addition, 26% of participants age 2–5 years and 14% of those age 6–11 years took supplements; rates increased with age from 10% of those age 12–19 years to 49% of men and 59% of women age 60 and older. Total vitamin D intakes were three times higher with supplement use than with diet alone; the mean intake from foods and beverages alone for individuals age 2 and older was 4.8 mcg (192 IU) but increased to 19.9 mcg (796 IU) when dietary supplements were included.

Some people take very high doses of vitamin D supplements. In 2013–2014, an estimated 3.2% of the U.S. adult population took supplements containing 100 mcg (4,000 IU) or more vitamin D [41].

One might expect a large proportion of the U.S. population to have vitamin D inadequacy on the basis of vitamin D intakes from foods, beverages, and even dietary supplements. However, comparing

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vitamin D intakes to serum 25(OH)D levels is problematic. One reason is that sun exposure affects vitamin D status, so serum 25(OH)D levels are usually higher than would be predicted on the basis of vitamin D dietary intakes alone [1]. Another reason is that animal foods contain some 25(OH)D. This form of vitamin D is not included in intake surveys and is considerably more potent than vitamins D_2 or D_3 at raising serum 25(OH)D levels [42].

An analysis of NHANES 2011–2014 data on serum 25(OH)D levels found that most people in the United States age 1 year and older had sufficient vitamin D intakes according to the FNB thresholds [43]. However, 18% were at risk of inadequacy (levels of 30–49 nmol/L [12–19.6 ng/mL]), and 5% were at risk of deficiency (levels below 30 nmol/L [12 ng/mL]). Four percent had levels higher than 125 nmol/L (50 ng/mL). Proportions at risk of deficiency were lowest among children age 1–5 years (0.5%), peaked at 7.6% in adults age 20–39 years, and fell to 2.9% among adults age 60 years and older; patterns were similar for risks of inadequacy. Rates of deficiency varied by race and ethnicity: 17.5% of non-Hispanic Blacks were at risk of vitamin D deficiency, as were 7.6% of non-Hispanic Asians, 5.9% of Hispanics, and 2.1% of non-Hispanic White people. Again, the pattern was similar for the risk of inadequacy. Vitamin D status in the United States remained stable in the decade between 2003–2004 and 2013–2014.

Vitamin D Deficiency

People can develop vitamin D deficiency when usual intakes are lower over time than recommended levels, exposure to sunlight is limited, the kidneys cannot convert 25(OH)D to its active form, or absorption of vitamin D from the digestive tract is inadequate. Diets low in vitamin D are more common in people who have milk allergy or lactose intolerance and those who consume an ovo-vegetarian or vegan diet [1].

In children, vitamin D deficiency is manifested as rickets, a disease characterized by a failure of bone tissue to become properly mineralized, resulting in soft bones and skeletal deformities [44]. In addition to bone deformities and pain, severe rickets can cause failure to thrive, developmental delay, hypocalcemic seizures, tetanic spasms, cardiomyopathy, and dental abnormalities [45,46].

Prolonged exclusive breastfeeding without vitamin D supplementation can cause rickets in infants, and, in the United States, rickets is most common among breastfed Black infants and children [47]. In one Minnesota county, the incidence rate of rickets in children younger than 3 years in the decade beginning in 2000 was 24.1 per 100,000 [48]. Rickets occurred mainly in Black children who were breastfed longer, were born with low birthweight, weighed less, and were shorter than other children. The incidence rate of rickets in the infants and children (younger than 7) seen by 2,325 pediatricians throughout Canada was 2.9 per 100,000 in 2002–2004, and almost all patients with rickets had been breastfed [49].

The fortification of milk (a good source of calcium) and other staples, such as breakfast cereals and margarine, with vitamin D beginning in the 1930s along with the use of cod liver oil made rickets rare in the United States [28,50]. However, the incidence of rickets is increasing globally, even in the United

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States and Europe, especially among immigrants from African, Middle-Eastern, and Asian countries [51]. Possible explanations for this increase include genetic differences in vitamin D metabolism, dietary preferences, and behaviors that lead to less sun exposure [45,46].

In adults and adolescents, vitamin D deficiency can lead to osteomalacia, in which existing bone is incompletely or defectively mineralized during the remodeling process, resulting in weak bones [46]. Signs and symptoms of osteomalacia are similar to those of rickets and include bone deformities and pain, hypocalcemic seizures, tetanic spasms, and dental abnormalities [45].

Screening for vitamin D status is becoming a more common part of the routine laboratory bloodwork ordered by primary-care physicians, irrespective of any indications for this practice [6,52-54]. No studies have examined whether such screening for vitamin D deficiency results in improved health outcomes [55]. The U.S. Preventive Services Task Force (USPSTF) found insufficient evidence to assess the benefits and harms of screening for vitamin D deficiency in asymptomatic adults [6]. It added that no national professional organization recommends population screening for vitamin D deficiency.

Groups at Risk of Vitamin D Inadequacy

Obtaining sufficient vitamin D from natural (nonfortified) food sources alone is difficult. For many people, consuming vitamin D-fortified foods and exposing themselves to some sunlight are essential for maintaining a healthy vitamin D status. However, some groups might need dietary supplements to meet their vitamin D requirements. The following groups are among those most likely to have inadequate vitamin D status.

Breastfed infants

Consumption of human milk alone does not ordinarily enable infants to meet vitamin D requirements, because it provides less than 0.6 to 2.0 mcg/L (25 to 78 IU/L) [1,56,57]. The vitamin D content of human milk is related to the mother's vitamin D status; studies suggest that the breastmilk of mothers who take daily supplements containing at least 50 mcg (2,000 IU) vitamin D_3 have higher levels of the nutrient [57,58].

Although UVB exposure can produce vitamin D in infants, the American Academy of Pediatrics (AAP) advises parents to keep infants younger than 6 months out of direct sunlight, dress them in protective clothing and hats, and apply sunscreen on small areas of exposed skin when sun exposure is unavoidable [59]. The AAP recommends 10 mcg (400 IU)/day vitamin D supplements for exclusively and partially breastfed infants starting shortly after birth and lasting until they are weaned and consume at least 1,000 mL/day vitamin D-fortified formula or whole milk [57]. The AAP also recommends 10 mcg (400 IU)/day supplemental vitamin D for all infants who are not breastfed and ingest less than 1,000 mL/day vitamin D-fortified formula or milk. An analysis of NHANES 2009–2016 data found that only 20.5% of breastfed infants and 31.1% of infants who were not breastfed ingested these recommended amounts of supplements [60].

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Older adults

Older adults are at increased risk of developing vitamin D insufficiency, partly because the skin's ability to synthesize vitamin D declines with age [1,61]. In addition, older adults are likely to spend more time than younger people indoors, and they might have inadequate dietary intakes of the vitamin [1].

People with limited sun exposure

Homebound individuals; people who wear long robes, dresses, or head coverings for religious reasons; and people with occupations that limit sun exposure are among the groups that are unlikely to obtain adequate amounts of vitamin D from sunlight [62]. The use of sunscreen also limits vitamin D synthesis from sunlight. However, because the extent and frequency of sunscreen use are unknown, the role that sunscreen may play in reducing vitamin D synthesis is unclear [1].

People with dark skin

Greater amounts of the pigment melanin in the epidermal layer of the skin result in darker skin and reduce the skin's ability to produce vitamin D from sunlight [1]. Black Americans, for example, typically have lower serum 25(OH)D levels than White Americans. However, whether these lower levels in persons with dark skin have significant health consequences is not clear [14]. Those of African American ancestry, for example, have lower rates of bone fracture and osteoporosis than do Whites (see the section below on bone health and osteoporosis).

People with conditions that limit fat absorption

Because vitamin D is fat soluble, its absorption depends on the gut's ability to absorb dietary fat [4]. Fat malabsorption is associated with medical conditions that include some forms of liver disease, cystic fibrosis, celiac disease, Crohn's disease, and ulcerative colitis [1,63]. In addition to having an increased risk of vitamin D deficiency, people with these conditions might not eat certain foods, such as dairy products (many of which are fortified with vitamin D), or eat only small amounts of these foods. Individuals who have difficulty absorbing dietary fat might therefore require vitamin D supplementation [63].

People with obesity or who have undergone gastric bypass surgery

Individuals with a body mass index (BMI) of 30 or more have lower serum 25(OH)D levels than individuals without obesity. Obesity does not affect the skin's capacity to synthesize vitamin D. However, greater amounts of subcutaneous fat sequester more of the vitamin [1]. People with obesity might need greater intakes of vitamin D to achieve 25(OH)D levels similar to those of people with normal weight [1,64,65].

Individuals with obesity who have undergone gastric bypass surgery can also become vitamin D deficient. In this procedure, part of the upper small intestine, where vitamin D is absorbed, is bypassed, and vitamin D that is mobilized into the bloodstream from fat stores might not raise 25(OH)D to

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adequate levels over time [66,67]. Various expert groups—including the American Association of Metabolic and Bariatric Surgery, The Obesity Society, and the British Obesity and Metabolic Surgery Society—have developed guidelines on vitamin D screening, monitoring, and replacement before and after bariatric surgery [66,68]

Vitamin D and Health

The FNB committee that established DRIs for vitamin D found that the evidence was inadequate or too contradictory to conclude that the vitamin had any effect on a long list of potential health outcomes (e.g., on resistance to chronic diseases or functional measures), except for measures related to bone health. Similarly, in a review of data from nearly 250 studies published between 2009 and 2013, the Agency for Healthcare Research and Quality concluded that no relationship could be firmly established between vitamin D and health outcomes other than bone health [69]. However, because research has been conducted on vitamin D and numerous health outcomes, this section focuses on seven diseases, conditions, and interventions in which vitamin D might be involved: bone health and osteoporosis, cancer, cardiovascular disease (CVD), depression, multiple sclerosis (MS), type 2 diabetes, and weight loss.

Most of the studies described in this section measured serum 25(OH)D levels using various methods that were not standardized by comparing them to the best methods. Use of unstandardized 25(OH)D measures can raise questions about the accuracy of the results and about the validity of conclusions drawn from studies that use such measures and, especially, from meta-analyses that pool data from many studies that use different unstandardized measures [5,9,70]. More information about assay standardization is available from the Vitamin D Standardization Program webpage.

Bone health and osteoporosis

Bone is constantly being remodeled. However, as people age—and particularly in women during menopause—bone breakdown rates overtake rates of bone building. Over time, bone density can decline, and osteoporosis can eventually develop [71].

More than 53 million adults in the United States have or are at risk of developing osteoporosis, which is characterized by low bone mass and structural deterioration of bone tissue that increases bone fragility and the risk of bone fractures [72]. About 2.3 million osteoporotic fractures occurred in the United States in 2015 [73]. Osteoporosis is, in part, a long-term effect of calcium and/or vitamin D insufficiency, in contrast to rickets and osteomalacia, which result from vitamin D deficiency. Osteoporosis is most often associated with inadequate calcium intakes, but insufficient vitamin D intakes contribute to osteoporosis by reducing calcium absorption [1].

Bone health also depends on support from the surrounding muscles to assist with balance and postural sway and thereby reduce the risk of falling. Vitamin D is also needed for the normal development and growth of muscle fibers. In addition, inadequate vitamin D levels can adversely affect muscle strength and lead to muscle weakness and pain (myopathy) [1].

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Most trials of the effects of vitamin D supplements on bone health also included calcium supplements, so isolating the effects of each nutrient is difficult. In addition, studies provided different amounts of nutrients and used different dosing schedules.

Clinical trial evidence on older adults

Among postmenopausal women and older men, many clinical trials have shown that supplements of both vitamin D and calcium result in small increases in bone mineral density throughout the skeleton [1,74]. They also help reduce fracture rates in institutionalized older people. However, the evidence on the impact of vitamin D and calcium supplements on fractures in community-dwelling individuals is inconsistent.

The USPSTF evaluated 11 randomized clinical trials of vitamin D and/or calcium supplementation in a total of 51,419 healthy, community-dwelling adults age 50 years and older who did not have osteoporosis, vitamin D deficiency, or prior fractures [75,76]. It concluded that the current evidence was insufficient to evaluate the benefits and harms of supplementation to prevent fractures. In addition, the USPSTF recommended against supplementation with 10 mcg (400 IU) or less of vitamin D and 1,000 mg or less of calcium to prevent fractures in this population, but it could not determine the balance of benefits and harms from higher doses.

The USPSTF also reviewed the seven published studies on the effects of vitamin D supplementation (two of them also included calcium supplementation) on the risk of falls in community-dwelling adults age 65 years or older who did not have osteoporosis or vitamin D deficiency. It concluded with moderate certainty that vitamin D supplementation does not reduce the numbers of falls or injuries, such as fractures, resulting from falls [77,78]. Another recent systematic review also found that vitamin D and calcium supplements had no beneficial effects on fractures, falls, or bone mineral density [79,80]. In contrast, a meta-analysis of six trials in 49,282 older adults found that daily vitamin D (10 or 20 mcg [400 IU or 800 IU]/day) and calcium (800 or 1,200 mg/day) supplementation for a mean of 5.9 years reduced the risk of any fracture by 6% and of hip fracture by 16% [81].

One systematic review and meta-analysis of 11 randomized, controlled trials published through 2018 of vitamin D supplementation alone (10–20 mcg [400–800 IU]/day or more at least every week or as rarely as once a year) for 9 months to 5 years found that the supplements provided no protection from fractures in 34,243 older adults [81].

More recently, a 2022 ancillary study of the Vitamin D and Omega-3 Trial (VITAL; described in the Cancer section below) investigated whether supplemental vitamin D3 (50 mcg [2,000 IU]/day) would lower the risk of fractures in 25,871 generally healthy men age 50 years and older and women age 55 years and older over a median follow-up of 5.3 years [82]. The mean age of all participants was 67.1 years; 50.6% were women and 20.2% were Black. Most participants were vitamin D sufficient; at baseline, only 2.4% had serum 25(OH)D levels less than 30 nmol/L (12 ng/mL), and 12.9% less than 50 nmol/L (20 ng/mL). Vitamin D supplementation did not lower the risk of total fractures, hip fractures, or nonvertebral fractures as compared with placebo. No substantial between-group differences in fracture incidence were found by race, ethnic group, BMI, age, baseline 25(OH)D levels, or whether

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participants took supplemental calcium, were at high fracture risk, or had a history of fragility fractures.

Vitamin D supplements for bone health in minority populations

Bone mineral density, bone mass, and fracture risk are correlated with serum 25(OH)D levels in White Americans and Mexican Americans, but not in Black Americans [14,83]. Factors such as adiposity, skin pigmentation, vitamin D binding protein polymorphisms, and genetics contribute to differences in 25(OH)D levels between Black and White Americans.

One clinical trial randomized 260 Black women age 60 years and older (mean age 68.2 years) to receive 60 to 120 mcg (2,400 to 4,800 IU) per day vitamin D_3 supplementation to maintain serum 25(OH)D levels above 75 nmol/L (30 ng/mL) for 3 years [84]. The results showed no association between 25(OH)D levels or vitamin D dose and the risk of falling in the 184 participants who completed the study. In fact, Black Americans might have a greater risk than White Americans of falls and fractures with daily vitamin D intakes of 50 mcg (2,000 IU) or more [14]. Furthermore, the bone health of older Black American women does not appear to benefit from raising serum 25(OH)D levels beyond 50 nmol/L (20 ng/mL) [84].

Vitamin D supplements and muscle function

Studies examining the effects of supplemental vitamin D on muscle strength and on rate of decline in muscle function have had inconsistent results [55]. One recent clinical trial, for example, randomized 78 frail and near-frail adults age 65 years and older to receive 20 mcg (800 IU) vitamin D_3, 10 mcg 25(OH)D, or placebo daily for 6 months. The groups showed no significant differences in measures of muscle strength or performance [85]. Another study randomized 100 community-dwelling men and women age 60 years and older (most were White) with serum 25(OH)D levels of 50 nmol/L (20 ng/ml) or less to 800 IU vitamin D_3 or placebo for 1 year [86]. Participants in the treatment group whose serum 25(OH)D level was less than 70 nmol/L (28 ng/ml) after 4 months received an additional 800 IU/day vitamin D_3. Despite increasing serum 25(OH)D levels to an average of more than 80 nmol/L (32 ng/ml), vitamin D supplementation did not affect lower-extremity power, strength, or lean mass.

Conclusions about vitamin D supplements and bone health

All adults should consume recommended amounts of vitamin D and calcium from foods and supplements if needed. Older women and men should consult their health care providers about their needs for both nutrients as part of an overall plan to maintain bone health and to prevent or treat osteoporosis.

Cancer

Laboratory and animal studies suggest that vitamin D might inhibit carcinogenesis and slow tumor progression by, for example, promoting cell differentiation and inhibiting metastasis. Vitamin D might also have anti-inflammatory, immunomodulatory, proapoptotic, and antiangiogenic effects [1,87]. Observational studies and clinical trials provide mixed evidence on whether vitamin D intakes or serum levels affect cancer incidence, progression, or mortality risk.

Total cancer incidence and mortality

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Some observational studies show associations between low serum levels of 25(OH)D and increased risks of cancer incidence and death. In a meta-analysis of 16 prospective cohort studies in a total of 137,567 participants who had 8,345 diagnoses of cancer, 5,755 participants died from cancer [88]. A 50 nmol/L (20 ng/mL) increase in 25(OH)D levels was associated with an 11% reduction in total cancer incidence rates and, in women but not men, a 24% reduction in cancer mortality rates. A meta-analysis of prospective studies that evaluated the association between serum 25(OH)D levels and cancer incidence (8 studies) or cancer mortality (16 studies) found that cancer risk decreased by 7% and cancer mortality rates decreased by 2% with each 20 nmol/L (8 ng/mL) increase in serum 25(OH)D levels [89]. Importantly, not all observational studies found higher vitamin D status to be beneficial, and the studies varied considerably in study populations, baseline comorbidities, and measurement of vitamin D levels.

Clinical trial evidence provides some support for the observational findings. For example, three meta-analyses of clinical trial evidence found that vitamin D supplementation does not affect cancer incidence but does significantly reduce total cancer mortality rates by 12%–13% [90-92]. In the most recent meta-analysis, 10 randomized clinical trials (including the VITAL trial described below) that included 6,537 cancer cases provided 10 to 50 mcg (400 to 2,000 IU) vitamin D_3 daily (six trials) or 500 mcg (20,000 IU)/week to 12,500 mcg (500,000 IU)/year boluses of vitamin D_3 (four trials) [91]. The study reports included 3–10 years of follow-up data. The vitamin D supplements were associated with serum 25(OH)D levels of 54 to 135 nmol/L (21.6 to 54 ng/mL). Vitamin D supplementation reduced cancer mortality rates by 13%, and most of the benefit occurred with daily supplementation.

The largest clinical trial, VITAL, to investigate the effects of vitamin D supplementation on the primary prevention of cancer in the general population gave 50 mcg (2,000 IU)/day vitamin D_3 supplements with or without 1,000 mg/day marine omega-3 fatty acids or a placebo for a median of 5.3 years [93]. The study included 25,871 men age 50 years and older and women age 55 years and older who had no history of cancer, and most had adequate serum 25(OH)D levels at baseline. Rates of breast, prostate, and colorectal cancer did not differ significantly between the vitamin D and placebo groups. However, normal-weight participants had greater reductions in cancer incidence and mortality rates than those with overweight or obesity.

A few studies have examined the effect of vitamin D supplementation on specific cancers. Below are brief descriptions of studies of vitamin D and its association with, or effect on, breast, colorectal, lung, pancreatic, and prostate cancers.

Breast cancer

Some observational studies support an inverse association between 25(OH)D levels and breast cancer risk and mortality, but others do not [94-97]. The Women's Health Initiative clinical trial randomized 36,282 postmenopausal women to receive 400 IU vitamin D_3 plus 1,000 mg calcium daily or a placebo for a mean of 7 years [98]. The vitamin D_3 and calcium supplements did not reduce breast cancer incidence, and 25(OH)D levels at the start of the study were not associated with breast cancer risk [99].

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In a subsequent investigation for 4.9 years after the study's end, women who had taken the vitamin D and calcium supplements (many of whom continued to take them) had an 18% lower risk of in situ (noninvasive) breast cancer [100]. However, women with vitamin D intakes higher than 15 mcg (600 IU)/day at the start of the trial and who received the supplements experienced a 28% increased risk of invasive (but not in situ) breast cancer.

Colorectal cancer

A large case-control study included 5,706 individuals who developed colorectal cancer and whose 25(OH)D levels were assessed a median of 5.5 years from blood draw to cancer diagnosis and 7,105 matched controls [101]. The results showed an association between 25(OH)D levels lower than 30 nmol/L (12 ng/mL) and a 31% higher colorectal cancer risk. Levels of 75 to less than 87.5 nmol/L (30 to less than 35 ng/mL) and 87.5 to less than 100 nmol/L (35 to less than 40 ng/mL) were associated with a 19% and 27% lower risk, respectively. The association was substantially stronger in women.

In the Women's Health Initiative clinical trial (described above), vitamin D_3 and calcium supplements had no effect on rates of colorectal cancer. In a subsequent investigation for 4.9 years after the study's end, women who had taken the vitamin D and calcium supplements (many of whom continued to take them) still had the same colorectal cancer risk as those who received placebo [100].

Another study included 2,259 healthy individuals age 45 to 75 years who had had one or more serrated polyps (precursor lesions to colorectal cancer) that had been removed [102]. These participants were randomized to take 25 mcg (1,000 IU) vitamin D_3, 1,200 mg calcium, both supplements, or a placebo daily for 3–5 years, followed by an additional 3–5 years of observation after participants stopped the treatment. Vitamin D alone did not significantly affect the development of new serrated polyps, but the combination of vitamin D with calcium increased the risk almost fourfold. The VITAL trial found no association between vitamin D supplementation and the risk of colorectal adenomas or serrated polyps [103].

Lung cancer

A study of cohorts that included 5,313 participants who developed lung cancer and 5,313 matched controls found no association between serum 25(OH)D levels and risk of subsequent lung cancer, even when the investigators analyzed the data by sex, age, race and ethnicity, and smoking status [104].

Pancreatic cancer

One study comparing 738 men who developed pancreatic cancer to 738 matched controls found no relationship between serum 25(OH)D levels and risk of pancreatic cancer [105]. Another study that compared 200 male smokers in Finland with pancreatic cancer to 400 matched controls found that participants in the highest quintile of 25(OH)D levels (more than 65.5 nmol/L [26.2 ng/mL]) had a threefold greater risk of developing pancreatic cancer over 16.7 years than those in the lowest quintile (less than 32 nmol/L [12.8 ng/mL]) [106]. An investigation that pooled data from 10 studies of cancer in 12,205 men and women found that concentrations of 25(OH)D greater than 75 nmol/L (30 ng/mL) but less than 100 nmol/L (40 ng/mL) did not reduce the risk of pancreatic cancer. However, the results did show an increased risk of pancreatic cancer with 25(OH)D levels of 100 nmol/L (40 ng/mL) or

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above [107].

Prostate cancer

Research to date provides mixed evidence on whether levels of 25(OH)D are associated with the development of prostate cancer. Several studies published in 2014 suggested that high levels of 25(OH)D might increase the risk of prostate cancer. For example, a meta-analysis of 21 studies that included 11,941 men with prostate cancer and 13,870 controls found a 17% higher risk of prostate cancer for participants with higher levels of 25(OH)D [108]. What constituted a higher level varied by study but was typically at least 75 nmol/L (30 ng/mL). In a cohort of 4,733 men, of which 1,731 had prostate cancer, those with 25(OH)D levels of 45–70 nmol/L (18–28 ng/mL) had a significantly lower risk of the disease than men with either lower or higher values [109]. This U-shaped association was most pronounced for men with the most aggressive forms of prostate cancer. A case-control analysis of 1,695 cases of prostate cancer and 1,682 controls found no associations between 25(OH)D levels and prostate cancer risk [110]. However, higher serum 25(OH)D levels (at a cut point of 75 nmol/L [30 ng/mL]) were linked to a modestly higher risk of slow-growth prostate cancer and a more substantial lower risk of aggressive disease.

Since 2014, however, several published studies and meta-analyses have found no relationship between 25(OH)D levels and prostate cancer risk [111,112]. For example, an analysis was conducted of 19 prospective studies that provided data on prediagnostic levels of 25(OH)D for 13,462 men who developed prostate cancer and 20,261 control participants [113]. Vitamin D deficiency or insufficiency did not increase the risk of prostate cancer, and higher 25(OH)D concentrations were not associated with a lower risk.

Several studies have examined whether levels of 25(OH)D in men with prostate cancer are associated with a lower risk of death from the disease or from any cause. One study included 1,119 men treated for prostate cancer whose plasma 25(OH)D levels were measured 4.9 to 8.6 years after their diagnosis. Among the 198 participants who died (41 deaths were due to prostate cancer), 25(OH)D levels were not associated with risk of death from prostate cancer or any cause [114]. However, a meta-analysis of seven cohort studies that included 7,808 men with prostate cancer found higher 25(OH)D levels to be significantly associated with lower mortality rates from prostate cancer or any other cause [115]. A dose-response analysis found that each 20 nmol/L [8 ng/mL] increase in 25(OH)D was associated with a 9% lower risk of both all-cause and prostate cancer-specific mortality.

For men with prostate cancer, whether vitamin D supplementation lengthens cancer-related survival is not clear. A meta-analysis of three randomized controlled trials in 1,273 men with prostate cancer found no significant differences in total mortality rates between those receiving vitamin D supplementation (from 10 mcg [400 IU]/day for 28 days to 45 mcg [1,800 IU] given in three doses total at 2-week intervals) and those receiving a placebo [116].

Conclusions about vitamin D and cancer

The USPSTF stated that, due to insufficient evidence, it was unable to assess the balance of benefits and harms of supplemental vitamin D to prevent cancer [117]. Taken together, studies to date do not

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indicate that vitamin D with or without calcium supplementation reduces the incidence of cancer, but adequate or higher 25(OH)D levels might reduce cancer mortality rates. Further research is needed to determine whether vitamin D inadequacy increases cancer risk, whether greater exposure to the nutrient can prevent cancer, and whether some individuals could have an increased risk of cancer because of their vitamin D status over time.

Cardiovascular disease

Vitamin D helps regulate the renin-angiotensin-aldosterone system (and thereby blood pressure), vascular cell growth, and inflammatory and fibrotic pathways [118]. Vitamin D deficiency is associated with vascular dysfunction, arterial stiffening, left ventricular hypertrophy, and hyperlipidemia [119]. For these reasons, vitamin D has been linked to heart health and risk of CVD.

Observational studies support an association between higher serum 25(OH)D levels and a lower risk of CVD incidence and mortality. For example, a meta-analysis included 34 observational studies that followed 180,667 participants (mean age greater than 50 years) for 1.3 to more than 32 years. The results showed that baseline serum 25(OH)D levels were inversely associated with total number of CVD events (including myocardial infarction, ischemic heart disease, heart failure, and stroke) and mortality risk [120]. Overall, the risk of CVD events was 10% lower for each 25 nmol/L (10 ng/mL) increase in serum 25(OH)D.

Another large observational study that followed 247,574 adults from Denmark for 0–7 years found that levels of 25(OH)D that were low (about 12.5 nmol/L [5 ng/mL]) and high (about 125 nmol/L [50 ng/mL]) were associated with a greater risk of mortality from CVD, stroke, and acute myocardial infarction [121]. Other meta-analyses of prospective studies have found associations between lower vitamin D status measured by serum 25(OH)D levels or vitamin D intakes and an increased risk of ischemic stroke, ischemic heart disease, myocardial infarction, and early death [122,123].

In contrast to the observational studies, clinical trials have provided little support for the hypothesis that supplemental vitamin D reduces the risk of CVD or CVD mortality. For example, a 3-year trial in New Zealand randomized 5,110 adults (mean age 65.9 years) to a single dose of 5,000 mcg (200,000 IU) vitamin D_3 followed by 2,500 mcg (100,000 IU) each month or a placebo for a median of 3.3 years [124]. Vitamin D supplementation had no effect on the incidence rate of myocardial infarction, angina, heart failure, arrhythmia, arteriosclerosis, stroke, venous thrombosis, or death from CVD. Similarly, the VITAL clinical trial described above found that vitamin D supplements did not significantly decrease rates of heart attacks, strokes, coronary revascularization, or deaths from cardiovascular causes [93]. Moreover, the effects did not vary by baseline serum 25(OH)D levels or whether participants took the trial's omega-3 supplement in addition to vitamin D.

However, another clinical trial designed to investigate bone fracture risk found that 800 IU/day vitamin D_3 (with or without calcium) or a placebo in 5,292 adults age 70 years and older for a median of 6.2 years offered protection from cardiac failure, but not myocardial infarction or stroke [125].

High serum cholesterol levels and hypertension are two of the main risk factors for CVD. The data on

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supplemental vitamin D and cholesterol levels are mixed, as shown in one meta-analysis of 41 clinical trials in a total of 3,434 participants (mean age 55 years). The results of this analysis showed that 0.5 mcg (20 IU) to 214 mcg (8,570 IU)/day vitamin D supplementation (mean of 2,795 IU) for 6 weeks to 3 years reduced serum total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels, but not high-density lipoprotein cholesterol levels [126].

Studies of the effects of vitamin D supplements on hypertension have also had mixed findings. In one meta-analysis of 46 clinical trials that included 4,541 participants, vitamin D supplements (typically 40 mcg [1,600 IU]/day or less) for a minimum of 4 weeks had no significant effects on systolic or diastolic blood pressure [127]. In contrast, another meta-analysis of 30 clinical trials in 4,744 participants (mean age 54.5 years) that administered 5 mcg (200 IU) to 300 mcg (12,000 IU)/day vitamin D_3 for a mean of 5.6 months showed that more than 20 mcg (800 IU)/day significantly reduced systolic and diastolic blood pressure in normal-weight participants who had hypertension [128]. However, more than 20 mcg (800 IU)/day vitamin D_3, when taken with calcium supplements, significantly increased blood pressure in participants with overweight and obesity. Another meta-analysis of genetic studies in 146,581 participants (primarily adults) found that a low vitamin D status increased blood pressure and hypertension risk in people with genetic variants associated with low endogenous production of 25(OH)D [129].

Overall, clinical trials show that vitamin D supplementation does not reduce CVD risk, even for people with low 25(OH)D status (below 20 nmol/L [12 ng/mL]) at baseline [93,124].

Depression

Vitamin D is involved in various brain processes, and vitamin D receptors are present on neurons and glia in areas of the brain thought to be involved in the pathophysiology of depression [130].

A systematic review and meta-analysis of 14 observational studies that included a total of 31,424 adults (mean age ranging from 27.5 to 77 years) found an association between deficient or low levels of 25(OH)D and depression [130].

Clinical trials, however, do not support these findings. For example, a meta-analysis of nine trials with a total of 4,923 adult participants diagnosed with depression or depressive symptoms found no significant reduction in symptoms after supplementation with vitamin D [131]. The trials administered different amounts of vitamin D (ranging from 10 mcg [400 IU]/day to 1,000 mcg [40,000 IU]/week). They also had different study durations (5 days to 5 years), mean participant ages (range, 22 years to 75 years), and baseline 25(OH)D levels; furthermore, some but not all studies administered concurrent antidepressant medications.

Three trials conducted since that meta-analysis also found no effect of vitamin D supplementation on depressive symptoms. One trial included 206 adults (mean age 52 years) who were randomized to take a bolus dose of 2,500 mcg (100,000 IU) vitamin D_3 followed by 500 mcg (20,000 IU)/week or a placebo for 4 months [132]. Most participants had minimal or mild depression, had a low mean baseline 25(OH) level of 33.8 nmol/L (13.5 ng/mL), and were not taking antidepressants. The second

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trial included 155 adults age 60–80 years who had clinically relevant depressive symptoms, no major depressive disorder, and serum 25(OH)D levels less than 50 to 70 nmol/L (20 to 28 ng/mL) depending on the season; in addition, they were not taking antidepressants [133,134]. Participants were randomized to take either 30 mcg (1,200 IU)/day vitamin D_3 or a placebo for 1 year. In the VITAL trial described above, 16,657 men and women 50 years of age and older with no history of depression and 1,696 with an increased risk of recurrent depression (that had not been medically treated for the past 2 years) were randomized to take 50 mcg (2,000 IU)/day vitamin D_3 (with or without fish oil) or a placebo for a median of 5.3 years [135]. The groups showed no significant differences in the incidence and recurrent rates of depression, clinically relevant depressive symptoms, or changes in mood scores.

Overall, clinical trials did not find that vitamin D supplements helped prevent or treat depressive symptoms or mild depression, especially in middle-age to older adults who were not taking prescription antidepressants. No studies have evaluated whether vitamin D supplements may benefit individuals under medical care for clinical depression who have low or deficient 25(OH)D levels and are taking antidepressant medication.

Multiple sclerosis

MS is an autoimmune disease of the central nervous system that damages the myelin sheath surrounding and protecting nerve cells in the brain and spinal cord. This damage hinders or blocks messages between the brain and body, leading to clinical features, such as vision loss, motor weakness, spasticity, ataxia, tremor, sensory loss, and cognitive impairment [136,137]. Some people with MS eventually lose the ability to write, speak, or walk.

The geographical distribution of MS around the world is unequal. Few people near the equator develop the disease, whereas the prevalence is higher further north and south. This uneven distribution has led to speculation that lower vitamin D levels in people who have less sunlight exposure might predispose them to the disease [137].

Many epidemiological and genetic studies have shown an association between MS and low 25(OH)D levels before and after the disease begins [137]. Observational studies suggest that adequate vitamin D levels might reduce the risk of contracting MS and, once MS is present, decrease the risk of relapse and slow the disease's progression [138]. One study, for example, tested 25(OH)D levels in 1,092 women in Finland an average of 9 years before their MS diagnosis and compared their outcomes with those of 2,123 similar women who did not develop MS [139]. More than half the women who developed MS had deficient or insufficient vitamin D levels. Women with 25(OH)D levels of less than 30 nmol/L (12 ng/mL) had a 43% higher MS risk than women with levels of 50 nmol/L (20 ng/mL) or higher. Among the women with two or more serum 25(OH)D samples taken before diagnosis (which reduced random measurement variation), a 50 nmol/L increase in 25(OH)D was associated with a 41% reduced risk of MS, and 25(OH)D levels less than 30 nmol/L were associated with an MS risk that was twice as high as levels of 50 nmol/L or higher.

Two earlier prospective studies of similar design—one in the United States with 444 non-Hispanic White individuals [140] and the other with 576 individuals in northern Sweden [141]—found that levels

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of 25(OH)D greater than 99.1 nmol/L (39.6 ng/mL) and at least 75 nmol/L (30 ng/mL), respectively, were associated with a 61%–62% lower risk of MS.

No clinical trials have examined whether vitamin D supplementation can prevent the onset of MS, but several have investigated whether supplemental vitamin D can help manage the disease. A 2018 Cochrane Review analyzed 12 such trials that had a total of 933 participants with MS; the reviewers judged all of these trials to be of low quality [137]. Overall, vitamin D supplementation, when compared with placebo administration, had no effect on relevant clinical outcomes, such as recurrent relapse or worsened disability.

Experts have reached no firm consensus on whether vitamin D can help prevent MS given the lack of clinical trial evidence [142]. In addition, studies have not consistently shown that vitamin D supplementation tempers the signs and symptoms of active MS or reduces rates of relapse.

Type 2 diabetes

Vitamin D plays a role in glucose metabolism. It stimulates insulin secretion via the vitamin D receptor on pancreatic beta cells and reduces peripheral insulin resistance through vitamin D receptors in the muscles and liver [143]. Vitamin D might be involved in the pathophysiology of type 2 diabetes through its effects on glucose metabolism and insulin signaling as well as its ability to reduce inflammation and improve pancreatic beta-cell function [1443,145].

Observational studies have linked lower serum 25(OH)D levels to an increased risk of diabetes, but their results might have been confounded by the fact that many participants were overweight or had obesity and were therefore more predisposed to developing diabetes and having lower 25(OH)D levels [1]. A review of 71 observational studies in adults with and without type 2 diabetes from 16 countries found a significant inverse relationship between vitamin D status and blood sugar levels in participants who did and did not have diabetes [146].

In contrast to observational studies, clinical trials provide little support for the benefits of vitamin D supplementation for glucose homeostasis. One trial included 65 adult men and women (mean age 32 years) with overweight or obesity who were otherwise healthy, did not have diabetes, and had low serum vitamin D levels (at or below 50 nmol/L [20 ng/mL]) [147]. The investigators randomly assigned participants to receive either a bolus oral dose of 2,500 mcg (100,000 IU) vitamin D_3 followed by 100 mcg (4,000 IU)/day or a placebo for 16 weeks. In the 54 participants who completed the study, vitamin D supplementation did not improve insulin sensitivity or insulin secretion in comparison with placebo.

One systematic review and meta-analysis evaluated 35 clinical trials that included 43,407 adults with normal glucose tolerance, prediabetes, or type 2 diabetes who received a median of 83 mcg (3,332 IU)/day vitamin D supplements or placebo for a median of 16 weeks [148]. Vitamin D had no significant effects on glucose homeostasis, insulin secretion or resistance, or hemoglobin A1c levels (a measure of average blood sugar levels over the previous 2–3 months), irrespective of the study population, vitamin D dose, or trial quality.

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Several trials have investigated whether vitamin D supplementation can prevent the transition from prediabetes to diabetes in patients with adequate 25(OH)D levels, and all have had negative results. In a trial in Norway, 511 men and women age 25–80 years (mean age 62 years) with prediabetes received 500 mcg (20,000 IU) vitamin D_3 or a placebo each week for 5 years [149]. The results showed no significant differences in rates of progression to type 2 diabetes; in serum glucose, insulin, or hemoglobin A1c levels; or in measures of insulin resistance. At baseline, participants had an adequate mean serum 25(OH)D level of 60 nmol/L (24 ng/mL).

The largest trial to date of vitamin D supplements for diabetes prevention randomized 2,423 men and women age 25 years and older (mean age 60 years) with prediabetes and overweight or obesity (mean BMI of 32.1) to 100 mcg (4,000 IU)/day vitamin D_3 or placebo for a median of 2.5 years [145]. Most participants (78%) had adequate serum levels of vitamin D at baseline (at least 50 nmol/L [20 ng/mL]). Vitamin D did not significantly prevent the development of diabetes in comparison with placebo. However, a post hoc analysis showed a 62% lower incidence of diabetes among participants with low baseline serum 25(OH)D levels (less than 30 nmol/L [12 ng/mL]) who took the vitamin D supplement than among those who took the placebo [145,150].

Studies have also assessed the value of vitamin D supplementation for managing diabetes, and they have found that the vitamin offers limited benefits. One meta-analysis of 20 clinical trials compared the effects of 0.5 mcg (20 IU)/day to 1,250 mcg (50,000 IU)/week vitamin D supplementation for 2–6 months with those of placebo on glycemic control in 2,703 adults from around the world who had diabetes [143]. The vitamin D reduced insulin resistance to a small but significant degree, especially in people taking more than 50 mcg (2,000 IU)/day who were vitamin D deficient at baseline, had good glycemic control, did not have obesity, and were of Middle Eastern ethnicity. However, the supplementation had no significant effects on fasting blood glucose, hemoglobin A1c, or fasting insulin levels.

Clinical trials to date provide little evidence that vitamin D supplementation helps maintain glucose homeostasis, reduces the risk of progression from prediabetes to type 2 diabetes, or helps manage the disease, particularly in vitamin D-replete individuals.

Weight loss

Observational studies indicate that greater body weights are associated with lower vitamin D status, and individuals with obesity frequently have marginal or deficient circulating 25(OH)D levels [151]. However, clinical trials do not support a cause-and-effect relationship between vitamin D and weight loss.

A systematic review and meta-analysis of 15 weight-loss intervention studies that used caloric restriction, exercise, or both, but not necessarily vitamin D supplementation or other treatments, found that people who lost weight had significantly greater increases in serum 25(OH)D levels than those who maintained their weight [152]. In another study, 10 mcg (400 IU)/day vitamin D and 1,000 mg/day calcium supplementation slightly, but significantly, reduced weight gain amounts in comparison with placebo in postmenopausal women, especially those with a baseline total calcium intake of less than

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1,200 mg/day [153]. However, a meta-analysis of 12 vitamin D supplementation trials (including 5 in which body composition measurements were primary outcomes) found that vitamin D supplements without calorie restriction did not affect body weight or fat mass when the results were compared with those of placebo [154].

Overall, the available research suggests that consuming higher amounts of vitamin D or taking vitamin D supplements does not promote weight loss.

Health Risks from Excessive Vitamin D

Excess amounts of vitamin D are toxic. Because vitamin D increases calcium absorption in the gastrointestinal tract, vitamin D toxicity results in marked hypercalcemia (total calcium greater than 11.1 mg/dL, beyond the normal range of 8.4 to 10.2 mg/dL), hypercalciuria, and high serum 25(OH)D levels (typically greater than 375 nmol/l [150 ng/mL]) [155]. Hypercalcemia, in turn, can lead to nausea, vomiting, muscle weakness, neuropsychiatric disturbances, pain, loss of appetite, dehydration, polyuria, excessive thirst, and kidney stones.

In extreme cases, vitamin D toxicity causes renal failure, calcification of soft tissues throughout the body (including in coronary vessels and heart valves), cardiac arrhythmias, and even death. Vitamin D toxicity has been caused by consumption of dietary supplements that contained excessive vitamin D amounts because of manufacturing errors, that were taken inappropriately or in excessive amounts, or that were incorrectly prescribed by physicians, [155-157].

Experts do not believe that excessive sun exposure results in vitamin D toxicity because thermal activation of previtamin D_3 in the skin gives rise to various non-vitamin D forms that limit formation of vitamin D_3. Some vitamin D_3 is also converted to nonactive forms [1]. However, frequent use of tanning beds, which provide artificial UV radiation, can lead to 25(OH)D levels well above 375–500 nmol/L (150–200 ng/mL) [158-160].

The combination of high intakes of calcium (about 2,100 mg/day from food and supplements) with moderate amounts of vitamin D (about 19 mcg [765 IU]/day from food and supplements) increased the risk of kidney stones by 17% over 7 years among 36,282 postmenopausal women who were randomly assigned to take 1,000 mg/day calcium and 10 mcg (400 IU)/day vitamin D or a placebo [161]. However, other, shorter (from 24 weeks to 5 years) clinical trials of vitamin D supplementation alone or with calcium in adults found greater risks of hypercalcemia and hypercalciuria, but not of kidney stones [162,163].

The FNB established ULs for vitamin D in 2010 (Table 4) [1]. While acknowledging that signs and symptoms of toxicity are unlikely at daily intakes below 250 mcg (10,000 IU), the FNB noted that even vitamin D intakes lower than the ULs might have adverse health effects over time. The FNB recommended avoiding serum 25(OH)D levels above approximately 125–150 nmol/L (50–60 ng/mL), and it found that even lower serum levels (approximately 75–120 nmol/L [30–48 ng/mL]) are associated with increases in rates of all-cause mortality, risk of cancer at some sites (e.g., pancreas), risk of cardiovascular events, and number of falls and fractures among older adults.

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Table 4: Tolerable Upper Intake Levels (ULs) for Vitamin D [1]

Age	Male	Female	Pregnancy	Lactation
0–6 months	25 mcg (1,000 IU)	25 mcg (1,000 IU)		
7–12 months	38 mcg (1,500 IU)	38 mcg (1,500 IU)		
1–3 years	63 mcg (2,500 IU)	63 mcg (2,500 IU)		
4–8 years	75 mcg (3,000 IU)	75 mcg (3,000 IU)		
9–18 years	100 mcg (4,000 IU)	100 mcg (4,000 IU)	100 mcg (4,000 IU)	100 mcg (4,000 IU)
19+ years	100 mcg (4,000 IU)	100 mcg (4,000 IU)	100 mcg (4,000 IU)	100 mcg (4,000 IU)

Interactions with Medications

Vitamin D supplements may interact with several types of medications. A few examples are provided below. Individuals taking these and other medications on a regular basis should discuss their vitamin D intakes and status with their health care providers.

Orlistat

The weight-loss drug orlistat (Xenical and alli), together with a reduced-fat diet, can reduce the absorption of vitamin D from food and supplements, leading to lower 25(OH)D levels [164-167].

Statins

Statin medications reduce cholesterol synthesis. Because endogenous vitamin D is derived from cholesterol, statins may also reduce vitamin D synthesis [167]. In addition, high intakes of vitamin D, especially from supplements, might reduce the potency of atorvastatin (Lipitor), lovastatin (Altoprev and Mevacor), and simvastatin (FloLipid and Zocor), because these statins and vitamin D appear to compete for the same metabolizing enzyme [167-170].

Steroids

Corticosteroid medications, such as prednisone (Deltasone, Rayos, and Sterapred), are often prescribed to reduce inflammation. These medications can reduce calcium absorption and impair vitamin D metabolism [171-173]. In the NHANES 2001–2006 survey, 25(OH)D deficiency (less than 25 nmol/L [10 ng/mL]) was more than twice as common among children and adults who reported oral steroid use (11%) than in nonusers (5%) [174].

Thiazide diuretics

Thiazide diuretics (e.g., Hygroton, Lozol, and Microzide) decrease urinary calcium excretion. The combination of these diuretics with vitamin D supplements (which increase intestinal calcium absorption) might lead to hypercalcemia, especially among older adults and individuals with compromised renal function or hyperparathyroidism [167,175,176].

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Vitamin D and Healthful Diets

The federal government's 2020–2025 *Dietary Guidelines for Americans* notes that "Because foods provide an array of nutrients and other components that have benefits for health, nutritional needs should be met primarily through foods. … In some cases, fortified foods and dietary supplements are useful when it is not possible otherwise to meet needs for one or more nutrients (e.g., during specific life stages such as pregnancy)."

For more information about building a healthy dietary pattern, refer to the *Dietary Guidelines for Americans (https://www.dietaryguidelines.gov)* and the USDA's *MyPlate. (https://www.choosemyplate.gov/)*

The *Dietary Guidelines for Americans* describes a healthy dietary pattern as one that

- Includes a variety of vegetables; fruits; grains (at least half whole grains); fat-free and low-fat milk, yogurt, and cheese; and oils.
    - Milk, many ready-to-eat cereals, and some brands of yogurt and orange juice are fortified with vitamin D. Cheese naturally contains small amounts of vitamin D. Vitamin D is added to some margarines.
- Includes a variety of protein foods such as lean meats; poultry; eggs; seafood; beans, peas, and lentils; nuts and seeds; and soy products.
    - Fatty fish, such as salmon, tuna, and mackerel, are very good sources of vitamin D. Beef liver and egg yolks have small amounts of vitamin D.
- Limits foods and beverages higher in added sugars, saturated fat, and sodium.
- Limits alcoholic beverages.
- Stays within your daily calorie needs.

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Disclaimer

This fact sheet by the National Institutes of Health (NIH) Office of Dietary Supplements (ODS) provides information that should not take the place of medical advice. We encourage you to talk to your health

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care providers (doctor, registered dietitian, pharmacist, etc.) about your interest in, questions about, or use of dietary supplements and what may be best for your overall health. Any mention in this publication of a specific product or service, or recommendation from an organization or professional society, does not represent an endorsement by ODS of that product, service, or expert advice.

Updated: September 18, 2023 History of changes to this fact sheet

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Exhibit E

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Archived Content

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FDA Letter to Industry Concerning Liquid Vitamin D Dietary Supplements

[Insert inside address]

Dear _____,

Over the past several months it has come to FDA's attention that there is an increase in the number of liquid vitamin D dietary supplements being marketed that could lead to infants receiving an unsafe amount of vitamin D. As a result, FDA believes industry should provide the necessary safeguards to ensure that infants using these products would not receive an unsafe amount of vitamin D.

Most liquid vitamin D products marketed today use a dropper to measure and deliver the vitamin D. FDA has concerns regarding any liquid vitamin D products that could deliver a significantly excessive amount of vitamin D. The concern is that the droppers can hold a considerably greater amount of liquid vitamin D than an infant should receive. This could lead to a parent inadvertently or mistakenly giving an unsafe amount of vitamin D to an infant.

To reduce the likelihood of dosing errors, FDA recommends that 400 units be clearly and accurately marked on the dropper accompanying your product. In addition, for products intended for infants, FDA recommends that the dropper hold no more than 400 units.

We are providing the following additional information to industry on Vitamin D and its safety:

Vitamin D is a generic term that is used to refer to the secosterols, ergocalciferol or Vitamin D2, and cholecalciferol or Vitamin D3 and their metabolites and analogues. Vitamin D is normally produced in the skin from a pro-vitamin using ultraviolet B (UV-B), and with adequate sun exposure, Vitamin D is not required in daily dietary intake. Vitamin D is the principal regulator of calcium, and is therefore important for skeletal development, bone mineralization and bone homeostasis, among other functions.

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The Institute of Medicine's (IOM) recommended Upper Limit (UL) for chronic Vitamin D intake for infants (children less than 1 year of age) is 25 mcg/day (1,000 IU/d), and for children age 1 year and older the recommended UL is 50 mcg/day (2,000 IU/d), based upon the specific vitamin D molecule being D3 from naturally occurring substances, such as fatty fish and fish liver oils. The UL for pregnancy and lactation is the same as for non-pregnant adults at 50 mcg/day (2000 IU/d). The UL value for adults ages 18 and older was based on a safety factor of 1.2 applied to a no adverse effect level (NOAEL) of 60 mcg/d. The lowest adverse effect level (LOAEL) in the IOM analysis was 95 mcg/d. The adverse effect used in the risk assessment was hypercalcemia, serum calcium elevated above the normal range, specified as above 2.75 mmol/liter (11 mg/dL)[1].

In 2008 the American Academy of Pediatrics (AAP) recommended that all breast-fed and partially breast-fed infants (beginning in the first 2 months after birth) consume 400 IU/day of Vitamin D . Initial symptoms of Vitamin D toxicity are usually associated with resultant high serum calcium and include weakness, fatigue, lassitude, headache, nausea, vomiting, and diarrhea; mental status changes and coma may also develop. Renal function may be affected early. Cardiac arrhythmias may also ensue. Prolonged hypercalcemia can lead to soft tissue deposition of calcium; favored areas include the kidney, resulting in other associated problems such as hypertension. Additionally, other soft tissues that are affected include blood vessels, heart, lungs, and skin. Osteomalacia may also occur. Blood chemistry changes include elevated calcium and urea, with inconsistent elevation of phosphorus. If the problem is caught early, treatment may completely reverse the symptoms. Residual sequelae may include permanent renal impairment, and osteoporosis, among others[2].

FDA appreciates your attention to this matter. If you have any questions, please contact Lesley Maloney, Pharm.D., Senior Advisor for Business and Industry Relations, in FDA's Office of External Relations. Her email address is **Lesley.Maloney@fda.hhs (http://wayback.archive-it.org/7993/20170722024258/mailto:Lesley.Maloney@fda.hhs)**.gov, and her telephone number is 301-796-8236.

Sincerely,

Joshua M. Sharfstein, M.D.
Principal Deputy Commissioner
of Food and Drugs

[1]Institute of Medicine, National Academy of Sciences, Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington, DC: National Academy Press, 1997.

[2]Daniel D. Federman, M.D., Elizabeth G. Nabel, MD, eds. 2010. ACP Medicine. New York, NY. BC Decker Inc. ISBN 0-9703902-9-7. ISSN 1548-9345. STAT!Ref Online Electronic Medical Library. http://online.statref.com/document.aspx?fxid=48&docid=630. 4/9/2010 12:41:40 PM CDT (UTC -05:00).

Resources for You

- **Infant Overdose Risk With Liquid Vitamin D (/7993/20170722024258/https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm214343.htm)**

More in Dietary Supplements (/7993/20170722024258/https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/DietarySupplements/default.htm)

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Exhibit F

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN™

CLINICAL REPORT

Prevention of Rickets and Vitamin D Deficiency in Infants, Children, and Adolescents

Guidance for the Clinician in Rendering Pediatric Care

Carol L. Wagner, MD, Frank R. Greer, MD, and the Section on Breastfeeding and Committee on Nutrition

This Clinical Report was retired October 2012.

ABSTRACT

Rickets in infants attributable to inadequate vitamin D intake and decreased exposure to sunlight continues to be reported in the United States. There are also concerns for vitamin D deficiency in older children and adolescents. Because there are limited natural dietary sources of vitamin D and adequate sunshine exposure for the cutaneous synthesis of vitamin D is not easily determined for a given individual and may increase the risk of skin cancer, the recommendations to ensure adequate vitamin D status have been revised to include all infants, including those who are exclusively breastfed and older children and adolescents. It is now recommended that all infants and children, including adolescents, have a minimum daily intake of 400 IU of vitamin D beginning soon after birth. The current recommendation replaces the previous recommendation of a minimum daily intake of 200 IU/day of vitamin D supplementation beginning in the first 2 months after birth and continuing through adolescence. These revised guidelines for vitamin D intake for healthy infants, children, and adolescents are based on evidence from new clinical trials and the historical precedence of safely giving 400 IU of vitamin D per day in the pediatric and adolescent population. New evidence supports a potential role for vitamin D in maintaining innate immunity and preventing diseases such as diabetes and cancer. The new data may eventually refine what constitutes vitamin D sufficiency or deficiency. *Pediatrics* 2008;122:1142–1152

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The guidance in this report does not indicate an exclusive course of treatment or serve as a standard of medical care. Variations, taking into account individual circumstances, may be appropriate.

Key Words
vitamin D, vitamin D deficiency, rickets, vitamin D requirements, infants, children, adolescents, 25-hydroxyvitamin D, vitamin D supplements

Abbreviations
AAP—American Academy of Pediatrics
25-OH-D—25-hydroxyvitamin D
1,25-OH_2-D—1,25-dihydroxyvitamin D
PTH—parathyroid hormone

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INTRODUCTION

This statement is intended to replace a 2003 clinical report from the American Academy of Pediatrics (AAP),[1] which recommended a daily intake of 200 IU/day of vitamin D for all infants (beginning in the first 2 months after birth), children, and adolescents. The new recommended daily intake of vitamin D is 400 IU/day for all infants, children, and adolescents beginning in the first few days of life.

BACKGROUND

Rickets attributable to vitamin D deficiency is known to be a condition that is preventable with adequate nutritional intake of vitamin D.[2–6] Despite this knowledge, cases of rickets in infants attributable to inadequate vitamin D intake and decreased exposure to sunlight continue to be reported in the United States and other Western countries, particularly with exclusively breastfed infants and infants with darker skin pigmentation.[4,7–14] Rickets, however, is not limited to infancy and early childhood, as evidenced by cases of rickets caused by nutritional vitamin D deficiency being reported in adolescents.[15]

Rickets is an example of extreme vitamin D deficiency, with a peak incidence between 3 and 18 months of age. A state of deficiency occurs months before rickets is obvious on physical examination, and the deficiency state may also present with hypocalcemic seizures,[16–18] growth failure, lethargy, irritability, and a predisposition to respiratory infections during infancy.[16–22] In a retrospective review of children presenting with vitamin D deficiency in the United Kingdom,[16] there were 2 types of presentations. The first was symptomatic hypocalcemia (including seizures) occurring during periods of rapid growth, with increased metabolic demands, long before any physical findings or radiologic evidence of vitamin D deficiency occurred. The second clinical presentation was that of a more chronic disease, with rickets and/or decreased bone mineralization and either normocalcemia or asymptomatic hypocalce-

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TABLE 1 Vitamin D Deficiency: Stages and Clinical Signs

1. Stages of vitamin D deficiency

Stage I

25-OH-D level decreases, resulting in hypocalcemia and euphosphatemia; 1,25-OH_2-D may increase or remain unchanged

Stage II

25-OH-D level continues to decrease; PTH acts to maintain calcium through demineralization of bone; the patient remains eucalcemic and hypophosphatemic and has a slight increase in the skeletal alkaline phosphatase level

Stage III

Severe 25-OH-D deficiency with hypocalcemia, hypophosphatemia, and increased alkaline phosphatase; bones have overt signs of demineralization

2. Clinical signs of vitamin D deficiency

- Dietary calcium absorption from the gut decreases from 30%–40% to 10%–15% when there is vitamin D deficiency
- Low concentrations of 25-OH-D trigger the release of PTH in older infants, children, and adolescents in an inverse relationship not typically seen with young infants; the increase in PTH mediates the mobilization of calcium from bone, resulting in a reduction of bone mass; as bone mass decreases, the risk of fractures increases
  - Rickets

    Enlargement of the skull, joints of long bones, and rib cage; curvature of spine and femurs; generalized muscle weakness
  - Osteomalacia and osteopenia
  - Abnormal immune function with greater susceptibility to acute infections and other long-latency disease states (see below)

3. Potential latent disease processes associated with vitamin D deficiency

- Dysfunction of the innate immune system is noted with vitamin D deficiency
  - Immunomodulatory actions may include
    - Potent stimulator of innate immune system acting through Toll-like receptors on monocytes and macrophages
    - Decrease threshold for long-latency diseases such as cancers (including leukemia and colon, prostate, and breast cancers), psoriasis, diabetes mellitus, and autoimmune diseases (eg, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosis)

mia. (For a more complete review of nutritional rickets and its management, please refer to the recent publication in *Endocrinology and Metabolism Clinics of North America* on the topic.[23])

There are 2 forms of vitamin D: D_2 (ergocalciferol, synthesized by plants) and D_3 (cholecalciferol, synthesized by mammals). The main source of vitamin D for humans is vitamin D_3 through its synthesis in the skin when UV-B in the range of 290 to 315 nm converts 7-dehydrocholesterol into previtamin D_3. Through the heat of the skin, previtamin D_3 is further transformed into vitamin D_3, which then binds to the vitamin D–binding protein and is transported to the liver and converted to 25-hydroxyvitamin D (25-OH-D) by the action of 25-hydroxylase. 25-OH-D, the nutritional indicator of vitamin D, undergoes a second hydroxylation in the kidney and other tissues to become 1,25-dihydroxyvitamin D (1,25-OH_2-D). Vitamin D is an important prehormone with active metabolites (25-OH-D and 1,25-OH_2-D) that are involved in many metabolic processes beyond bone integrity and calcium homeostasis.[24] More-detailed reviews of vitamin D physiology and metabolism are available from Hathcock et al,[25] Holick,[26] Webb,[27] and Misra et al.[23]

It is important to note that measuring the concentration of 1,25-OH_2-D instead of 25-OH-D for assessment of vitamin D status can lead to erroneous conclusions, because 1,25-OH_2-D concentrations will be normal or even elevated in the face of vitamin D deficiency as a result of secondary hyperparathyroidism (see Table 1). Prevention of vitamin D deficiency and achieving adequate intake of vitamin D and calcium throughout childhood may reduce the risk of osteoporosis as well as other long-latency disease processes that have been associated with vitamin D–deficiency states in adults.[28–31]

The presence of vitamin D as a natural ingredient in food in most diets is limited, occurring in relatively significant amounts only in fatty fish and certain fish oils, liver and fat from aquatic mammals, and egg yolks of chickens fed vitamin D.[32] In adults, new evidence suggests that vitamin D plays a vital role in maintaining innate immunity[33] and has been implicated in the prevention of certain disease states including infection,[34,35] autoimmune diseases (multiple sclerosis,[28,33,36,37] rheumatoid arthritis[38]), some forms of cancer (breast, ovarian, colorectal, prostate),[24,30,39–42] and type 2 diabetes mellitus.[43–45] Results from prospective observational studies also suggest that vitamin D supplements in infancy and early childhood may decrease the incidence of type 1 diabetes mellitus.[46–50]

RECOMMENDED DAILY INTAKE OF VITAMIN D FOR INFANTS AND CHILDREN

In partnership with the Institute of Medicine, the National Academy of Sciences Panel for Vitamin D recommended in 1997 a daily intake of 200 IU vitamin D to prevent vitamin D deficiency in normal infants, children and adolescents.[51] This recommendation was endorsed by the AAP in a previous clinical report.[1] The National Academy of Sciences guidelines for infants were based on data primarily from the United States, Norway, and China, which showed that an intake of at least 200 IU/day of vitamin D prevented physical signs of vitamin D deficiency and maintained the concentration of 25-OH-D at or above 27.5 nmol/L (11 ng/mL).† These recommendations were made despite 50 years of clinical experience demonstrating that 400 IU of vitamin D (the concentration measured in a teaspoon of cod liver oil) not only prevented rickets but also treated it.[52–55] Primarily on the basis of new information in adults linking

†Universal units of measure for 25-OH-D and 1,25-OH_2-D are nmol/L. Conversion to ng/mL is made by dividing the value expressed in nmol/L by 2.496. Thus, 80 nmol/L becomes 32 ng/mL.

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other biomarkers (parathyroid hormone [PTH], insulin resistance, bone mineralization, and calcium absorption studies) to vitamin D deficiency, there is a growing concern that the previous recommendation of 200 IU/day as an adequate intake of vitamin D is not sufficient, even for infants and children.[53,56–61]

This new information has resulted in defining vitamin D deficiency in adults as a 25-OH-D concentration of <50 nmol/L and vitamin D insufficiency as a 25-OH-D concentration of 50 to 80 nmol/L.[25,26,62–67] At the present time, however, consensus has not been reached with regard to the concentration of 25-OH-D to define vitamin D insufficiency for infants and children.[66–69] Although there may not be a precise definition of what constitutes vitamin D insufficiency in infants and children, it is known that 200 IU/day of vitamin D will not maintain 25-OH-D concentrations at >50 nmol/L in infants, the concentration attributed to vitamin D sufficiency in adults.[62,67,70–74] On the other hand, 400 IU/day of vitamin D has been shown to maintain serum 25-OH-D concentrations at >50 nmol/L in exclusively breastfed infants.[73] It is also of note that liquid vitamins and vitamin D–only preparations available in the United States conveniently supply 400 IU/day, not 200 IU/day, in either drop or milliliter preparations.

SUNLIGHT EXPOSURE AND VITAMIN D

Historically, the main source of vitamin D has been via synthesis in the skin from cholesterol after exposure to UV-B light. Full-body exposure during summer months for 10 to 15 minutes in an adult with lighter pigmentation will generate between 10 000 and 20 000 IU of vitamin D_3 within 24 hours; individuals with darker pigmentation require 5 to 10 times more exposure to generate similar amounts of vitamin D_3.[75–78] The amount of UV exposure available for the synthesis of vitamin D depends on many factors other than just time spent outdoors. These factors include the amount of skin pigmentation, body mass, degree of latitude, season, the amount of cloud cover, the extent of air pollution, the amount of skin exposed, and the extent of UV protection, including clothing and sunscreens.[56,77,79–81] The Indoor Air Quality Act of 1989 reported that Americans spent an average of 93% of their time indoors,[82] supporting the higher prevalence of lower 25-OH-D concentrations among adult Americans.[83,84] More recently, vitamin D deficiency (as defined by concentrations of 25-OH-D < 25 nmol/L) among school-aged children and adolescents has been reported, reflecting modern-day lifestyle changes.[3,6,9,58,85–96]

The multitude of factors that affect vitamin D synthesis by the skin,[27] the most important of which is degree of skin pigmentation, make it difficult to determine what is adequate sunshine exposure for any given infant or child.[97–99] Furthermore, to limit exposure to UV light, the Centers for Disease Control and Prevention, with the support of many organizations including the AAP and the American Cancer Society, launched a major public health campaign in 1998 to increase public awareness about sunlight exposure and the risks of various skin cancers.[100] Indirect epidemiologic evidence now suggests that the age at which direct sunlight exposure is initiated is even more important than the total sunlight exposure over a lifetime in determining the risk of skin cancer.[101–105] Among dermatologists, there is active discussion about the risks and potential benefits of sun exposure and/or oral vitamin D supplementation[97,99,106]; however, the vast majority would agree with the current AAP guidelines for decreasing sunlight exposure, which include the advice that infants younger than 6 months should be kept out of direct sunlight. Although the AAP encourages physical activity and time spent outdoors, children's activities that minimize sunlight exposure are preferred, and when outdoors, protective clothing as well as sunscreens should be used.[105] In following these guidelines, vitamin D supplements during infancy, childhood, and adolescence are necessary.

PREGNANCY, VITAMIN D, AND THE FETUS

The Institute of Medicine in 1997[51] and a Cochrane review in 2002[107] concluded that there are few data available regarding maternal vitamin D requirements during pregnancy, despite the fact that maternal vitamin D concentrations largely determine the vitamin D status of the fetus and newborn infant. With restricted vitamin D intake and sunlight exposure, maternal deficiency may occur, as has been documented in a number of studies.[107–113]

Recent work has demonstrated that in men and nonpregnant women, oral vitamin D intake over a 4- to 5-month period will increase circulating 25-OH-D concentrations by approximately 0.70 nmol/L for every 40 IU of vitamin D ingested,[114,115] which is consistent with earlier work performed in pregnant women. In those studies, as predicted by vitamin D kinetics, supplements of 1000 IU/day of vitamin D to pregnant women resulted in a 12.5 to 15.0 nmol/L increase in circulating 25-OH-D concentrations in both maternal and cord serum compared with nonsupplemented controls.[108–110] Maternal 25-OH-D concentrations ranged from a mean of approximately 25 nmol/L at baseline to 65 ± 17.5 nmol/L at 230 days of gestation in the group of women who received 1000 IU of vitamin D per day during the last trimester. In comparison, 25-OH-D concentrations were 32.5 ± 20.0 nmol/L in the unsupplemented control group. These data suggest that doses exceeding 1000 IU of vitamin D per day are necessary to achieve 25-OH-D concentrations of >50 nmol/L in pregnant women.[108–115] The significance of these findings for those who care for the pediatric population is that when a woman who has vitamin D deficiency gives birth, her neonate also will be deficient.

It is important to note that women with increased skin pigmentation or who have little exposure of their skin to sunlight are at a greater risk of vitamin D deficiency and may need additional vitamin D supplements, especially during pregnancy and lactation.[71] In a study by van der Meer et al,[116] >50% of pregnant women with darker pigmentation in the Netherlands were vitamin D

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deficient, as defined by a 25-OH-D concentration of <25 nmol/L.

Studies in human subjects have shown a strong relationship between maternal and fetal circulating (cord blood) 25-OH-D concentrations.[117–120] With severe maternal vitamin D deficiency, the fetus may rarely develop rickets in utero and manifest this deficiency at birth.[71] Supplementation with 400 IU of vitamin D per day during the last trimester of pregnancy has minimal effect on circulating 25-OH-D concentrations in the mother and her infant at term.[112] An unsupplemented infant born to a vitamin D–deficient mother will reach a state of deficiency more quickly than an infant whose mother was replete during pregnancy.[71]

Adequate nutritional vitamin D status during pregnancy is important for fetal skeletal development, tooth enamel formation, and perhaps general fetal growth and development.[121] There is some evidence that the vitamin D status of the mother has long-term effects on her infant. In a recent Canadian study by Mannion et al comparing growth parameters in newborn infants with the maternal intakes of milk and vitamin D during pregnancy, investigators found an association between vitamin D intake during pregnancy and birth weight but not infant head circumference or length at birth.[122] With every additional 40 IU of maternal vitamin D intake, there was an associated 11-g increase in birth weight. Another study of the intrauterine effect of maternal vitamin D status revealed a significant association between umbilical cord 25-OH-D concentrations and head circumference at 3 and 6 months' postnatal age that persisted after adjustment for confounding factors.[109,111] A study performed in the United Kingdom during the 1990s demonstrated that higher maternal vitamin D status during pregnancy was associated with improved bone-mineral content and bone mass in children at 9 years of age.[123]

Given the growing evidence that adequate maternal vitamin D status is essential during pregnancy, not only for maternal well-being but also for fetal development,[71,122,124,125] health care professionals who provide obstetric care should consider assessing maternal vitamin D status by measuring the 25-OH-D concentrations of pregnant women. On an individual basis, a mother should be supplemented with adequate amounts of vitamin D_3 to ensure that her 25-OH-D levels are in a sufficient range (>80 nmol/L).[25,26,64,66,67] The knowledge that prenatal vitamins containing 400 IU of vitamin D_3 have little effect on circulating maternal 25-OH-D concentrations, especially during the winter months, should be imparted to all health care professionals involved in the care of pregnant women.[26,64,71,115]

THE EFFECT OF MATERNAL VITAMIN D SUPPLEMENTATION DURING LACTATION ON THE VITAMIN D STATUS OF THE BREASTFED INFANT

The vitamin D content of human milk (parental vitamin D compound plus 25-OH-D) is related to the lactating mother's vitamin D status.[71–74,126] In a lactating mother supplemented with 400 IU/day of vitamin D, the vitamin D content of her milk ranges from <25 to 78 IU/L.[73,74,126–129] Infants who are exclusively breastfed but who do not receive supplemental vitamin D or adequate sunlight exposure are at increased risk of developing vitamin D deficiency and/or rickets.[7,10–12,14,18,81,130] Infants with darker pigmentation are at greater risk of vitamin D deficiency,[131] a fact explained by the greater risk of deficiency at birth[132] and the decreased vitamin D content in milk from women who themselves are deficient.[127]

A small number of studies have examined the effect of higher maternal supplements of vitamin D on the 25-OH-D concentrations in breastfed infants. Supplements of 1000 to 2000 IU of vitamin D per day to nursing mothers have little effect on the breastfeeding infant's vitamin D status as measured by infant 25-OH-D concentrations.[81,133,134] In 2 recent pilot studies that involved lactating women supplemented with high-dose vitamin D (up to 6400 IU/day), the vitamin D content of the mothers' milk increased to concentrations as high as 873 IU/L without any evidence of maternal vitamin D toxicity.[73,74] The 25-OH-D concentrations in breastfed infants of mothers who received 6400 IU/day of vitamin D increased from a mean concentration of 32 to 115 nmol/L. These results compared favorably with infants receiving 300 to 400 IU of vitamin D per day, whose 25-OH-D concentrations increased from a mean of 35 to 107 nmol/L. Although vitamin D concentrations can be increased in milk of lactating women by using large vitamin D supplements, such high-dose supplementation studies in lactating women must be validated and demonstrated to be safe in larger, more representative populations of women across the United States. Recommendations to universally supplement breastfeeding mothers with high-dose vitamin D cannot be made at this time. Therefore, supplements given to the infant are necessary.

VITAMIN D SUPPLEMENTATION FOR BREASTFEEDING INFANTS

Although it is clear and incontrovertible that human milk is the best nutritive substance for infants during the first year,[135–137] there has been concern about the adequacy of human milk in providing vitamin D.[70,138] As such, the AAP published its 2003 vitamin D supplementation statement,[1] recommending that all breastfed infants start to receive 200 IU of vitamin D per day within the first 2 months after delivery.

With improved understanding of the detrimental effects of insufficient vitamin D status before the appearance of rickets, studies in North America are continuing to examine the vitamin D status of children and appropriate 25-OH-D serum concentrations. A 2003 report of serum 25-OH-D status in healthy 6- to 23-month-old children in Alaska revealed that 11% had concentrations of <37 nmol/L and 20% had concentrations of 37 to 62 nmol/L.[139,140] Thirty percent of the infants were still breastfeeding, and these infants were more likely to have serum 25-OH-D concentrations of <37 nmol/L. After this study, the Alaskan Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) began an initiative to actively identify breastfeeding chil-

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TABLE 2 Oral Vitamin D Preparations Currently Available in the United States (in Alphabetical Order)

Preparation[a]	Dosage
Bio-D-Mulsion (Biotics Research Laboratory, Rosenberg, TX; www.bioticsresearch.com)	1 drop contains 400 IU[b]; also comes in a preparation of 2000 IU per drop[b]; corn oil preparation
Carlson Laboratories (Arlington Heights, IL; www.carlsonlabs.com)	1 gel cap contains 400 IU; also comes in 2000-IU and 4000-IU gel caps and in single-drop preparations of 400-IU, 1000-IU, and 2000-IU[b]; safflower oil preparation
Just D (Sunlight Vitamins Inc [Distributed by UnitDrugCo, Centennial, CO]; www.sunlightvitamins.com)	1 mL contains 400 IU; corn oil preparation
Multivitamin preparations: polyvitamins (A, D, and C vitamin preparations)[c]	1 mL contains 400 IU; variable preparations that include glycerin and water; may also contain propylene glycol and/or polysorbate 80

Note that higher-dose oral preparations may be necessary for the treatment of those with rickets in the first few months of therapy or for patients with chronic diseases such as fat malabsorption (cystic fibrosis) or patients chronically taking medications that interfere with vitamin D metabolism (such as antiseizure medications).

[a] A study by Martinez et al[162] showed that newborn and older infants preferred oil-based liquid preparations to alcohol-based preparations.

[b] Single-drop preparation may be better tolerated in patients with oral aversion issues, but proper instruction regarding administration of these drops must be given to the parents or care provider, given the increased risk of toxicity, incorrect dosing, or accidental ingestion.

[c] The cost of vitamin D–only preparations may be more than multivitamin preparations and could be an issue for health clinics that dispense vitamins to infants and children. The multivitamin preparation was the only preparation available until recently; therefore, there is a comfort among practitioners in dispensing multivitamins to all age groups.

dren and provide free vitamin supplements for them and a vitamin D fact sheet for their mothers. Another recent study by Ziegler et al[141] assessed the vitamin D status of 84 breastfeeding infants in Iowa (latitude 41°N). In the 34 infants who received no supplemental vitamin D, 8 (23%) infants had a serum 25-OH-D concentration of <27 nmol/L at 280 days of age. Of these 8 low measurements, 7 were made in the winter months (November through April). Thus, at this time it is prudent to recommend that all breastfed infants be given supplemental vitamin D_3.

The 2003 AAP statement recommended supplements of 200 IU of vitamin D per day to all breastfed infants within the first 2 months of life, after breastfeeding was well established.[1] This was in agreement with a 1997 report from the Institute of Medicine.[51] This report's recommendation of 200 IU/day was largely based on a study that showed that among breastfed infants in northern China supplemented with 100 or 200 IU of vitamin D per day, there were no cases of rickets.[142] However, 17 of 47 infants and 11 of 37 infants receiving 100 or 200 IU of vitamin D per day, respectively, had serum concentrations of 25-OH-D at <27 nmol/L. Although corollary maternal serum concentrations were not measured, on the basis of vitamin D pharmacokinetics, maternal vitamin D status is assumed to have been abnormally low, thereby preventing adequate transfer of vitamin D in human milk. When the breastfeeding mother has marginal vitamin D status or frank deficiency, infant 25-OH-D concentrations are very low in unsupplemented infants, particularly in the winter months in latitudes further from the equator. It is clear that 25-OH-D concentrations of >50 nmol/L can be maintained in exclusively breastfed infants with supplements of 400 IU/day of vitamin D, which is the amount contained in 1 teaspoon of cod liver oil[52,54] and for which there is historic precedence of safety and prevention and treatment of rickets.[5,6,143]

Thus, given the evidence that (1) vitamin D deficiency can occur early in life, especially when pregnant women are deficient, (2) 25-OH-D concentrations are very low in unsupplemented breastfeeding infants, particularly in the winter months when mothers have marginal vitamin D status or are deficient, (3) that the amount of sunshine exposure necessary to maintain an adequate 25-OH-D concentration in any given infant at any point in time is not easy to determine, and (4) serum 25-OH-D concentrations are maintained at >50 nmol/L in breastfed infants with 400 IU of vitamin D per day, the following recommendation is made: A supplement of 400 IU/day of vitamin D should begin within the first few days of life and continue throughout childhood. Any breastfeeding infant, regardless of whether he or she is being supplemented with formula, should be supplemented with 400 IU of vitamin D, because it is unlikely that a breastfed infant would consume 1 L (~1 qt) of formula per day, the amount that would supply 400 IU of vitamin D.

FORMS OF VITAMIN D SUPPLEMENTS

There are 2 forms of vitamin D that have been used as supplements: vitamin D_2 (ergocalciferol, which is plant derived) and vitamin D_3 (cholecalciferol, which is fish derived). It has been shown that vitamin D_3 has greater efficacy in raising circulating 25-OH-D concentrations under certain physiological situations.[144] Most fortified milk products and vitamin supplements now contain vitamin D_3. Vitamin D–only preparations are now available in the United States, in addition to the multivitamin liquids supplements, to provide the appropriate concentrations of 400 IU/mL (see Table 2). Some also contain 400 IU per drop, but such preparations must be prescribed with caution; explicit instruction and demonstration of use are essential because of the greater potential for a vitamin D overdose if several drops are administered at once.

The new vitamin D–only preparations are particularly appropriate for the breastfed infant who has no need for multivitamin supplements. The cost of purchase and administration of vitamin D either alone or in combination with vitamins A and C (as it is currently constituted) is minimal. Pediatricians and other health care professionals should work with the Special Supplemental Nutrition Program for Women, Infants, and Children

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clinics to make vitamin D supplements available for breastfeeding infants. Current preparations, assuming correct administration of dosage by caregivers, place the infant at little risk of overdosage and vitamin D toxicity, although this must be considered. Care must be taken by health care professionals to provide explicit instructions regarding the correct dosage and administration.[145] Preparations that contain higher concentrations of vitamin D should only be prescribed in the setting of close surveillance of vitamin D status and for those who have such a demonstrated requirement (eg, those who suffer from fat malabsorption or who must chronically take antiseizure medication).

FORMULA-FED INFANTS AND VITAMIN D SUPPLEMENTS

All infant formulas sold in the United States must have a minimum vitamin D concentration of 40 IU/100 kcal (258 IU/L of a 20 kcal/oz formula) and a maximum vitamin D_3 concentration of 100 IU/100 kcal (666 IU/L of a 20 kcal/oz formula).[146] All formulas sold in the United States have at least 400 IU/L of vitamin D_3.[147] Because most formula-fed infants ingest nearly 1 L or 1 qt of formula per day after the first month of life, they will achieve a vitamin D intake of 400 IU/day. As mentioned earlier, infants who receive a mixture of human milk and formula also should get a vitamin D supplement of 400 IU/day to ensure an adequate intake. As infants are weaned from breastfeeding and/or formula, intake of vitamin D–fortified milk should be encouraged to provide at least 400 IU/day of vitamin D. Any infant who receives <1 L or 1 qt of formula per day needs an alternative way to get 400 IU/day of vitamin D, such as through vitamin supplements.

VITAMIN D SUPPLEMENTS DURING LATER CHILDHOOD AND ADOLESCENCE

As was mentioned earlier, there is active debate among vitamin D experts as to what constitutes vitamin D "sufficiency," "insufficiency," and "deficiency" in adults and children as defined by 25-OH-D serum concentrations.‡ Vitamin D deficiency is not limited to infancy and early childhood but covers the life span, with periods of vulnerability that mirror periods of accelerated growth or physiologic change. In fact, vitamin D deficiency in older children and adolescents continues to be reported worldwide.§ Recent studies of vitamin D status have shown that 16% to 54% of adolescents have serum 25-OH-D concentrations of ≤50 nmol/L.[9,85–88,90,94,150–152] In 1 study that used the adult definition of insufficiency of a serum 25-OH-D concentration of <80 nmol/L, 73.1% of adolescents demonstrated values below this concentration.[153] In examining the prevalence of vitamin D deficiency in adolescents, studies across North America have shown that serum 25-OH-D concentrations of <30 nmol/L occur in as few as 1% to as many as 17% of adolescents, depending on the subjects themselves and the latitude and season of measurement.[3,86,87,151,152] All of these studies found black adolescents to have significantly lower 25-OH-D status than individuals who are not black. Although there have been no large series of adolescents with vitamin D–deficiency rickets, cases continue to occur.[15]

The inverse relationship of increasing PTH with decreasing 25-OH-D concentrations has been demonstrated in older children and adolescents.[9,152] A study of vitamin D insufficiency in 6- to 10-year-old preadolescent black children in Pittsburgh, PA, revealed that serum PTH concentrations decreased with increasing serum 25-OH-D concentrations and reached a plateau when the serum 25-OH-D concentration was ≥75 nmol/L.[150] In Boston, MA, Gordon et al[152] found that 24.1% of healthy teenagers in their cross-sectional cohort were vitamin D deficient (25-OH-D concentration ≤ 37 nmol/L), of whom 4.6% were severely deficient (25-OH-D concentration ≤ 20 nmol/L) and 42% were vitamin D insufficient (25-OH-D concentration ≤ 50 nmol/L). There was an inverse correlation between serum 25-OH-D and PTH concentrations ($R = -0.29$). Concentrations of 25-OH-D also were related to season, ethnicity, milk and juice consumption, BMI, and physical activity, which were independent predictors of vitamin D status.

Similar results were found by Cheng et al[89] in their cohort of pubertal and prepubertal Finnish girls. These investigators also found a significantly lower cortical volumetric bone-mineral density of the distal radius and tibial shaft in girls with vitamin D deficiency (as defined by 25-OH-D concentrations ≤ 25 nmol/L). These results are supported by the work of Viljakainen et al[58] in their study of 212 Finnish early-adolescent (aged 11–12 years) girls who were randomly assigned to receive 0, 200, or 400 IU of vitamin D per day for 12 months. After 1 year, bone-mineral augmentation of the femur was 14.3% and 17.2% higher in the girls receiving 200 and 400 IU of vitamin D, respectively, compared with those in the placebo group.

The extent of vitamin D deficiency has been suggested by reports from other regions of the world, including children and adolescents living in northern Greece[94] and Germany[57] and adolescents in Beijing,[153] Turkey,[88] Finland,[58] and Ireland.[95] With lower 25-OH-D concentrations correlating with increased PTH concentrations, vitamin D deficiency could result in secondary hyperparathyroidism. This condition would deplete the bone of mineral, especially during periods of accelerated bone growth, and lead to long-term detrimental effects.

In evaluating bone mineralization as a function of vitamin D status in adolescents, several studies in the United States and Europe have demonstrated an unfavorable effect of lower 25-OH-D concentrations on bone health.[58,89,154,155] Adolescent girls with serum 25-OH-D concentrations of >40 nmol/L have demonstrated increased radial, ulnar, and tibial bone-mineral densities,[152] although studies have demonstrated inconsistent findings in other body sites.[154] Additional studies are needed to identify the serum 25-OH-D status that promotes optimal bone health in older children and adolescents.

Although consuming 1 qt (32 oz) of vitamin D–forti-

‡Refs 6, 9, 56, 64, 66, 67, 94, 132, and 148–150.
§Refs 9, 57, 58, 85–89, 94–96, and 150–154.

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fied milk will provide 400 IU of vitamin D_3 per day, it is clear that in the adolescent population, the intake of vitamin D–fortified milk is much less.[155–157] In the United States, milk intake decreased by 36% among adolescent girls from 1977–1978 to 1994–1998.[156] Fortified cereals (½-cup dry) and 1 egg (yolk) will each provide approximately 40 IU of vitamin D_3. Given the dietary practices of many children and adolescents, a dietary intake of 400 IU of vitamin D is difficult to achieve.[157] Thus, for older children and adolescents, a daily multivitamin or vitamin D–only preparation containing 400 IU of vitamin D would be warranted. Additional studies are needed to evaluate what the optimal vitamin D status in older children and adolescents is and whether this level can be achieved consistently through diet and a vitamin D supplement of 400 IU/day.

Along with adequate vitamin D intake, dietary calcium intake to achieve optimal bone formation and modeling must be ensured.[87] A dietary history is essential in assessing the adequacy of dietary intake for various vitamins, minerals, and nutrients, including vitamin D and calcium.[3,91] Children and adolescents at increased risk of developing rickets and vitamin D deficiency, including those with increased skin pigmentation, decreased sunlight exposure, chronic diseases characterized by fat malabsorption (cystic fibrosis, etc), and those who require anticonvulsant medications (which induce cytochrome P450 and other enzymes that may lead to catabolism of vitamin D) may require even higher doses than 400 IU/day of vitamin D.[158–161]

SUMMARY GUIDELINES

To prevent rickets and vitamin D deficiency in healthy infants, children, and adolescents, a vitamin D intake of at least 400 IU/day is recommended. To meet this intake requirement, we make the following suggestions:

1. Breastfed and partially breastfed infants should be supplemented with 400 IU/day of vitamin D beginning in the first few days of life. Supplementation should be continued unless the infant is weaned to at least 1 L/day or 1 qt/day of vitamin D–fortified formula or whole milk. Whole milk should not be used until after 12 months of age. In those children between 12 months and 2 years of age for whom overweight or obesity is a concern or who have a family history of obesity, dyslipidemia, or cardiovascular disease, the use of reduced-fat milk would be appropriate.[163]
2. All nonbreastfed infants, as well as older children who are ingesting <1000 mL/day of vitamin D–fortified formula or milk, should receive a vitamin D supplement of 400 IU/day. Other dietary sources of vitamin D, such as fortified foods, may be included in the daily intake of each child.
3. Adolescents who do not obtain 400 IU of vitamin D per day through vitamin D–fortified milk (100 IU per 8-oz serving) and vitamin D–fortified foods (such as fortified cereals and eggs [yolks]) should receive a vitamin D supplement of 400 IU/day.
4. On the basis of the available evidence, serum 25-OH-D concentrations in infants and children should be ≥50 nmol/L (20 ng/mL).
5. Children with increased risk of vitamin D deficiency, such as those with chronic fat malabsorption and those chronically taking antiseizure medications, may continue to be vitamin D deficient despite an intake of 400 IU/day. Higher doses of vitamin D supplementation may be necessary to achieve normal vitamin D status in these children, and this status should be determined with laboratory tests (eg, for serum 25-OH-D and PTH concentrations and measures of bone-mineral status). If a vitamin D supplement is prescribed, 25-OH-D levels should be repeated at 3-month intervals until normal levels have been achieved. PTH and bone-mineral status should be monitored every 6 months until they have normalized.
6. Pediatricians and other health care professionals should strive to make vitamin D supplements readily available to all children within their community, especially for those children most at risk.

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Exhibit G

Human Vitamin and Mineral Requirements

Report of a joint FAO/WHO expert consultation Bangkok, Thailand



Food and Agriculture Organization of the United Nations



World Health Organization

Food and Nutrition Division
FAO Rome

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Foreword

The report of this *joint FAO/WHO expert consultation on human vitamin and mineral requirements* has been long in coming. The consultation was held in Bangkok in September 1998, and much of the delay in the publication of the report has been due to controversy related to final agreement about the recommendations for some of the micronutrients. *A priori* one would not anticipate that an evidence based process and a topic such as this is likely to be controversial. But the reality is that there is surprisingly little data on specific health status indicators on which to draw conclusions, whereas there is more information in relation to overt deficiency disease conditions. The recommended intakes are therefore largely based on the interpretation of the best available scientific information, and this leaves the door open for differences in interpretation.

A fundamental point is that when we look at recommended nutrient intakes (RNIs) in industrialized countries over the last 25 years, the levels for some of the micronutrients have been gradually increasing. The question is whether this comes from better scientific knowledge and understanding of the biochemical role of the nutrients, or whether the criteria for setting the levels of the requirements have changed. Even if the scientific knowledge base has expanded, it appears that the basic criteria for deciding on levels to recommend may bear more of the responsibility. Whereas RNIs for vitamins and minerals were initially established on the understanding that they are meant to meet the basic nutritional needs of over 97 percent of the population, a fundamental criterion in industrialized countries has become one of presumptive role these nutrients may play in the "prevention" against an increasing range of disease conditions that characterise these populations. The latter approach implies the notion of "optimal nutrition", and this may be insidiously pushing requirements to higher levels time and again.

This shift in the goal for setting RNIs is not without reason. The populations that are targeted for prevention through "optimal nutrition" are characterised by sedentary lifestyles and longer life expectations. The populations of developed countries are ageing, and the concern for the health of the ageing has become prominent. By contrast the micronutrient needs of developing countries as a whole have not really changed, and are more appropriately described as those that will satisfy basic needs of younger populations that are physically active. This, nevertheless, is not to deny the double burden of malnutrition, which is rapidly rising in many developing countries, and one needs to also bear that in mind.

The concern raised about possible differences in micronutrient needs of populations with different lifestyles is for a very practical reason. The logic behind the establishment of micronutrient needs of industrialized nations has come about at the same time that there is a large and growing demand for a variety of supplements of all kinds, and manufacturers have responded quickly to meet this market. This phenomenon could be skewing our strategy for nutritional development, with a tendency to want to resolve micronutrient deficiency problems of developing countries through the use of supplements and fortification strategies, rather than through increasing the consumption of an adequate and varied diet. Higher levels of RNIs in developed countries can easily be supported because they can be met with supplementation in addition to food. But when it becomes difficult to meet some of the micronutrient needs of developing countries by consuming locally available food, there is a problem.

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The nutrients of concern currently are, first, calcium, for which the RNI may be difficult to meet without dairy products. The recently revised US/Canada Dietary Reference Intakes (DRIs) only propose an Acceptable Intake (AI) for calcium, instead of a Recommended Daily Allowance (RDA), in recognition of the fact that intake data is out of step with the relatively high intake requirements observed with experimentally derived values. Another is iron, particularly during pregnancy, where supplementation appears to be essential during the second half of pregnancy. Folic acid requirements are doubled for women of child-bearing age to prevent the incidence of neural tube defects in the foetus. Conversion factors for carotenoids are under review, with the looming conclusion that servings of green leafy vegetables needed to meet vitamin A requirements would probably need to be at least doubled. In view of this uncertainty, we have only provided for "recommended safe intakes" rather than RNIs. Selenium is undergoing growing interest because of its properties as an antioxidant. The RNIs recommended from the FAO/WHO process for this micronutrient are in a lower range than that provided by the US/Canada process because the latter is calculated on a cellular basis, whereas the former are based on the more traditional whole body estimates.

Are these "developments" or "new understandings" appropriate and applicable for developing countries? There is no clear answer based on our current knowledge, but the time may be coming when RNIs will need to be seen differently for developing countries, and based on *developing* country data. There may be a need to identify some biomarkers that are specific to developing country conditions. There is therefore an urgent need for research to be carried out in developing countries about *their* nutrient needs. The current situation also implies that the RNIs for the micronutrients of concern discussed above will need to be re-evaluated as soon as significant additional data becomes available.

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Chapter 1
Introduction

A Joint Food and Agriculture Organization of the United Nations/World Food Organization of the United Nations *(FAO/WHO) expert consultation on human vitamin and mineral requirements* was held in the FAO Regional Office for Asia and the Pacific, Bangkok, Thailand, from 21 to 30 September 1998. The purpose of the consultation was to complement the information, which had been provided through previous consultations for different nutrients, in order to provide current knowledge on all essential nutrients as the first step towards the production of a new edition of the *manual on human nutritional requirements.*

Background

The Secretariat of the FAO and WHO organized the *joint FAO/WHO expert consultation on human vitamin and mineral requirements* to review the FAO/WHO micronutrient requirements and to develop recommended nutrient intakes. The report of the Expert Consultation (this report) is being published as a technical document, and this will later serve as the basis for developing a revised edition of the *FAO/WHO handbook of human nutrition.*

Consultations such as this one are part of a continuing commitment by both FAO and WHO to promote a reliable, nutritious, and safe food supply and to provide scientifically sound nutritional advice to Member Nations. This commitment was recently reaffirmed by the World Food Summit in November 1996 in Rome.

Written documents providing the criteria that were used in the past to develop the FAO/WHO RNIs were made available to the experts. These documents included the 1974 *FAO/WHO handbook on human nutritional requirements*, the 1988 *FAO/WHO expert consultation report on the requirements for vitamin A, iron, folate, and vitamin* B_{12}, and the 1996 *WHO/FAO/IAEA report on trace elements in human nutrition and health*.

For the purpose of preparation of the background papers, the following working definition of a recommended nutrient intake (RNI) was used. The RNI is the intake level sufficient to meet the daily nutrient requirements of most individuals in a specific life-stage and gender group and is based on an estimated average nutrient requirement (EAR) plus two standard deviations above the mean: $RNI = EAR + 2SD_{EAR}$.

Terms of reference and process

The terms of reference for the Expert Panel were the following:

- To review the full scope of vitamin and minerals requirements, including their role in normal human physiology and metabolism and in deficiency disease conditions. To focus on the requirements of the essential vitamins and minerals, including vitamins A, C, D, E, and K; the B vitamins; calcium; iron; magnesium; zinc; selenium; and iodine.
- To draft and adopt a report which would provide recommended nutrient intakes for vitamins A, C, D, E, and K; the B vitamins; calcium; iron; magnesium; zinc; selenium; and iodine. The report would provide practical advice and recommendations which will constitute an authoritative source of information to all those from member countries who work in the area of nutrition, agriculture, food production and distribution, and health

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promotion. This report will form, in large part, the basis for a new edition of the FAO/WHO Handbook on Human Nutritional Requirements, which was published in 1974 and last re-issued in 1980.

- To identify key issues for future research and make preliminary recommendations for the handbook.

The presentations addressed changes in the science base for each of the essential vitamin and mineral nutrients from the time of the most recent FAO/WHO review of those nutrients. The Expert Panel made recommendations for the nutrient requirements, identified key issues for future research, and made preliminary recommendations for the handbook.

Definitions of terms used

The following definitions relate to the nutrient intake from food (including water) that is required to prevent deficiency conditions. Upper limits of nutrient intake are defined for specific vitamins and minerals where there is a potential problem with excess.

Requirement

A requirement is an intake level, which will meet specified criteria of adequacy, preventing risk of deficit or excess. These criteria include a gradient of biological effects related to the nutrient intake. This dose response will be assumed to have a Gaussian distribution unless it is known to be otherwise. A risk function (a probability of 0 to 1) of deficiency and excess can be derived (***Figure 1***).

The relevance of the biological effects starts with the most extreme case, that is, the prevention of death. For nutrients where sufficient data on mortality are not available, the nutrient intake that prevents clinical disease or sub-clinical pathological conditions, identified by biochemical or functional assays, is used. The next sets of biomarkers that are used to define requirements include measures of nutrient stores or critical tissue pools. Intakes to assure replete body stores are important when deficiency conditions are highly prevalent. Presently, approaches to define requirements of most nutrients use several criteria examined in combination, functional assays of sub-clinical conditions are considered the most relevant. These biomarkers ideally should be sensitive to changes in nutritional state while at the same time be specific in terms of identifying sub-clinical deficiency conditions. The use of nutrient balance to define requirements has been avoided whenever possible. However, in the absence of other criteria it has been used. In most cases, balance based on input-output measurements are greatly influenced by level of intake, that is, subjects adjust to high intakes by increasing output, conversely they lower output when intake is low. Thus, if sufficient time is provided balance can be achieved at multiple levels of intake. The same can be said of nutrient blood levels, they usually will reflect level of intake and absorption rather than functional state. Unless balance or plasma level is related to abnormal function or disease conditions, they are inadequate for use as a criteria to support the definition of requirements. Where relevant, requirement estimates should include allowance for variations in bio-availability.

Recommended nutrient intake

Recommended nutrient intake (RNI) is the daily intake, which meets the nutrient requirements of almost all (97.5 percent) apparently healthy individuals in an age and sex-specific population group. Daily intake corresponds to the average over a period of time. Criteria to establish requirements used in this report will be nutrient specific. The estimation of RNI starts with the definition of the criteria for requirement and adds corrections for physiologic and dietary factors. The average requirement value obtained from a group of individuals is then adjusted for inter-individual variability. If the distribution of values is not

known, a Gaussian distribution is assumed, that is, a mean plus 2 SD is expected to cover 97.5 percent of the population. If the SD is not known, a value based on each nutrient's physiology is used. In most cases a variation in the range of 10-12.5 percent was assumed; exceptions are noted within chapters. The definition of RNI used in this report is equivalent to that of recommended dietary allowance (RDA) as used by the Food and Nutrition Board of the US National Academy of Sciences (*1*).

Apparently healthy

Apparently healthy refers to the absence of disease based on clinical signs and symptoms and function, normally assessed by routine laboratory methods and physical evaluation.

Upper tolerable nutrient intake level

Upper tolerable nutrient intake levels (ULs) have been defined for some nutrients. ULs are the maximum intake from food that is unlikely to pose risk of adverse health effects from excess in almost all (97.5 percent) apparently healthy individuals in an age and sex-specific population group. ULs should be based on long-term exposure from food, including fortified food products. For most nutrients no adverse effects are anticipated when they are consumed as foods, because their absorption and or excretion are regulated. The special situation of consumption of nutritional supplements which when added to the nutrient intake from food may exceed the UL will be addressed in the specific chapters. The ULs as presented here do not meet the strict definition of no observed effect level used in health risk assessment by toxicologists because in most cases a dose-response curve for risk from total exposure to a nutrient will not be available. For more details on how to derive ULs, see the model presented in Nutrition Reviews (2).

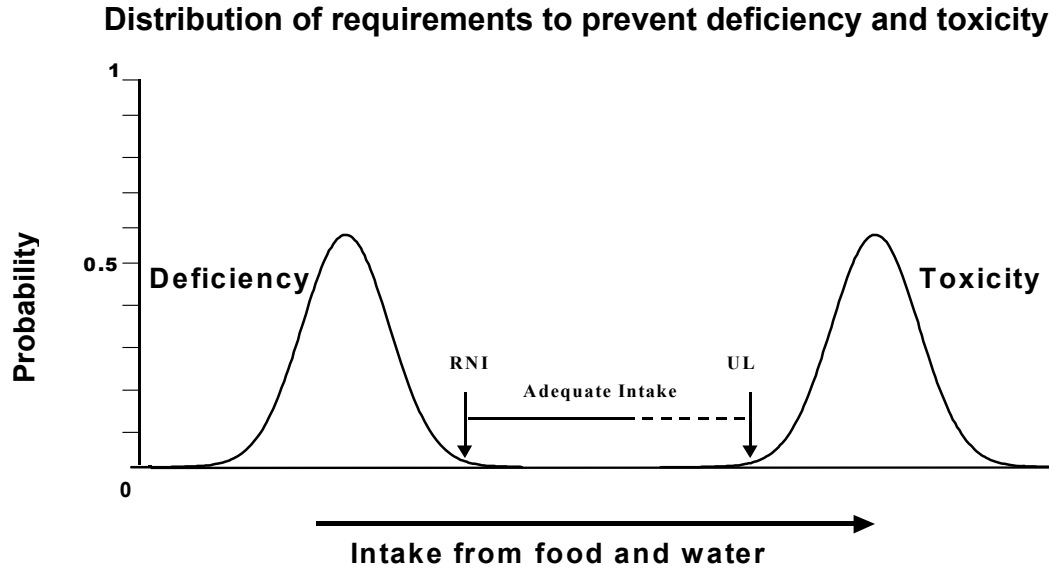
The range of intakes encompassed by the RNI and UL should be considered sufficient to prevent deficiency while avoiding toxicity. If no UL can be derived from experimental or observational data in humans, the UL can be defined from available data on upper range of observed dietary intake of apparently healthy populations.

Protective nutrient intake

The concept of protective nutrient intake has been introduced in some cases to refer to an amount greater than the RNI, which may be protective against a specified health or nutritional risk of public health relevance (e.g., vitamin C intake with a meal to promote iron absorption or folic acid to lower the risk of neural tube defects). The text will indicate when existing data provide justifiable differences between RNI values and protective intake levels. These intakes are expressed as a daily value or as an amount to be consumed within a meal.

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Figure 1



Report of the consultation

In welcoming the participants, Dr Nath, Assistant Director General, Regional Office for Asia and the Pacific, (FAO), recalled the previous Consultation and publication on this subject from 1974. That and the present Consultation are part of a long series of such expert consultations, which have as a primary objective; a) the review of the state of knowledge on the role of various nutrients in the human diet; and, b) the formulation of practical recommendations where interpretation is needed or controversy exists. The most recent in this series was the *joint FAO/WHO expert consultation on carbohydrates in human nutrition* held in Rome in 1997.

Dr Nath spoke of the increasing evidence for the important role which vitamins and minerals play in preventing disease and promoting overall health. Indeed, the understanding of the role of micronutrients in foods and nutrition has significantly increased over the past 24 years. For many years the basic assumption – which still may be the best assumption – on which nutritionists make their projections has been that all nutrients can be obtained from a diet containing a variety of foods from a variety of sources. Some of the challenges to this assumption rest in the complexities and diversity of worldwide realities, culture, and traditions.

Dr Nath pointed out that for many people with access to an adequate energy intake, an extensive freedom of choice exists in the selection of food. However, the existence of widespread poverty in the majority of the UN member countries precludes the opportunity to consume adequate energy let alone a diet balanced in micronutrients. He observed that the increase in the availability of a wide variety of foods and especially “fast foods” in almost every country in the world, coupled with the increasing pace of urban lifestyles across all cultures and countries, does not necessarily result in adequate vitamin and mineral intake.

The existence of the dichotomies in lifespan was also mentioned by Dr Nath. In different parts of the world and in different segments of society within the same countries, there are broad ranges of life spans in part due to nutritional adequacy. Especially in urban populations, as lifespan increases as a result of nutritional adequacy and despite improved

access to health care, an increase in obesity, diabetes, some forms of cancer, and cardiovascular disease has been recorded in all regions of the globe. Although epidemiological studies do not provide us with cause-and-effect explanations, they do provide impetus for future research into the role of vitamins and minerals in the prevention and management of some non-communicable diseases.

Dr Nath reminded the participants that they had been invited to the Consultation as independent experts and that their participation in the Consultation was to be in their individual capacity and not as a representative of any organization, affiliation, or government. He underscored the importance of drawing conclusions and making recommendations based on science, which is traceable to studies conducted largely in humans. This is necessary for correct food labelling and relevant health claims and for the better use of foods in the dietary management and prevention of non-communicable diseases. These issues have economic implications for agricultural production, the food industry, and public health policy.

Dr Sultana Khanum, Regional Adviser/Nutrition, SEARO (South-East Asia Regional Office), WHO, added her welcome on behalf of the Director-General of WHO, Dr Gro Harlem Brundtland, and the Regional Director for South-East Asia Region, Dr Uton Muchtar Raffle. Dr Khanum noted that the choice of South-East Asia as the site of the Expert Consultation was significant because some of the most tangible successes and achievements have occurred within this geographical region in the realm of identifying, preventing, reducing, and eliminating many forms of malnutrition.

Dr Khanum noted that the FAO and WHO have a long history of collaboration at the country, regional, and global levels towards combating food and nutritional problems. She underlined the importance of using science as the basis of the standard setting process, which took place during the consultation.

Dr Graeme Clugston, Director, WHO Nutrition Programmes, added his welcome to the participants on behalf of the Director-General of WHO. Dr Clugston pointed out that the formulation and implementation of science-based dietary guidelines have become a central issue for the nutritional sciences as well as a major challenge for governments world wide, especially since the *International Conference on Nutrition* held in Rome, December 1992.

Dr Clugston expressed confidence that this Expert Consultation would lead to scientifically sound up-to-date recommendations for vitamin and mineral requirements in human nutrition. FAO and WHO would then ensure that these recommendations would be passed on to all Member States world wide, providing them with the best possible guidance for developing their own appropriate dietary guidelines for health promotion, good nutrition, and disease prevention.

The Consultation elected Dr Donald McCormick as chairperson and Professor Chen Chunming as vice-chair. Dr Glenville Jones and Dr Colin Mills were appointed jointly as rapporteurs. Dr McCormick in his response indicated the importance of this Consultation and outlined the scope of the issues that would be discussed and on which the two agencies, FAO and WHO, were seeking expert guidance from the Consultation.

Recommended nutrient intakes

Appendix 1 at the end of the report provides two composite tables summarising the recommended nutrient intakes (RNIs) for each of the vitamins and minerals. For the purposes of preparing these tables the recommendations made by the experts were adjusted so that the tables could be based on common body weights and age groups. Details are provided in the footnote at the bottom of the tables.

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Chapter 2

Food-based approaches to meeting vitamin and mineral needs

Dietary patterns have varied over time depending on the agricultural practices and the climatic, ecologic, cultural, and socio-economic factors, which determine available foods. At present, virtually all dietary patterns adequately satisfy or even exceed the nutritional needs of population groups. This is true except where socio-economic conditions limit the capacity to produce and purchase food or aberrant cultural practices restrict the choice of foods. It is thought that if people have access to a sufficient quantity and variety of foods, they will meet their nutritional needs. The current practice of evaluating nutritive value of diets should include not only energy and protein adequacy but also the micronutrient density of the diet.

A healthy diet can be attained in more than one way because of the variety of foods, which can be combined. It is thus difficult to define the ranges of intake for a specific food, which should be included in a given combination to comply with nutritional adequacy. In practice, the set of food combinations which is compatible with nutritional adequacy is restricted by the level of food production sustainable in a given ecologic and population setting. In addition, there are economic constraints, which limit food supply at household level. The development of food-based dietary guidelines (FBDGs) by the FAO and WHO (*1*) recognises this and focuses on the combination of foods that can meet nutrient requirements rather than on how each specific nutrient is provided in adequate amounts.

The first step in the process of setting dietary guidelines is defining the significant diet-related public health problems in a community. Once these are defined, the adequacy of the diet is evaluated by comparing the information available on dietary intake with recommended nutrient intakes (RNIs). Nutrient intake goals under this situation are specific for a given ecologic setting, and their purpose is to promote overall health, control specific nutritional diseases (whether they are induced by an excess or deficiency of nutrient intake), and reduce the risk of diet-related multi-factorial diseases. Dietary guidelines represent the practical way to reach the nutritional goals for a given population. They take into account the customary dietary pattern and indicate what aspects should be modified. They consider the ecologic setting, socio-economic and cultural factors, and biologic and physical environment in which the population lives.

The alternative approach to defining nutritional adequacy of diets is based on the biochemical and physiologic basis of human nutritional requirements in health and disease. The quantitative definition of nutrient needs and its expression as RNIs have been important instruments of food and nutrition policy in many countries and have focused the attention of international bodies. This nutrient-based approach has served many purposes but has not always fostered the establishment of nutritional and dietary priorities consistent with the broad public health priorities at the national and international levels. It has permitted a more precise definition of requirements for essential nutrients when establishing RNIs but unfortunately has often been narrowly focused, concentrating on the precise nutrient requirement amount and not on solving the nutritional problems of the world. In contrast to RNIs, FBDGs are based on the fact that people eat food, not nutrients. As illustrated in this chapter, the notion of nutrient density is

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helpful for defining FBDGs and evaluating the adequacy of diets. In addition, they serve to educate the public through the mass media and provide a practical guide to selecting foods by defining dietary adequacy (1).

Advice for a healthy diet should provide both a quantitative and qualitative description of the diet for it to be understood by individuals, who should be given information on both size and number of servings per day. The quantitative aspects include the estimation of the amount of nutrients in foods and their bio-availability in the form they are actually consumed. Unfortunately, available food composition data for most foods currently consumed in the world are incomplete, outdated, or insufficient for evaluating true bio-availability. The qualitative aspects relate to the biologic utilisation of nutrients in the food as consumed by humans and explore the potential for interaction among nutrients. Such an interaction may enhance or inhibit the bio-availability of a nutrient from a given food source.

Including foods in the diet, which have high micronutrient density – such as pulses or legumes, vegetables (including green leafy vegetables), and fruits – is the preferred way of ensuring optimal nutrition including micronutrient adequacy for most population groups. Most population groups afflicted by micronutrient deficiency largely subsist on refined cereal grain or tuber-based diets, which provide energy and protein (with improper amino acid balance) but are insufficient in critical micronutrients. Figures 2-5 and Tables 1-4 included at the end of this chapter illustrate how addition of a variety of foods to the basic four diets (white rice- ***Figure 2***, corn tortilla- ***Figure 3***, refined couscous- ***Figure 4***, and potato- ***Figure 5***) can increase the nutrient density of a cereal or tuber-based diet. There is a need for broadening the food base and diversification of diets. Much can be gained from adding reasonable amounts of these foods, which will add micronutrient density to the staple diet (***Table 1, 2, 3*** *and* ***4***).

The recent interest in the role of phyto-chemicals and antioxidants on health and their presence in plant foods lend further support to the recommendation for increasing vegetables and fruit consumed in the diet. The need for dietary diversification is supported by the knowledge of the interrelationships of food components, which may enhance the nutritional value of foods and prevent undesirable imbalances, which may limit the utilisation of some nutrients. For example, fruits rich in ascorbic acid will enhance the absorption of ionic iron.

If energy intake is low (<8.368 MJ/day), for example, in the case of young children, sedentary women, or the elderly, the diet may not provide vitamin and mineral intakes sufficient to meet the RNIs. This situation may be of special relevance to the elderly, who are inactive, have decreased lean body mass, and typically decrease their energy intake. Young children, pregnant women, and lactating women, who have greater micronutrient needs relative to their energy needs, will also require increased micronutrient density.

The household is the basic unit for food consumption under most settings, and if there is sufficient food, individual members of the household can consume a diet with the recommended nutrient densities and meet their specific RNIs. However, appropriate food distribution within the family must be considered to ensure that children and women receive adequate food with high micronutrient density. Household food distribution must be considered when establishing general dietary guidelines and addressing the needs of vulnerable groups in the community. In addition, education detailing the appropriate storage and processing of foods to prevent micronutrient losses at the household level is important.

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Dietary diversification when consuming cereal and tuber-based diets (rice, corn, wheat, potato, and cassava)

Dietary diversification is important to improve the intake of critical nutrients. The micronutrients selected discussed here, although limited in number, are of public health relevance or serve as markers for overall micronutrient intake. The chapters on individual nutrients will provide further details on food-related considerations for micronutrient adequacy. The nutrients selected for discussion below include some of the nutrients, which are most difficult to obtain in cereal and tuber-based diets. Nutrient deficiencies of vitamin A, iron, and zinc are widespread.

Vitamin A

The vitamin A content of most staple diets can be significantly improved with the addition of a relatively small portion of plant foods rich in carotenoids, the precursors of vitamin A. For example, a usual portion of cooked carrots (50 g) added to a daily diet, or 21 g of carrots per 4.184 MJ, provides 500 μg retinol equivalents, which is the recommended nutrient density for this vitamin. The biologic activity of pro-vitamin A varies among different plant sources, and fruits and vegetables such as carrots, mango, papaya, and melon contain large amounts of nutritionally active carotenoids, (*2, 3*). Green leafy vegetables such as ivy gourd have been successfully used in Thailand as a source of vitamin A, and carotenoid-rich red palm oil serves as an easily available and excellent source of vitamin A in other countries. Consequently, a regular portion of these foods included in an individual's diet may provide 100 percent or more of the daily requirement for retinol equivalents. Vitamin A is also present in animal food sources in a highly bio-available form. Therefore it is important to consider the possibility of meeting vitamin A needs by including animal foods in the diet. For example, providing minor amounts of fish or chicken liver (20–25 g) in the diet provides more than the recommended vitamin A nutrient density for virtually all age and sex groups.

Vitamin C

A real gain in vitamin C intake can be achieved by including citrus fruit or other foods rich in ascorbic acid in the diet. For example, an orange or a small amount of other vitamin C-rich fruit (60 g of edible portion) provides the recommended ascorbic acid density. Adding an orange to a potato-based diet increases the level of vitamin C threefold. Other good vitamin C food sources are guava, amla, kiwi, cranberries, strawberries, papaya, mango, melon, cantaloupe, spinach, Swiss chard, tomato, asparagus, and Brussels sprouts. All these foods, when added to a diet or meal in regular portion sizes, will significantly improve the vitamin C density. Because ascorbic acid is heat labile, minimal cooking (steaming or stir-frying) is recommended to maximise the bio-available nutrient. The significance of consuming vitamin C with meals will be discussed relative to iron absorption (see ***Chapter 13***).

Folate

Folate is now considered significant not only for the prevention of macrocytic anaemia, but also for normal foetal development. Recently, this vitamin was implicated in the maintenance of cardiovascular health and cognitive function in the elderly. Staple diets consisting largely of cereal grains and tubers are very low in folate but can be improved by the addition of legumes or green leafy vegetables. For example, a regular portion of cooked lentils (95 g) added to a rice-based diet can provide an amount of folate sufficient to meet the desirable nutrient density for this vitamin. Other legumes such as beans and peas are also good sources of this vitamin, but larger portions are needed for folate sufficiency (100 g beans and 170 g peas). Cluster bean and colacasia leaves are excellent folate sources used in the Indian diet.

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Another good source of folate is chicken liver; only one portion (20–25 g) is sufficient to meet the desirable nutrient density for folate and vitamin A simultaneously. The best sources of folate are organ meats, green leafy vegetables, and sprouts. However, 50 percent or more of food folate is destroyed during cooking. Prolonged heating in large volumes of water should be avoided, and it is advisable to consume the water used in the cooking of vegetables.

Iron and zinc

Minerals such as iron and zinc are low in cereal and tuber-based diets, but the addition of legumes can slightly improve the iron content of those diets. However, the bio-availability of this non-heme iron source is low. Therefore, it is not possible to meet the recommended levels of iron and zinc in the staple-based diets through a food-based approach unless some meat, poultry, or fish is included. For example adding a small portion (50 g) of meat, poultry, or fish will increase the total iron content as well as the amount of bio-available iron. For zinc the presence of a small portion (50 g) of meat, poultry, or fish will secure dietary sufficiency of most staple diets.

The consumption of ascorbic acid along with the food rich in iron will enhance absorption. There is a critical balance between enhancers and inhibitors of iron absorption. Nutritional status can be improved significantly by educating households on food preparation practices, which minimise the consumption of inhibitors of iron absorption; for example, the fermentation of phytate-containing grains before the baking of breads to enhance iron absorption.

How to accomplish dietary diversity in practice

It is essential to work on strategies, which promote and facilitate dietary diversification to achieve complementarity of cereal or tuber-based diets with foods rich in micronutrients in populations with limited economics or limited access to food. A recent FAO and International Life Sciences Institute (*4*) publication proposed strategies to promote dietary diversification within the implementation of food-based approaches. These strategies, which follow, have been adapted or modified based on the discussions held in this consultation:

1. Community or home vegetable and fruit gardens. These projects should lead to increased production and consumption of micronutrient-rich foods (legumes, green leafy vegetables, and fruits) at the household level. The success of such projects requires a good knowledge and understanding of local conditions as well as the involvement of women and the community in general. These are key elements for supporting, achieving, and sustaining beneficial nutritional change at the household level. Land availability and water supply may present common constraints, which require local government intervention or support before they are overcome. The educational effort should be directed towards securing appropriate within-family distribution, which considers the needs of the most vulnerable members of the family, especially infants and young children. Separate FBDGs for vulnerable groups, such as pregnant and lactating women, children, and the elderly, should be developed.

2. Production of fish, poultry, and small animals (rabbits, goats, and guinea pigs). These are excellent sources of highly bio-available essential micronutrients such as vitamin A, iron, and zinc. The production of animal foods at the local level may permit communities to access foods which otherwise are not available because of their high costs. These types of projects also need some support from local governments or non-governmental organizations to overcome cost constraints of programme implementation, including the training of producers.

3. Implementation of large-scale commercial vegetable and fruit production. The objective of this initiative is to provide micronutrient-rich foods at reasonable prices through effective

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and competitive markets, which lower consumer prices without reducing producer prices. This will serve predominantly the urban and non-food-producing rural areas.

4. Reduction of post-harvest losses of the nutritional value of micronutrient-rich foods, such as fruits and vegetables. Improvement of storage and food-preservation facilities significantly reduces post-harvest losses. At the household level, the promotion of effective cooking methods and practical ways of preserving foods (solar drying of seasonal micronutrient-rich foods such as papaya, grapes, mangoes, peaches, tomatoes, and apricots) may significantly increase the access to bio-available micronutrient-rich foods. At the commercial level, grading, packing, transport, and marketing practices reduce losses, stimulate economic growth, and optimise income generation.

5. Improvement of micronutrient levels in soils and plants, which will improve the composition of plant foods and enhance yields. Current agricultural practices can improve the micronutrient content of foods through correcting soil quality and pH and increasing soil mineral content depleted by erosion and poor soil conservation. Long-term food-based solutions to micronutrient deficiencies will require improvement of agricultural practices, seed quality, and plant breeding (by means of a classical selection process or genetic modification).

The green revolution made important contributions to cereal supplies, and it is time to address the need for improvements in the production of legumes, vegetables, fruits, and other micronutrient-rich foods. FBDGs can serve to reemphasise the need for these crops.

It is well recognised that the strategies proposed to promote dietary diversity need a strong community-level commitment. For example, the increase in price of legumes associated with decreased production and lower demand needs to be corrected. The support of local authorities and government may facilitate the implementation of such projects because these actions require economic resources, which sometimes are beyond the reach of the most needy.

Practices which will enhance the success of food-based approaches

To achieve dietary adequacy of vitamin A, vitamin C, folate, iron, and zinc by using food-based approaches, food preparation and dietary practices must be considered. For example, it is important to recommend that vegetables rich in vitamin C, folate, and other water-soluble or heat-labile vitamins be minimally cooked in small amounts of water. For iron bio-availability it is essential to reduce the intake of inhibitors of iron absorption and to increase the intake of enhancers of absorption in a given meal. Following this strategy, it is recommended to increase the intake of: germinated seeds, fermented cereals, heat-processed cereals, meats, and fruits and vegetables rich in vitamin C and to encourage the consumption of tea, coffee, chocolate, or herbal teas at times other than with meals (see ***Chapter 13*** and ***Chapter 16***). Consumption of flesh foods improves zinc absorption whereas it is inhibited by consumption of diets high in phytate, such as diets based on unrefined cereal. Zinc availability can be estimated according to the phytate-to-zinc (molar) ratio of the meal (*5*).

This advice is particularly important for people who consume cereal and tuber-based diets. These foods constitute the main staples for most populations of the world, populations that are also most at risk for micronutrient deficiencies. Other alternatives – fortification and supplementation – have been proposed as stopgap measures when food-based approaches are not feasible or are still in progress. There is a definite role for fortification in meeting iron, folate, iodine, and zinc needs. Fortification and supplementation should be seen as complementary to food-based strategies and not as a replacement. Combined, all these strategies can go a long way toward stabilising the micronutrient status of populations at risk.

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Food-based approaches usually take longer to implement but once established are truly sustainable.

Delineating the role of supplementation and food fortification for nutrients which cannot be supplied by regular foods

Under ideal conditions of food access and availability, food diversity should satisfy micronutrient and energy needs of the general population. Unfortunately, for many people in the world, the access to a variety of micronutrient-rich foods is not possible. As demonstrated in our analysis of cereal and tuber-based diets (***see appendixes***), micronutrient-rich foods including small amount of flesh foods and a variety of plant foods (vegetables and fruits) are needed daily. This may not be realistic at present for many communities living under conditions of poverty. Food fortification and food supplementation are important alternatives that complement food-based approaches to satisfy the nutritional needs of people in developing and developed countries.

Fortification

Fortification refers to the addition of nutrients to a commonly eaten food (the vehicle). It is possible for a single nutrient or group of micronutrients (the fortificant) to be added to the vehicle, which has been identified through a process in which all stakeholders have participated. This strategy is accepted as sustainable under most conditions and often is cost effective on a large scale when successfully implemented. Iron fortification of wheat flour and iodine fortification of salt is examples of fortification strategies with excellent results (*6*).

There are at least three essential conditions that must be met in any fortification programme(*6, 7*): the fortificant should be effective, bio-available, acceptable, and affordable; the selected food vehicle should be easily accessible and a specified amount of it should be regularly consumed in the local diet; and detailed production instructions and monitoring procedures should be in place and enforced by law.

Iron fortification

Food fortification with iron is recommended when dietary iron is insufficient or the dietary iron is of poor bio-availability, which is the reality for most people in the developing world and for vulnerable population groups in the developed world. Moreover, the prevalence of iron deficiency and anaemia in vegetarians and in populations of the developing world which rely on cereal or tuber foods is significantly higher than in omnivore populations.

Iron is present in foods in two forms, as heme iron, which is derived from flesh foods (meats, poultry, and fish), and as non-heme iron, which is the inorganic form present in plant foods such as legumes, grains, nuts, and vegetables (*8, 9*). Heme iron is highly (20–30 percent) absorbed and its bio-availability is relatively unaffected by dietary factors. Non-heme iron has a lower rate of absorption (2–10 percent), depending on the balance between iron absorption inhibitors (phytates, polyphenols, calcium, and phosphate) and iron absorption enhancers (ascorbic and citric acids, cysteine-containing peptides, ethanol, and fermentation products) present in the diet (*8, 9*). Because staple foods around the world provide predominantly non-heme iron sources of low bio-availability, the traditionally eaten staple foods represent an excellent vehicle for iron fortification. Examples of foods, which have been fortified, are wheat flour, corn (maize) flour, rice, salt, sugar, cookies, curry powder, fish sauce, and soy sauce (*8*). Nevertheless the beneficial effects of consumption of iron absorption enhancers have been extensively proven and should always be promoted (i.e., consumption of vitamin C–rich food together with the non-heme iron source).

Iodine fortification

Iodine is sparsely distributed in the Earth's surface and foods grown in soils with little or no iodine lack an adequate amount of this micronutrient. This situation had made iodine deficiency disorders exceedingly common in most of the world and highly prevalent in many countries before the introduction of salt iodisation (*10*). Only foods of marine origin are naturally rich sources of iodine. Salt is a common food used by most people worldwide, and the establishment of an well-implemented permanent salt-iodisation programme has been proven to eradicate iodine deficiency disorders (see ***Chapter 12***). Universal salt iodisation is the best way to virtually eliminate iodine deficiency disorders by the year 2000 (*4*).

However, salt iodisation is not simply a matter of legislating mandatory iodisation of salt. It is important to determine the best fortification technique, co-ordinate the implementation at all salt production sites, establish effective monitoring and quality control programmes, and measure iodine fortification level periodically. The difficulties in implementing salt iodisation programmes arise primarily when the salt industry is widely dispersed among many small producers. The level of iodine fortification usually lies between 25 and 50 mg/kg salt. The actual amount should be specified according to the level of salt intake and magnitude of deficit at the country level, because iodine must be added within safe and effective ranges. Additionally, it is very important to implement a monitoring plan to control the amount of iodine in the salt at the consumer's table, (*10, 11*). United Nations agencies responsible for assisting governments in establishing iodisation programmes should provide technical support for programme implementation, monitoring, and evaluation to ensure sustainability.

Zinc fortification

The body depends on a regular zinc supply provided by the daily diet because stores are quite limited. Food diversity analysis demonstrates that it is virtually impossible to achieve zinc adequacy in the absence of a flesh food source. Among flesh foods, beef is the best source of zinc and is followed by poultry and then fish. Zinc fortification programmes are being studied, especially for populations, which consume predominately plant foods. Fortification of cereal staple foods is a potentially attractive intervention, which could benefit the whole population as well as target the vulnerable population groups of children and pregnant women. Such addition of zinc to the diet would perhaps decrease the prevalence of stunting in many developing countries with low-zinc diets, because linear growth is affected by zinc supply.

Folic acid fortification

The recommended nutrient density by the developers of the FAO/WHO (*1*) FBDGs for folic acid is 200 μg/4.184 MJ. Although this reference value is higher than other standards of reference, the increase in folic acid consumption by women of childbearing age is very important: it may improve birth weight and reduce the prevalence of neural tube defects by 50 percent. Elevated plasma homo-cysteine levels are considered to be an independent risk factor for heart disease; a higher intake of folic acid may also benefit the rest of the population because it may lower homo-cysteine levels in adults (see ***Chapter 4***). In addition, folate may improve the mental condition of the elderly population (*12, 13*).

Although the desirable folic acid density may be achieved through dietary diversity, it requires the daily presence of organ meats, green leafy vegetables, pulses, legumes, or nuts in the diet (*14*). Most population groups may not easily reach the appropriate level of folic acid consumption; therefore, folic acid fortification has been recommended. The United States initiated mandatory folic acid fortification of cereal-grain products in January 1998. The

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fortification level approved in the United States is 140 μg/100 g product, which will increase the average woman's intake by only 100 μg/day. This amount is considered safe (a dose, which will not mask pernicious anaemia, which results from vitamin B12 deficiency,) but it may be ineffective in lowering the occurrence of neural tube defects (*15*).

Supplementation

Supplementation refers to periodic administration of pharmacologic preparations of nutrients as capsules or tablets or by injection when substantial or immediate benefits are necessary for the group at risk. As established at the *International Conference on Nutrition* (*16*), nutritional supplementation should be restricted to vulnerable groups, which cannot meet their nutrient needs through food (women of childbearing age, infants and young children, elderly people, low socio-economic groups, displaced people, refugees, and populations experiencing other emergency situations). For example, iron supplementation is recognised as the only option to control or prevent iron deficiency anaemia in pregnant women. Supplementation with folic acid should be considered for women of childbearing age who have had a child with neural tube defect to prevent recurrence.

Food-based dietary guidelines

Food-based dietary guidelines (FBDGs) are an instrument of and expression of food and nutrition policy and should be based directly on diet and disease relationships of particular relevance to the individual country. Their primary purpose is to educate healthcare professionals and consumers about health promotion and disease prevention. In this way priorities in establishing dietary guidelines can address the relevant public health concerns whether they are related to dietary insufficiency or excess. In this context, meeting the nutritional needs of the population takes its place as one of the components of food and nutrition policy goals along with the priorities included in the FBDGs for improved health and nutrition for a given population.

The world nutrition and health situation demonstrates that the major causes of death and disability have been traditionally related to undernutrition in developing countries and to the imbalance between energy intake and expenditure (which lead to obesity and other chronic diseases – diabetes, cardiovascular disease, hypertension, and stroke) in industrialized countries. The tragedy is that many suffer from too little food while others have diseases resulting from too much food, but both would benefit from a more balanced distribution of food and other resources. Although the nature of the health and nutrition problems in these two contrasting groups is very different, the dietary guidelines required to improve both situations are not. Most countries presently have the combined burden of malnutrition from deficit and increasing prevalence of obesity and other chronic diseases from over consumption. The approaches to address the problems, nevertheless, should be country and population specific.

Although two-thirds of the world's population depends on cereal or tuber-based diets, the other one-third consumes significant amounts of animal food products. The latter group places an undue demand on land, water, and other resources required for intensive food production, which makes the typical Western diet not only undesirable from the standpoint of health but also environmentally unsustainable. If we balance energy intake with the expenditure required for basal metabolism, physical activity, growth, and repair, we will find that the dietary quality required for health is essentially the same across population groups.

Efforts in nutrition education and health promotion should include a strong encouragement for active lifestyles. Improving energy balance for rural populations in developing countries may mean increasing energy intake to normalise low body mass index (BMI, weight/height^2, calculated as kg/m^2), ensuring adequate energy stores and energy for

appropriate social interactions. In sedentary urban populations, improving energy balance will mean increasing physical activity to decrease energy stores (body fat mass) and thus normalise BMI. Thus, the apparent conflicting goals – eradicating undernutrition while preventing overnutrition – are resolved by promoting sufficient energy for a normal BMI. Moreover, if we accept that FBDGs should be ecologically sustainable, the types and amounts of foods included in a balanced diet are not very different for promoting adequate nutrition in the undernourished and preventing overnutrition in the affluent.

This is well exemplified by the similarities in the FBDGs across countries, whether represented by pyramids, rainbows, dishes, pots, etc. It is obvious that consumption of excess energy will induce an increase in energy stores, which may lead to obesity and related health complications. Populations should consume nutritionally adequate and varied diets, based primarily on foods of plant origin with small amounts of added flesh foods. Households should select predominantly plant-based diets rich in a variety of vegetables and fruits, pulses or legumes, and minimally processed starchy staple foods. The evidence that such diets will prevent or delay a significant proportion of non-communicable chronic diseases is consistent. A predominantly plant-based diet has a low energy density, which may protect against obesity. This should not exclude small amounts of animal foods, which may make an important nutritional contribution to plant-food-based diets, as illustrated in the examples presented earlier. Inadequate diets occur when food is scarce or when food traditions change rapidly, as is seen in societies undergoing demographic transitions or rapid urbanisation. Traditional diets, when adequate and varied, are likely to be generally healthful and more protective against chronic non-communicable diseases than the typical Western diet, consumed predominantly in industrialized societies (*17)*.

Reorienting food production, agricultural research, and commercialisation policies needs to take into consideration FBDGs, which increase the demand for a variety of micronutrient-rich foods and thus stimulate production to meet the consumption needs. Prevailing agricultural policies encourage research on and production and importation of foods, which do not necessarily meet the requirements of FBDG implementation. For example, great emphasis is placed on cereals, horticultural crops for export, legumes for export, non-food cash crops, and large livestock. Necessary policy reorientation is required to ensure increased availability of micronutrient-rich foods within the local food system. Norway has successfully implemented agricultural and food production policies based on a National Nutrition Plan of Action, providing economic incentives for the producer and consumer in support of healthful diets. The results speak for themselves, as Norway has experienced a sustained improvement in life expectancy and a reduction in deaths from cardiovascular disease and other chronic non-communicable conditions.

Recommendations for the future

The Consultation acknowledged the limitations in our knowledge of these important aspects, which affect nutrient utilisation and recommended that the International Food Data System (INFoods) effort led by FAO/UNU be strengthened. Special emphasis should be placed on the micronutrient composition of local diets as affected by ecologic setting; analysis of food components (nutrients or bio-active components), which may affect the bio-availability and utilisation of critical micronutrients; and the analysis of cooked foods and typical food combinations as actually consumed by population groups. In addition the development of FBDGs at the country level should be supported by UN agencies.

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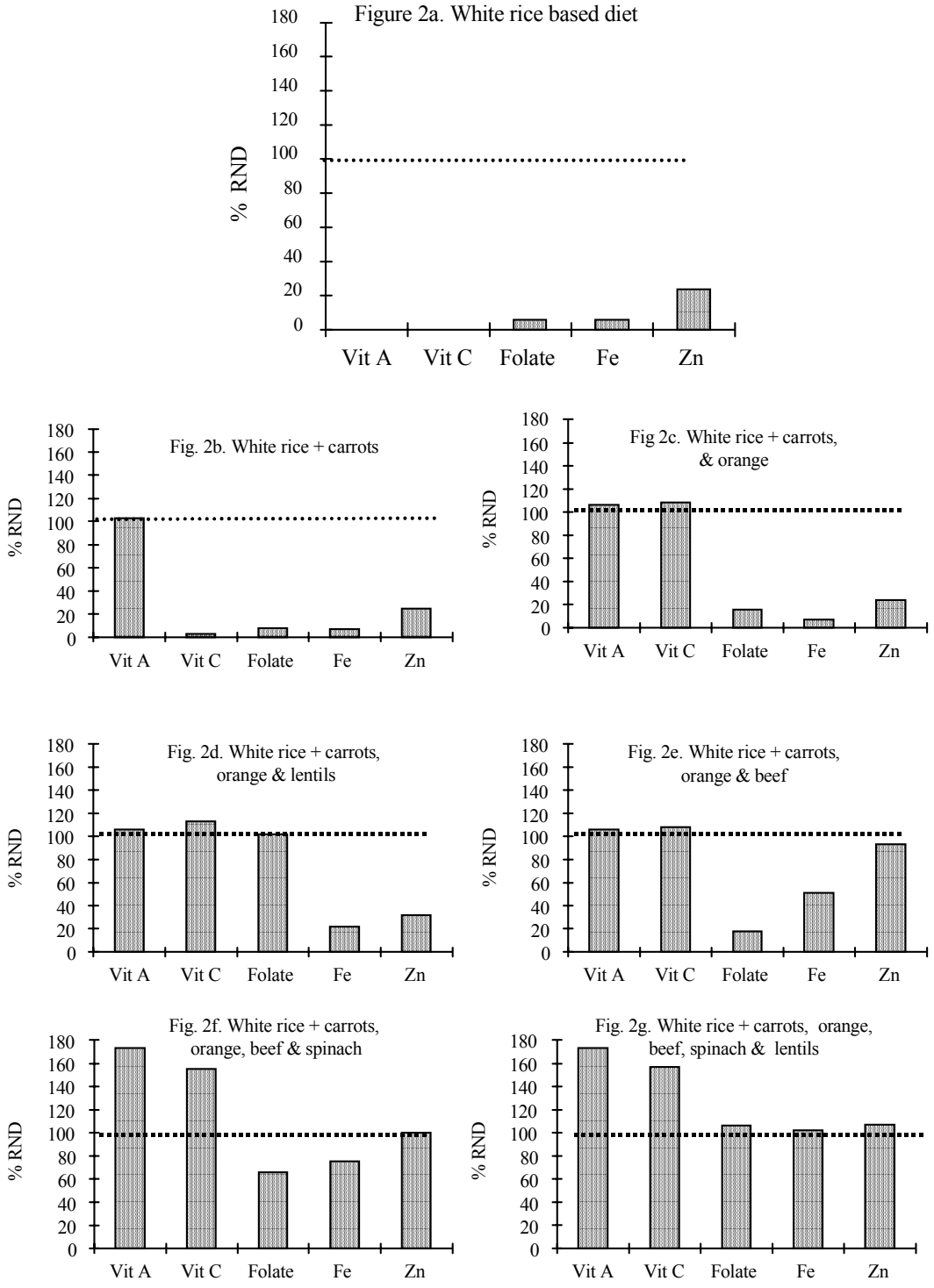
Future research

The following research needs were identified to facilitate the implementation of a food-based approach in the prevention of micronutrient deficiencies:

- food data system development, which includes development of methodology for micronutrient composition of foods, organizing data retrieval, and reporting and dissemination through electronic means; this effort should include phyto-chemicals, antioxidants, and other components which may affect health and nutrition, with special emphasis on local foods which may be important for given food cultures;
- identification and evaluation of optimal methods for cooking foods to preserve the nutrient value and enhance the bio-availability of micronutrients;
- development of better methods to preserve foods, especially micronutrients, at the household and community levels;
- identification and propagation of agricultural methods which will enhance the food yields, content, and biologic value of micronutrient-rich foods;
- identification of optimal food combinations and serving size which will be most effective in preventing micronutrient deficits and methods of promotion for these food combinations at the community level;
- development of agricultural research to support the implementation of FBDGs; and
- evaluation of the nutritional impact and cost benefit of food-based approaches in combating micronutrient deficiencies.

Figure 2

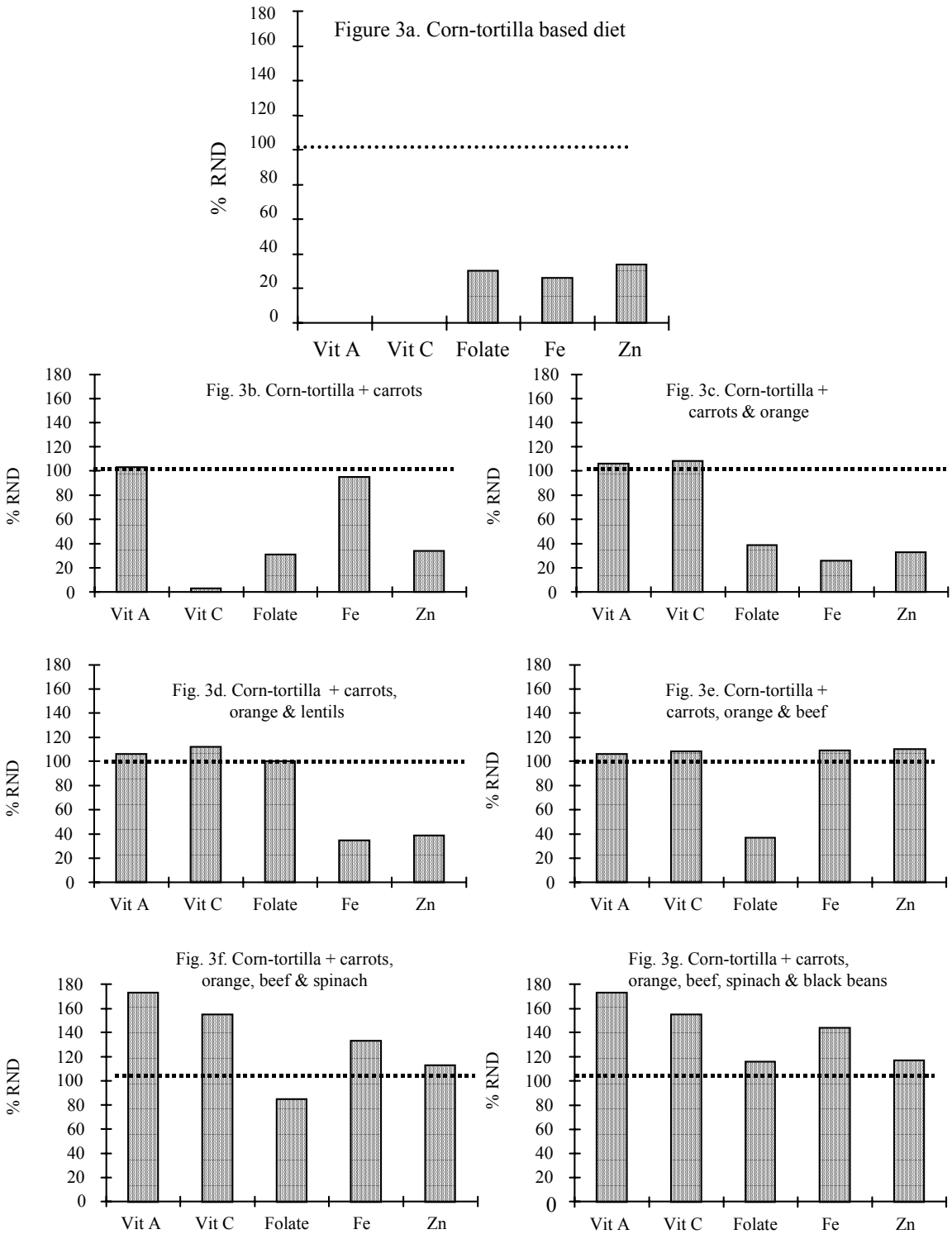
Percentage of recommended nutrient density (RND) for a diet of white rice and the addition of a variety of foods



Note: Data in ***Tables 1*** *and* ***3***

Figure 3

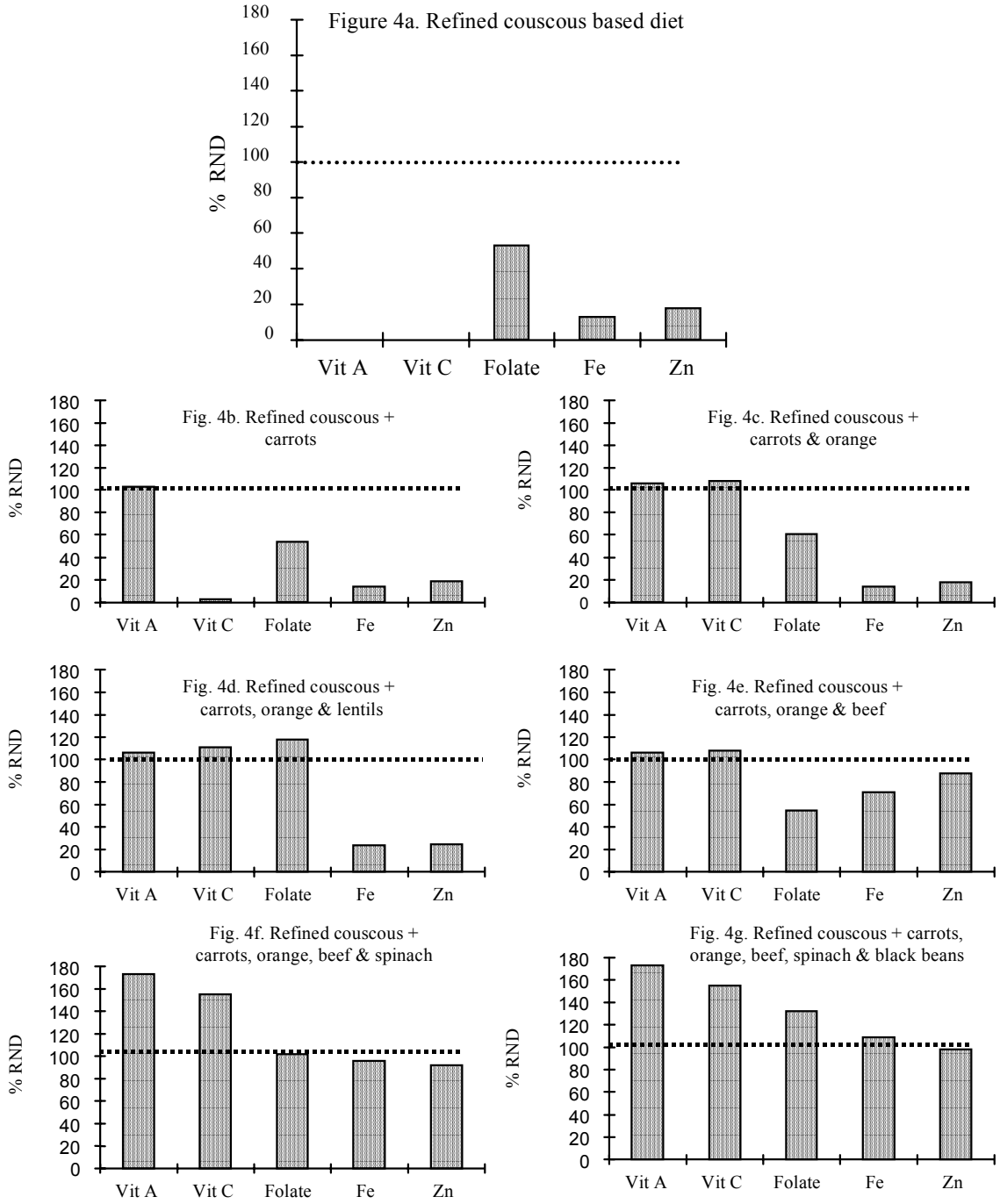
Percentage of recommended nutrient density (RND) for a diet of corn-tortilla and the addition of a variety of foods



Note: Data in ***Tables 1*** *and* ***3***

Figure 4

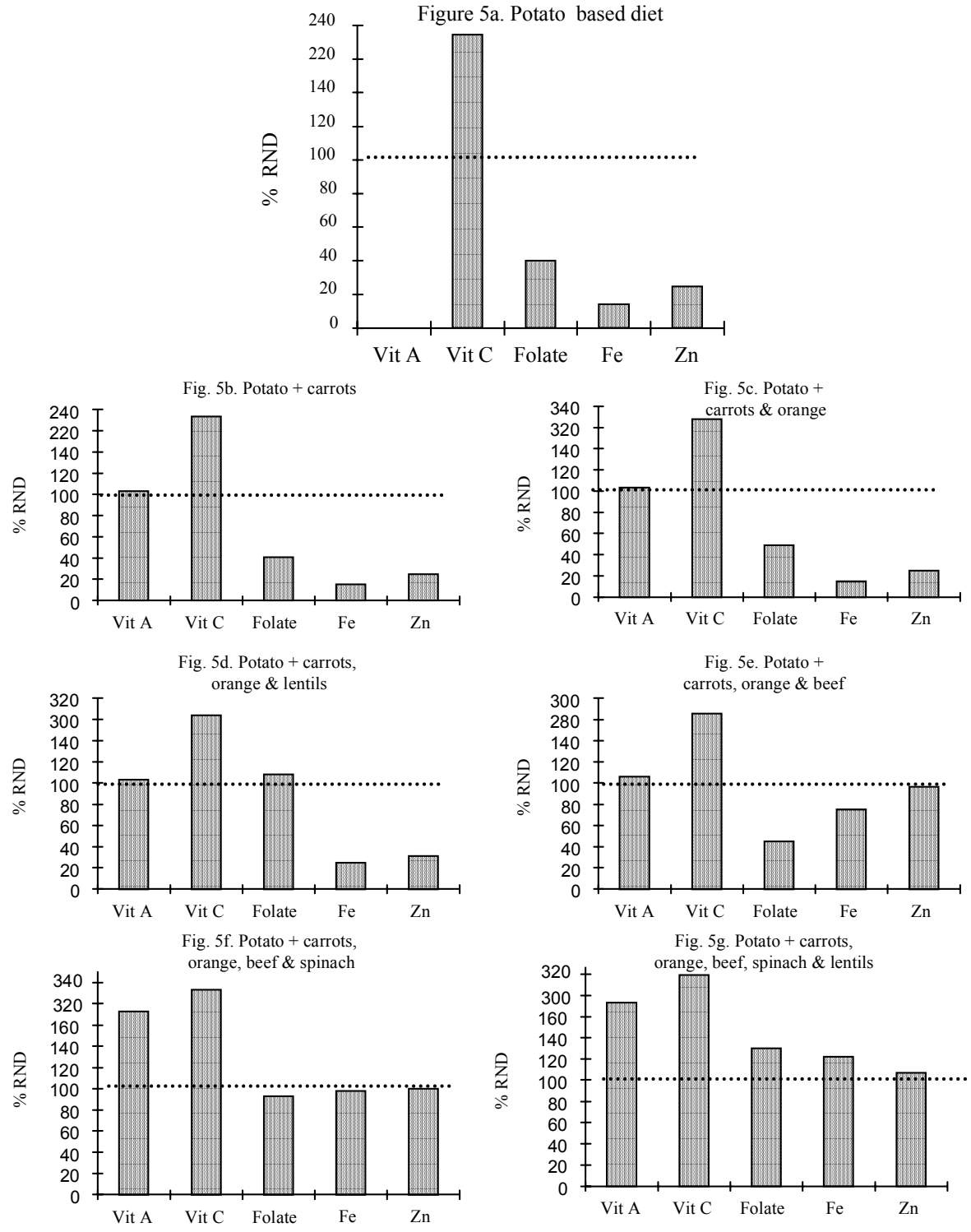
Percentage of recommended nutrient density (RND) for a diet of refined couscous and the addition of a variety of foods



Note: Data in ***Tables 2*** *and* ***4***

Figure 5

Percentage of recommended nutrient density (RND) for a diet of potatoes and the addition of a variety of foods



Note: Data in ***Tables 2*** *and* ***4***

Table 1: White rice and corn-tortilla based diets composition and nutrient density values per 1000 kcals for vitamin A, vitamin C, folate, iron and zinc

	WHITE RICE BASED DIET						
	White rice 598 g Vegetable oil 25 g	White rice 590g Vegetable oil 25 g Carrots 21 g	White rice 570 g Vegetable oil 25 g Carrots 21 g Orange 60 g	White rice 483 g Vegetable oil 25 g Carrots 21 g Orange 60 g Lentils 95g	White rice 477 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g	White rice 468 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	White rice 428 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Lentils 45 g
% E as Protein	5	6	6	8	11	12	13
% E as CHO	72	72	72	69	61	60	59
% E as Fat	23	22	22	23	28	28	28
Vitamin A (μg)	0	516	528	529	528	864	864
Vitamin C (mg)	0	0.5	32.5	33.9	32.5	46.5	47.0
Folate (μg)	12	15	32	203	35	131	212
Iron (mg)	1.2	1.3	1.3	4.3	2.8	4.1	5.6
Zinc (mg)	2.4	2.5	2.4	3.2	5.8	6.0	6.4
Calcium (mg)	18.0	24.2	47.7	62.8	49.4	98.6	105.8

	CORN-TORTILLA BASED DIET						
	Corn-tortilla 368 g Vegetable oil 20 g	Corn-tortilla 363 g Vegetable oil 20 g Carrots 21 g	Corn-tortilla 351 g Vegetable oil 20 g Carrots 21 g Orange 60 g	Corn-tortilla 314 g Vegetable oil 20 g Carrots 21 g Orange 60 g Lentils 71 g	Corn-tortilla 297 g Vegetable oil 20 g Carrots 21 g Orange 60 g Beef 55 g	Corn-tortilla 292g Vegetable oil 20 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	Corn-tortilla 266 g Vegetable oil 20 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Black beans 45 g
% E as Protein	8	8	8	10	13	14	15
% E as CHO	67	67	67	66	58	57	57
% E as Fat	25	25	25	24	29	29	28
Vitamin A (μg)	0	516	528	529	528	864	864
Vitamin C (mg)	0	0.5	32.5	33.5	32.5	46.5	46.5
Folate (μg)	59	61	77	200	73	169	232
Iron (mg)	5.2	5.2	5.1	6.9	6.0	7.3	7.9
Zinc (mg)	3.4	3.4	3.3	3.9	6.6	6.8	7.0
Calcium (mg)	647.7	645.4	648.3	596.5	557.8	598.5	565.0

Table 2: Refined Couscous and potato based diets composition and nutrient density values per 1000 kcal for vitamin A, vitamin C, folate, iron and zinc

	REFINED COUSCOUS BASED DIET						
	Ref. Couscous 697 g Vegetable oil 25 g	Ref. Couscous 690 g Vegetable oil 25 g Carrots 21 g	Ref. Couscous 665g Vegetable oil 25 g Carrots 21 g Orange 60 g	Ref. Couscous 590 g Vegetable oil 25 g Carrots 21 g Orange 60 g Lentils70 g	Ref. Couscous 555 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g	Ref. Couscous 546g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	Ref. Couscous 493 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Black beans 45 g
% E as Protein	11	11	10	12	15	16	17
% E as CHO	66	66	66	64	56	55	55
% E as Fat	23	23	24	24	29	29	28
Vitamin A (μg)	0	516	528	529	528	864	864
Vitamin C (mg)	0	0.5	32.5	33.5	32.5	46.5	46.5
Folate (μg)	105	107	121	236	109	204	263
Iron (mg)	2.6	2.8	2.7	4.7	3.9	5.3	6.0
Zinc (mg)	1.8	1.9	1.8	2.5	5.3	5.5	5.9
Calcium (mg)	55.7	61.6	83.7	90.8	79.4	128.2	136.2

	POTATO BASED DIET						
	Potato 907 g Vegetable oil 25 g	Potato 895 g Vegetable oil 25 g Carrots 21 g	Potato 865 g Vegetable oil 25 g Carrots 21 g Orange 60 g	Potato 770 g Vegetable oil 25 g Carrots 21 g Orange 60 g Lentils 70 g	Potato 723 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g	Potato 710 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	Potato 649 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Lentils 45 g
% E as Protein	6	6	6	8	12	12	13
% E as CHO	71	71	71	69	61	60	59
% E as Fat	23	23	23	23	27	28	28
Vitamin A (μg)	0	516	528	529	528	864	864
Vitamin C (mg)	67.2	67.0	96.5	90.5	86.0	99.1	95.2
Folate (μg)	80	82	97	216	89	185	261
Iron (mg)	2.8	2.9	2.9	4.9	4.1	5.4	6.7
Zinc (mg)	2.5	2.5	2.5	3.1	5.8	6.0	6.4
Calcium (mg)	67.2	72.8	94.6	100.7	88.7	137.1	141.0

Table 3: White rice and corn-tortilla based diets composition and percent of nutrient density values for vitamin A, vitamin C, folate, iron and zinc

	WHITE RICE BASED DIET						
	White rice 598 g Vegetable oil 25 g	White rice 590g Vegetable oil 25 g Carrots 21 g	White rice 570 g Vegetable oil 25 g Carrots 21 g Orange 60 g	White rice 483 g Vegetable oil 25 g Carrots 21 g Orange 60 g Lentils 95g	White rice 477 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g	White rice 468 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	White rice 428 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Lentils 45 g
% E as Protein	5	6	6	8	11	12	13
% E as CHO	72	72	72	69	61	60	59
% E as Fat	23	22	22	23	28	28	28
Vitamin A (μg)	0	103 - 147	106 - 151	106 - 151	106 - 151	173 - 247	173 - 247
Vitamin C (mg)	0	2	108	113	108	155	157
Folate	6 - 8	8 - 10	16 - 21	102 - 135	18 - 23	66 - 87	106 - 141
Iron	6	7	7	22	51	75	102
Zinc	24	25	24	32	97	100	107
Calcium	5 - 7	6 - 10	12 - 19	16 - 25	12 - 20	25 - 39	26 - 42

	CORN-TORTILLA BASED DIET						
	Corn-tortilla 368 g Vegetable oil 20 g	Corn-tortilla 363 g Vegetable oil 20 g Carrots 21 g	Corn-tortilla 351 g Vegetable oil 20 g Carrots 21 g Orange 60 g	Corn-tortilla 314 g Vegetable oil 20 g Carrots 21 g Orange 60 g Lentils 71 g	Corn-tortilla 297 g Vegetable oil 20 g Carrots 21 g Orange 60 g Beef 55 g	Corn-tortilla 292 g Vegetable oil 20 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	Corn-tortilla 266 g Vegetable oil 20 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Black beans 45 g
% E as Protein	8	8	8	10	13	14	15
% E as CHO	67	67	67	66	58	57	57
% E as Fat	25	25	25	24	29	29	28
Vitamin A (μg)	0	103 - 147	106 - 151	106 - 151	106 - 151	173 - 247	173 - 247
Vitamin C (mg)	0	2	108	112	108	155	155
Folate	30 - 39	31 - 41	39 - 51	100 - 133	37 - 49	85 - 113	116 - 155
Iron	26	26	26	35	109	133	144
Zinc	34	34	33	39	110	113	117
Calcium	162 - 259	161 - 258	162 - 259	149 - 239	139 - 223	150 - 239	141 - 226

***Table 4**:* Refined couscous and potato based diets composition and percent of nutrient density values for vitamin. A, vitamin C, folate, iron and zinc

	REFINED COUSCOUS BASED DIET						
	Ref. Couscous 697 g Vegetable oil 25 g	Ref. Couscous 690 g Vegetable oil 25 g Carrots 21 g	Ref. Couscous 665g Vegetable oil 25 g Carrots 21 g Orange 60 g	Ref. Couscous 590 g Vegetable oil 25 g Carrots 21 g Orange 60 g Lentils70 g	Ref. Couscous 555 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g	Ref. Couscous 546 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	Ref. Couscous 492g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Black beans 45 g
% E as Protein	11	11	10	12	15	16	17
% E as CHO	66	66	66	64	56	55	55
% E as Fat	23	23	24	24	29	29	28
Vitamin A (μg)	0	103 - 147	106 - 151	106 - 151	106 - 151	173 - 247	173 - 247
Vitamin C (mg)	0	2	108	112	108	155	155
Folate	53 - 70	54 - 71	61 - 81	118 - 157	55 - 73	102 - 136	132 - 175
Iron	13	14	14	24	71	96	109
Zinc	18	19	18	25	88	92	98
Calcium	14 - 22	15 - 25	21 - 33	23 - 36	20 - 32	32 - 51	34 - 54

	POTATO BASED DIET						
	Potato 907 g Vegetable oil 25 g	Potato 895 g Vegetable oil 25 g Carrots 21 g	Potato 865 g Vegetable oil 25 g Carrots 21 g Orange 60 g	Potato 770 g Vegetable oil 25 g Carrots 21 g Orange 60 g Lentils 70 g	Potato 725 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g	Potato 710 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g	Potato 649 g Vegetable oil 25 g Carrots 21 g Orange 60 g Beef 55 g Spinach raw 50 g Lentils 45 g
% E as Protein	6	6	6	8	12	12	13
% E as CHO	71	71	71	69	61	60	59
% E as Fat	23	23	23	23	27	28	28
Vitamin A (μg)	0	103 - 147	106 - 151	106 - 151	106 - 151	173 - 247	173 - 247
Vitamin C (mg)	224	223	322	302	287	330	317
Folate	40 - 53	41 - 55	49 - 65	108 - 144	45 - 59	93 - 123	131 - 174
Iron	14	15	15	25	75	98	122
Zinc	25	25	25	31	97	100	107
Calcium	17 - 27	18 - 29	24 - 38	25 - 40	22 - 35	34 - 55	35 - 56

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Chapter 3

Thiamin, riboflavin, niacin, vitamin B_6, pantothenic acid and biotin

The B-complex vitamins covered here are presented in ***Table 5*** along with the biochemical and physiologic roles of the co-enzyme forms and a brief description of clinical deficiency symptoms.

Table 5

Physiologic roles and deficiency signs of B-complex vitamins

Vitamin	Physiologic roles	Deficiency
Thiamin (B_1)	Co-enzyme functions in metabolism of carbohydrates and branched-chain amino acids	Beri-beri, polyneuritis, and Wernicke-Korsakoff syndrome
Riboflavin (B_2)	Co-enzyme functions in numerous oxidation and reduction reactions	Growth, cheilosis, angular stomatitis, and dermatitis
Niacin (nicotinic acid and nicotinamide)	Co-substrate/co-enzyme for hydrogen transfer with numerous dehydrogenases	Pellagra with diarrhoea, dermatitis, and dementia
Vitamin B_6 (pyridoxine, pyridoxamine, and pyridoxal)	Co-enzyme functions in metabolism of amino acids, glycogen, and sphingoid bases	Naso-lateral seborrhoea, glossitis, and peripheral neuropathy (epileptiform convulsions in infants)
Pantothenic acid	Constituent of co-enzyme A and phosphopantetheine involved in fatty acid metabolism	Fatigue, sleep disturbances, impaired coordination, and nausea
Biotin	Co-enzyme functions in bicarbonate-dependent carboxylations	Fatigue, depression, nausea, dermatitis, and muscular pains

Rice and wheat are the staples for many populations of the world. Excessive refining and polishing of cereals removes considerable proportions of B vitamins contained in these cereals. Clinical manifestations of deficiency of some B vitamins – such as beri-beri (cardiac and dry), peripheral neuropathies, pellagra, and oral and genital lesions (related to riboflavin deficiency) – were once major public health problems in parts of the world. These manifestations have now declined, the decline being brought about not through programmes,

which distribute synthetic vitamins but through changes in the patterns of food availability and consequent changes in dietary practices of the populations.

Although these clinical manifestations of B-vitamin deficiencies have decreased, there is evidence of widespread sub-clinical deficiency of these vitamins (especially of riboflavin and pyridoxine). These sub-clinical deficiencies, although less dramatic in their manifestations, exert deleterious metabolic effects. Despite the progress in reduction of large-scale deficiency in the world, there are periodic reports of outbreaks of B-complex deficiencies, which are linked to deficits of B vitamins in populations under various distress conditions.

Refugee and displaced population groups (20 million people by current United Nations estimates) are at risk for B-complex deficiency because most cereal foods used under emergency situations are not fortified with micronutrients (*1*). Recent reports have implicated the low B-complex content of diets as a factor in the outbreak of peripheral neuropathy and visual loss observed the adult population of Cuba (*2-4*). This deficiency in Cuba resulted from the consequences of an economic blockade (*4*).

Because of the extensive literature pertaining to the study of the B-complex vitamins, the references cited here were selected from those published after the FAO/WHO *handbook on human nutritional requirements* was published in 1974 (*5*). Greater weight has been given to studies which used larger numbers of subjects over longer periods, more thoroughly assessed dietary intake, varied the level of the specific vitamin being investigated, and used multiple indicators, including those considered functional in the assessment of status. These indicators have been the main basis for ascertaining requirements. Although extensive, the bibliographic search of recently published reports presented in this chapter most likely underestimates the extent of B-complex deficiency considering that many cases are not reported in the medical literature. Moreover, outbreaks of vitamin deficiencies in populations are usually not publicised because governments may consider the existence of these conditions to be politically sensitive information. Additional references are listed in the publication by the Food and Nutrition Board of the Institute of Medicine of the US National Academy of Sciences (*6*).

Thiamin

Background with requisite function in human metabolic processes

Deficiency

Thiamin (vitamin B_1, aneurin) deficiency results in the disease called beri-beri, which has been classically considered to exist in dry (paralytic) and wet (oedematous) forms (*7, 8*). Beri-beri occurs in human-milk-fed infants whose nursing mothers are deficient. It also occurs in adults with high carbohydrate intakes mainly from milled rice and with intakes of anti-thiamin factors. Beri-beri is still endemic in Asia. In relatively industrialized nations, the neurologic reflections of Wernicke-Korsakoff syndrome are frequently associated with chronic alcoholism with limited food consumption (*9*). Some cases of thiamin deficiency have been observed with patients who are hypermetabolic, are on parenteral nutrition, are undergoing chronic renal dialysis, or have undergone a gastrectomy. Thiamin deficiency has also been observed in Nigerians who ate silk worms, Russian schoolchildren (in Moscow), Thai rural elderly, Cubans, Japanese elderly, Brazilian Xavante Indians, French Guyanense, Southeast Asian schoolchildren who were infected with hookworm, Malaysian detention inmates, and people with chronic alcoholism.

Toxicity

Toxicity is not a problem with thiamin because renal clearance of levels conceivably ingested is rapid.

Functions

Thiamin functions as the co-enzyme thiamin pyrophosphate (TPP) in the metabolism of carbohydrates and branched-chain amino acids. Specifically the Mg^{2+}-coordinated TPP participates in the formation of α-ketols (e.g., among hexose and pentose phosphates) as catalysed by transketolase and in the oxidation of α-keto acids (e.g., pyruvate, α-ketoglutarate, and branched-chain α-keto acids) by dehydrogenase complexes (*10, 11*). Hence, when there is insufficient thiamin, the overall decrease in carbohydrate metabolism and its inter-connection with amino acid metabolism (via α-keto acids) have severe consequences, such as a decrease in the formation of acetylcholine for neural function.

Biochemical indicators

Indicators used to estimate thiamin requirements are urinary excretion, erythrocyte transketolase activity coefficient, erythrocyte thiamin, blood pyruvate and lactate, and neurologic changes. The excretion rate of the vitamin and its metabolites reflects intake, and the validity of the assessment of thiamin nutriture is improved with load test. Erythrocyte transketolase activity co-efficient reflects TPP levels and can indicate rare genetic defects. Erythrocyte thiamin is mainly a direct measure of TPP but also is a measure of thiamin and thiamin monophosphate by high performance liquid chromatography (HPLC) separation.

Thiamin status has been assessed by measuring urinary thiamin excretion under basal conditions or after thiamin loading, transketolase activity, and free and phosphorylated forms in blood or serum (*6, 9*). Although overlap with baseline values for urinary thiamin was found with oral doses below 1 mg, a correlation of 0.86 between oral and excreted amounts was found by Bayliss *et al.* (*12*). The erythrocyte transketolase assay, in which an activity coefficient based on a TPP stimulation of the basal level is given, continues to be a main functional indicator (*9*), but some problems have been encountered. Gans and Harper (*13*) found a wide range of TPP effect when thiamin intakes were adequate above 1.5 mg/day over a 3-day period. In some cases the activity coefficient may appear normal after prolonged deficiency (*14*). This measure seemed poorly correlated with dietary intakes estimated for a group of English adolescents (*15*). Certainly, there are both inter-individual and genetic factors affecting the transketolase (*16*). Baines and Davies (*17*) suggested that it is useful to determine erythrocyte TPP directly because the co-enzyme is less susceptible to factors that influence enzyme activity; however, there are also methods for determining thiamin and its phosphate esters in whole blood (*18*).

Factors affecting requirements

Because thiamin facilitates energy utilisation, its requirements have traditionally been expressed on the basis of energy intake, which can vary depending on activity levels. However, Fogeholm *et al.* (*19*) found no difference in activation coefficients for erythrocyte transketolase from a small group of skiers and from less physically active control subjects. Also, a study with thiamin-restricted Dutch males whose intake averaged 0.43 mg/day for 11 weeks did not reveal an association between short bouts of intense exercise and the decreases in indicators of thiamin status (*20*). Alcohol consumption may interfere with thiamin absorption (*9*).

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Findings by age and life stage

Recommendations for infants are based on adequate food intake. Mean thiamin content of human milk is 0.21 mg/l (0.62 μmol/l) (*21*), which corresponds to 0.16 mg (0.49μmol) thiamin per 0.75 l of secreted milk per day. The blood concentration for total thiamin averages 210 ± 53 nmol/l for infants up to 6 months but decreases over the first 12–18 months of life (*22*).

A study of 13–14-year-old children related dietary intake of thiamin to several indicators of thiamin status (*15*). Sauberlich *et al.* (*23*) concluded from a carefully controlled depletion-repletion study of seven healthy young men that 0.3 mg thiamin per 4184 kJ met their requirements. Intakes below this amount lead to irritability and other symptoms and signs of deficiency (*24*). Anderson *et al*. (*25*) reported thiamin intakes of 1.0 and 1.2 mg/day as minimal for women and men, respectively. Hoorn et al. (*26*) reported that 23 percent of 153 patients aged 65–93 years were deemed deficient based on a transketolase activation coefficient greater than 1.27, which was normalised after thiamin administration. Nichols and Basu (*27*) found that only 57 percent of 60 adults aged 65–74 years had TPP effects of less than 14 percent and suggested that ageing may increase thiamin requirements.

An average total energy cost of 230 MJ has been estimated for pregnancy (*28*). With an intake of 0.4 mg thiamin/4184 kJ, this amounts to 22 mg total, or 0.12 mg/day for an additional thiamin need for the second and third trimesters (180 days). Taking into account an increased growth in maternal and foetal compartments, an overall additional requirement of 0.3 mg/day is adequate (6).

Lactating women are estimated to transfer 0.2 mg thiamin in their milk each day, and an additional 0.2 mg is estimated as a need for the increased energy cost of lactation of about 2092 kJ/day.

Recommendations

The recommendations for thiamin are given in ***Table 6***.

Table 6

Recommended nutrient intakes for thiamin

Group	Recommended nutrient intake mg/day
Infants and children	
0–6 months	0.2
7–12 months	0.3
1–3 years	0.5
4–6 years	0.6
7–9 years	0.9
Adolescents, 10–18 years	
Females	1.1
Males	1.2
Adults	
Females, 19+ years	1.1
Males, 19+ years	1.2
Pregnancy	1.4
Lactation	1.5

Riboflavin

Background with requisite function in human metabolic processes

Deficiency

Riboflavin (vitamin B_2) deficiency results in the condition of hypo- or ariboflavinosis, with sore throat; hyperaemia; oedema of the pharyngeal and oral mucous membranes; cheilosis; angular stomatitis; glossitis; seborrheic dermatitis; and normochromic, normocytic bone marrow (*8, 29*). Because the deficiency almost invariably occurs combined with a deficiency of other B-complex vitamins, some of the symptoms (e.g., glossitis and dermatitis) may result from other complicating deficiencies. The major cause of hypo-riboflavinosis is inadequate dietary intake as a result of limited food supply, which is sometimes exacerbated by poor food storage or processing. Children in developing countries will commonly demonstrate clinical signs of riboflavin deficiency during periods of the year when gastrointestinal infections are prevalent. Decreased assimilation of riboflavin also results from abnormal digestion such as that which occurs with lactose intolerance. This condition is highest in African and Asian populations and can lead to a decreased intake of milk as well as an abnormal absorption of the vitamin. Absorption of riboflavin is also affected in some other conditions, for example, tropical sprue, celiac disease, malignancy and resection of the small bowel, and decreased gastrointestinal passage time. In relatively rare cases the causes of deficiency are inborn errors in which the genetic defect is in the formation of a flavoprotein (e.g., acyl-co-enzyme A [co-A] dehydrogenases). Also at risk are those receiving phototherapy for neonatal jaundice and perhaps those with inadequate thyroid hormone. Some cases of riboflavin deficiency were also observed in Russian schoolchildren (Moscow) and Southeast Asian schoolchildren (infected with hookworm).

Toxicity

Riboflavin toxicity is not a problem because of limited intestinal absorption.

Functions

Conversion of riboflavin to flavin mononucleotide (FMN) and further to the predominant flavin adenine dinucleotide (FAD) occurs before these flavins form complexes with numerous flavoprotein dehydrogenases and oxidases. These flavoco-enzymes (FMN and FASD) participate in oxidation-reduction reactions in metabolic pathways and in energy production via the respiratory chain (*10, 11*).

Biochemical indicators

Indicators used to estimate riboflavin requirements are urinary flavin excretion, erythrocyte glutathione reductase activity coefficient, and erythrocyte flavin. The urinary flavin excretion rate of vitamin and metabolites reflects intake; validity of assessment of riboflavin adequacy is improved with load test. Erythrocyte glutathione reductase activity coefficient reflects FAD levels; results are confounded by such genetic defects as glucose-6-phosphate dehydrogenase deficiency and heterozygous β thalassemia.

Erythrocyte flavin is mainly a measure of FMN and riboflavin after hydrolysis of labile FAD and HPLC separation.

Riboflavin status has been assessed by measuring urinary excretion of the vitamin in fasting, random, and 24-hour specimens or by load returns tests (amounts measured after a specific amount of riboflavin is given orally); erythrocyte glutathione reductase; or erythrocyte flavin concentration (*6, 9, 29*). The HPLC method with fluorometry gives lower values for urinary riboflavin than do fluorometric methods, which measure the additive fluorescence of similar flavin metabolites (*30*). The metabolites can comprise as much as one-third of total

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urinary flavin (*31, 32*) and in some cases may depress assays dependent on a biologic response because certain catabolites can inhibit cellular uptake (*33*). Under conditions of adequate riboflavin uptake (≈1.5 mg/day) by adults, an estimated 120 μg (320 nmol) total riboflavin or 80 μg/g of creatinine is excreted daily (*32*). The erythrocyte glutathione reductase assay, with an activity coefficient (AC) expressing the ratio of activities in the presence and absence of added FAD, continues to be used as a main functional indicator, but some limits have been noted. The reductase in erythrocytes from individuals with glucose-6-phosphate dehydrogenase deficiency (often present in blacks) has an increased avidity for FAD, which makes this test invalid (*34*). Sadowski (*35*) has set an upper limit of normality for the AC at 1.34 based on the mean value plus 2 standard deviations from several hundreds of apparently healthy individuals aged 60 years and over. Suggested guidelines for the interpretation of such enzyme ACs are as follows: less than 1.2, acceptable; 1.2–1.4, low; greater than 1.4, deficient (*9*). In general agreement with earlier findings on erythrocyte flavin, Ramsay *et al*. (*36*) found a correlation between cord blood and maternal erythrocyte deficiencies and suggested that values greater than 40 nmol/l are considered adequate.

Factors affecting requirements

Several studies reported modest effects of physical activity on the erythrocyte glutathione reductase AC (*37-41*). A slight increase in the AC and decrease in urinary flavin of weight-reducing women (*39*) and older women undergoing exercise training (*41*) were "normalised" with 20 percent additional riboflavin. However, riboflavin supplementation did not lead to an increase in work performance when such subjects were not clinically deficient (*42-45*).

Bio-availability of riboflavin in foods, mostly as digestible flavoco-enzymes, is excellent at nearly 95 percent (*6*), but absorption of the free vitamin is limited to about 27 mg per single meal or dose in an adult (*46*). No more than about 7 percent of food flavin is found as 8α-FAD covalently attached to certain flavoprotein enzymes. Although some portions of the 8α-(amino acid)-riboflavins are released by proteolysis of these flavoproteins, they do not have vitamin activity (*47*). A lower fat-to-carbohydrate ratio may decrease the riboflavin requirements of the elderly (*48*). Riboflavin interrelates with other B vitamins, notably niacin, which requires FAD for its formation from tryptophan, and vitamin B_6, which requires FMN for conversion of the phosphates of pyridoxine and pyridoxamine to the co-enzyme pyridoxal 5'-phosphate (PLP) (*49*). Contrary to earlier reports, no difference was seen in riboflavin status of women taking oral contraceptives when dietary intake was controlled by providing a single basic daily menu and meal pattern after 0.6 mg riboflavin/418 kJ was given in a 2-week acclimation period (*50*).

Findings by age and life stage

As reviewed by Thomas et al. (*51*), early estimates of riboflavin content in human milk showed changes during the post-partum period. More recent investigations of flavin composition of both human (*52*) and cow (*53*) milk have helped clarify the nature of the flavins present and provide better estimates of riboflavin equivalence. For human milk consumed by infants up to age 6 months, the riboflavin equivalence averages 0.35mg (931 nmol) /l (*6*) or 0.26 mg (691nmol) /0.75 l of milk per day. For low-income Indian women with erythrocyte glutathione reductase activity ratios averaging 1.80 and a milk riboflavin content of 0.22 mg/l, breast-fed infants averaged AC ratios near 1.36 (*54*). Hence, a deficiency sufficient to reduce human-milk riboflavin content by one-third can lead to a mild sub-clinical deficiency in infants.

Studies of riboflavin status in adults include those by Belko *et al.* (*38, 39*) in modestly obese young women on low-energy diets, by Bates *et al.* (*55*) on deficient Gambians, and by

Kuizon *et al.* (*56*) on Filipino women. Most of a 1.7-mg dose of riboflavin given to healthy adults consuming at least this amount was largely excreted in the urine (*32*). Such findings corroborate earlier work indicating a relative saturation of tissue with intakes above 1.1 mg/day. Studies by Alexander *et al.* (*57*) on riboflavin status in the elderly show that doubling the estimated riboflavin intakes of 1.7 mg/day for women aged 70 years and over, with a reductase AC of 1.8, led to a doubling of urinary riboflavin from 1.6–3.4 µg (4.2 to 9.0 nmol) /mg creatinine and a decrease in AC to 1.25. Boisvert *et al.* (*48*) obtained normalisation of the glutathione reductase AC in elderly Guatemalans with approximately 1.3 mg/day of riboflavin, with a sharp increase in urinary riboflavin occurring at intakes above 1.0–1.1 mg/day.

Pregnant women have an increased erythrocyte glutathione reductase AC (*58, 59*). Kuizon *et al.* (*56*) found that riboflavin at 0.7 mg /4184 kJ was needed to lower the AC of four of eight pregnant women to 1.3 within 20 days, whereas only 0.41 mg/4184 kJ was needed for five of the seven non-pregnant women. Maternal riboflavin intake was positively associated with foetal growth in a study of 372 pregnant women (*60*). The additional riboflavin requirement of 0.3 mg/day for pregnancy is an estimate based on increased growth in maternal and foetal compartments. For lactating women, an estimated 0.3 mg riboflavin is transferred in milk daily and, because utilisation for milk production is assumed to be 70 percent efficient, the value is adjusted upward to 0.4 mg/day.

Recommendations

The recommendations for riboflavin are given in ***Table 7***.

Table 7

Recommended nutrient intakes for riboflavin

Group	Recommended nutrient intake mg/day
Infants and children	
0–6 months	0.3
7–12 months	0.4
1–3 years	0.5
4–6 years	0.6
7–9 years	0.9
Adolescents, 10–18 years	
Females	1.0
Males	1.3
Adults	
Females, 19+ years	1.1
Males, 19+ years	1.3
Pregnancy	1.4
Lactation	1.6

Niacin

Background with requisite function in human metabolic processes

Deficiency

Niacin (nicotinic acid) deficiency classically results in pellagra, which is a chronic wasting disease associated with a characteristic erythematous dermatitis that is bilateral and symmetrical, a dementia after mental changes including insomnia and apathy preceding an overt encephalopathy, and diarrhoea resulting from inflammation of the intestinal mucous

surfaces (*8, 9, 61*). At present, pellagra occurs endemically in poorer areas of India, China, and Africa. Its cause has been mainly attributed to a deficiency of niacin; however, its biochemical inter-relationship to riboflavin and vitamin B_6, which are needed for the conversion of L-tryptophan to niacin equivalents (NEs), suggests that insufficiencies of these vitamins may also contribute to pellagra (*62*). Pellagra-like syndromes occurring in the absence of a dietary niacin deficiency are also attributable to disturbances in tryptophan metabolism (e.g., Hartnup disease with impaired absorption of the amino acid and carcinoid syndrome where the major catabolic pathway routes to 5-hydroxytryptophan) (*61*). Pellagra also occurs in people with chronic alcoholism (*61*). Cases of niacin deficiency have been found in people suffering from Crohn's disease (*61*).

Toxicity

Although therapeutically useful in lowering serum cholesterol, administration of chronic high oral doses of nicotinic acid can lead to hepatotoxicity as well as dermatologic manifestations. An upper limit (UL) of 35 mg/day as proposed by the US Food and Nutrition Board (*6*) was adopted by this consultation.

Functions

Niacin is chemically synonymous with nicotinic acid although the term is also used for its amide (nicotinamide). Nicotinamide is the other form of the vitamin, which does not have the pharmacologic action of the acid that is administered at high doses to lower blood lipids. It is the amide form that exists within the redox-active co-enzymes, nicotinamide adenine dinucleotide (NAD) and its phosphate (NADP), which function in dehydrogenase-reductase systems requiring transfer of a hydride ion (*10*, *11*). NAD is also required for non-redox adenosine diphosphate–ribose transfer reactions involved in DNA repair (*63*) and calcium mobilisation. NAD functions in intracellular respiration and with enzymes involved in the oxidation of fuel substrates such as glyceraldehyde 3-phosphate, lactate, alcohol, 3-hydroxybutyrate, and pyruvate. NADP functions in reductive biosyntheses such as fatty acid and steroid syntheses and in the oxidation of glucose-6-phosphate to ribose-5-phosphate in the pentose phosphate pathway.

Biochemical indicators

Indicators used to estimate niacin requirements are urinary excretion, plasma concentrations of metabolites, and erythrocyte pyridine nucleotides. The excretion rate of metabolites, mainly N'-methyl-nicotinamide and its 2- and 4-pyridones, reflects intake and is usually expressed as a ratio of the pyridones to N'-methyl-nicotinamide. Concentrations of metabolites, especially 2-pyridone, are measured in plasma after a load test. Erythrocyte pyridine nucleotides measure NAD concentration changes.

Niacin status has been monitored by daily urinary excretion of methylated metabolites, especially the ratio of the 2-pyridone to N'-methyl-nicotinamide; erythrocyte pyridine nucleotides; oral dose uptake tests; erythrocyte NAD; and plasma 2-pyridone (*6, 9*). Shibata and Matsuo (*64*) found that the ratio of urinary 2-pyridone to N'-methyl-nicotinamide was as much a measure of protein adequacy as it was a measure of niacin status. Jacob *et al.* (*65*) found this ratio too insensitive to marginal niacin intake. The ratio of the 2-pyridone to N'-methyl-nicotinamide also appears to be associated with the clinical symptoms of pellagra, principally the dermatitic condition (*66*). In plasma, 2-pyridone levels change in reasonable proportion to niacin intake (*65*). Similarly to the situation for erythrocyte pyridine nucleotide (nicotinamide co-enzymes), NAD concentration decreased 70 percent whereas NADP remained unchanged in adult males fed diets with only 6 or 10 mg NEs/day (*67*). Erythrocyte

NAD provided a marker at least as sensitive as urinary metabolites of niacin in this study (*67*) and in a niacin depletion study of elderly subjects (*68*).

Factors affecting requirements

The biosynthesis of niacin derivatives on the pathway to nicotinamide co-enzymes stems from tryptophan, an essential amino acid found in protein, and as such this source of NEs increases niacin intake. There are several dietary, drug, and disease factors that reduce the conversion of tryptophan to niacin (*61*) (e.g. the use of oral contraceptives [*69*]). Although a 60-to-1 conversion factor represents the average for human utilisation of tryptophan as NEs, there are substantial individual differences (*70, 71*). There is also an interdependence of enzymes within the tryptophan-to-niacin pathway where vitamin B_6 (as pyridoxal phosphate) and riboflavin (as FAD) are functional. Further, riboflavin (as FMN) is required for the oxidase that forms co-enzymic PLP from the alcohol and amine forms of phosphorylated vitamin B_6 (*49*).

Findings by age and life stage

Niacin content of human milk is approximately 1.5 mg (12.3 μmol) /l and the tryptophan content is 210 mg (1.0mmol) /l (*21*). Hence, the total content is approximately 5 mg NEs/l or 4 mg NEs/ 0.75 l secreted daily in human milk. Recent studies (*64, 70*) together with those reported in the 1950s suggest that 12.5 mg NEs, which corresponds to 5.6 mg NEs/4184 kJ, is minimally sufficient for niacin intake in adults.

For pregnant women, where 230 MJ is the estimated energy cost of pregnancy, calculated needs above those of non-pregnant women are 5.6 mg NEs/ 4186 kjoule (1,000 kcal) × 230,000 kjoule (55,000 kcal), or 308 mg NEs for the entire pregnancy or 1.7 mg NEs/day (308 mg NEs/180 days) for the second and third trimester, which is about a 10 percent increase. Also about 2 mg NEs/day is required for growth in maternal and foetal compartments (*6*).

For lactating women, an estimated 1.4 mg preformed niacin is secreted daily, and an additional requirement of less than 1 mg is needed to support the energy expenditure of lactation. Hence, 2.4 mg NEs/day is the added need attributable to lactation.

Recommendations

The recommendations for niacin are given in ***Table 8***.

Table 8

Recommended nutrient intakes for niacin

Group	Recommended nutrient intake, NEs/day[a]
Infants and children	
0–6 months	2[b]
7–12 months	4
1–3 years	6
4–6 years	8
7–9 years	12
Adolescents, 10–18 years	16
Adults	
Females, 19+ years	14
Males, 19+ years	16
Pregnancy	18
Lactation	17

[a] NEs, niacin equivalents. [b] Preformed.

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Vitamin B_6

Background with requisite function in human metabolic processes

Deficiency

A deficiency of vitamin B_6 alone is uncommon because it usually occurs in association with a deficit in other B-complex vitamins (*72*). Early biochemical changes include decreased levels of plasma PLP and urinary 4-pyridoxic acid. These are followed by decreases in synthesis of transaminases (aminotransferases) and other enzymes of amino acid metabolism such that there is an increased urinary xanthurenate and a decreased glutamate conversion to the anti-neurotransmitter γ-aminobutyrate. Hypovitaminosis B_6 may often occur with riboflavin deficiency, because riboflavin is needed for the formation of the co-enzyme PLP. Infants are especially susceptible to insufficient intakes, which can lead to epileptiform convulsions. Skin changes include dermatitis with cheilosis and glossitis. There is usually a decrease in circulating lymphocytes and possibly a normocytic, microcytic, or sideroblastic anaemia (*9*). The sensitivity of such systems as sulphur amino acid metabolism to vitamin B_6 availability is reflected in homo-cysteinemia. A decrease in the metabolism of glutamate in the brain, which is found in vitamin B_6 insufficiency, reflects a nervous system dysfunction. As is the case with other micronutrient deficiencies, vitamin B_6 deficiency results in an impairment of the immune system. A current concern is for the rather pandemic occurrence of somewhat low vitamin B_6 intakes in many people who eat poorly (e.g., people with eating disorders). Vitamin B_6 deficiency has also been observed in Russian schoolchildren (Moscow), Southeast Asian schoolchildren (infected with hookworm), elderly Europeans (Dutch), and in some individuals with hyperhomo-cysteinemia or on chronic hemodialysis. Several medical conditions can also affect vitamin B_6 metabolism and lead to deficiency symptoms.

Toxicity

Use of high doses of pyridoxine for dubious treatment of pre-menstrual syndrome, carpal tunnel syndrome, and some neurologic diseases has resulted in neurotoxicity. A UL of 100 mg/day as proposed by the US Food and Nutrition Board (*6*) was adopted by this consultation.

Functions

There are three natural vitamers (different forms of the vitamin) of vitamin B_6, namely pyridoxine, pyridoxamine, and pyridoxal. These must be phosphorylated and the 5'-phosphates of the first two oxidized to the functional PLP, which serves as a carbonyl-reactive co-enzyme to diverse enzymes involved in the metabolism of amino acids. Such enzymes include aminotransferases, decarboxylases, and dehydratases, δ-aminolevulinate synthase in heme biosynthesis, phosphorylase in glycogen breakdown, and sphingoid base biosynthesis, etc. (*10, 11*).

Biochemical indicators

Indicators used to estimate vitamin B_6 requirements are PLP, urinary excretion, erythrocyte aminotransferases activity coefficients, tryptophan catabolites, erythrocyte and whole blood PLP, and plasma homo-cysteine. PLP is the major vitamin B_6 form in tissue and reflects liver PLP; it changes fairly slowly in response to vitamin intake. The excretion rate of vitamin and particularly 4-pyridoxate reflects intake. Erythrocyte aminotransferases for aspartate and alanine reflect PLP levels and show large variations in activity coefficients. The urinary excretion of xanthurenate, a tryptophan catabolite, is used especially after a tryptophan load test.

Vitamin B_6 status is most appropriately evaluated by using a combination of indicators, including those considered direct (e.g., vitamer concentration in cells or fluids) and indirect or functional indicators (e.g., erythrocyte aminotransferase saturation by PLP or tryptophan metabolites) (*9*). Plasma PLP may be the best single indicator because it appears to reflect tissue stores (*73*). Kretsch *et al*. (*74*) found that diets containing less than 0.05 mg vitamin B_6 given to 11 young women led to abnormal electroencephalograph patterns in 2 of the women and a plasma PLP of approximately 9 nmol. Hence, a level of about 10 nmol is considered sub-optimal. A cut-off for plasma PLP of 20 nmol has been proposed as an index of adequacy (*6*) based on recent findings (*73, 75*). Plasma PLP levels have been reported to fall with age (*76*). Urinary 4-pyridoxic acid level changes promptly with changes in vitamin B_6 intake (*73*) and is therefore of questionable value in assessing status. However, a value higher than 3 µmol/day , achieved with an intake of ~1 mg/d, has been suggested to reflect adequate intake (*77*). Erythrocyte aminotransferases for aspartate and alanine are commonly measured before and after addition of PLP to ascertain amounts of apoenzymes, the proportion of which increases with vitamin B_6 depletion. Values of 1.5–1.6 for the aspartate aminotransferase and approximately 1.2 for the alanine aminotransferase have been suggested as being adequate (*9*, *77*). Catabolites from tryptophan and methionine have also been used to assess vitamin B_6 status. In a review of such literature, Leklem (*77*) suggested that a 24-hour urinary excretion less than 65 µmol xanthurenate after a 2-g oral dose of tryptophan indicates normal vitamin B_6 status.

Factors affecting requirements

A recent review by Gregory (*78*) shows that bio-availability of vitamin B_6 in a mixed diet is about 75 percent (*79*), with approximately 8 percent of this total contributed by pyridoxine β-D-glucoside, which is about half as effectively utilised (*78*) as free B vitamers or their phosphates. The amine and aldehyde forms of vitamin B_6 may be about 10 percent less effective than pyridoxine (*80*). Despite the involvement of PLP with many enzymes affecting amino acid metabolism, there seems to be only a slight effect of dietary proteins on vitamin B_6 status (*81*). Studies reported decreases in indicators of vitamin B_6 status in women receiving oral contraceptives (*82, 83*), but this probably reflects hormonal stimulation of tryptophan catabolism rather than any deficiency of vitamin B_6 per se. Subjects with pre-eclampsia or eclampsia have lowered plasma PLP levels than do healthy pregnant women (*84, 85*).

Findings by age and life stage

The average intake for infants, based on human-milk content, is 0.13 mg/l (*86*) or 0.1 mg/0.75 l/day. With an average maternal dietary intake of vitamin B_6 of 1.4 mg/day, human milk was found to contain 0.12 mg/l, and plasma PLP of nursing infants averaged 54 nmol (*87*). Extrapolation on the basis of metabolic body size, weight, and growth suggests 0.3 mg/day as an adequate intake for infants 6–12 months of age (*6*). Information on vitamin B_6 requirements for children is limited, but Heiskanen *et al*. (*88*) found an age-related decrease in erythrocyte PLP and an increase in the aspartate aminotransferase activation.

In a review of earlier studies of men with various protein intakes, Linkswiler (*89*) concluded that normalisation of a tryptophan load test required 1–1.5 mg vitamin B_6. Miller *et al.* (*90*) found that 1.6 mg vitamin B_6 led to plasma PLP levels above 30 nmol/l for young men with various protein intakes. From several investigations of young women (*91-94*), a requirement closer to 1–1.2 mg vitamin B_6 could be estimated.

Limited studies of the elderly indicate that requirements may be somewhat higher, at least to maintain plasma PLP above a 20-nmolar cut-off level (*95, 96*).

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For pregnancy it was confirmed that indicators of vitamin B_6 status decrease, especially in the third trimester (*85, 97, 98*). It is not clear, however, whether this is a normal physiologic phenomenon. For a maternal body store of 169 mg and foetal plus placental accumulation of 25 mg vitamin B_6, about 0.1 mg/day is needed on average over gestation (*6*). With additional allowances for the increased metabolic need and weight of the mother and about 75 percent of bio-availability, an additional average requirement in pregnancy of 0.25 mg can be assumed. Because most of this need is in the latter stages of pregnancy and vitamin B_6 is not stored to any significant extent, an extra 0.5 mg/day of vitamin B_6 may be justified to err on the side of safety.

For lactation, it may be prudent to add 0.6 mg vitamin B_6 to the base requirement for women because low maternal intakes could lead to a compromised vitamin B_6 status in the infant (*99*).

Recommendations

The recommendations for vitamin B_6 are given in ***Table 9***.

Table 9

Recommended nutrient intakes for vitamin B_6

Group	Recommended nutrient intake mg/day
Infants and children	
0–6 months	0.1
7–12 months	0.3
1–3 years	0.5
4–6 years	0.6
7–9 years	1.0
Adolescents, 10–18 years	
Females	1.2
Males	1.3
Adults	
Females, 19–50 years	1.3
Males, 19–50 years	1.3
Females, >50 years	1.5
Males, >50 years	1.7
Pregnancy	1.9
Lactation	2.0

Pantothenate

Background with requisite function in human metabolic processes

Deficiency

The widespread occurrence of releasable pantothenic acid in food makes a dietary deficiency unlikely (*8, 9, 100, 101*). If a deficiency occurs, it is usually accompanied by deficits of other nutrients. The use of experimental animals, an antagonistic analogue (ω-methyl-pantothenate) given to humans, and more recently the feeding of semi-synthetic diets virtually free of pantothenate (*102*) have all helped to define signs and symptoms of deficiency. Subjects become irascible; develop postural hypotension, rapid heart rate on exertion, epigastric distress with anorexia and constipation, numbness and tingling of the hands and feet ("burning feet" syndrome); and have hyperactive deep tendon reflexes and weakness of finger extensor

muscles. Some cases of pantothenate deficiency have been observed in patients with acne and other dermatitic conditions.

Toxicity

Toxicity is not a problem with pantothenate.

Functions

Pantothenic acid is a component of CoA, a cofactor that carries acyl groups for many enzymatic processes, and of phosphopantetheine within acyl carrier protein, a component of the fatty acid synthase complex (*10, 11*). Pantothenate is most especially involved in fatty acid metabolism but has a wide-ranging function as a prosthetic group that adds specificity to binding with appropriate enzymes.

Biochemical indicators

Indicators used to estimate pantothenate requirements are urinary excretion and blood levels. Excretion rate reflects intake. Whole blood, which contains vitamin and pantothenate-containing metabolites, has a general correlation with intake; erythrocyte levels seem more meaningful than plasma or serum levels.

Relative correspondence to pantothenate status has been reported for urinary excretion and for blood content of both whole blood and erythrocytes (*6, 9*). Fry *et al.* (*102*) reported a decline in urinary pantothenate levels from approximately 3–0.8 mg/day (13.7–3.6 μmol/day) in young men fed a deficient diet for 84 days. Urinary excretion for a typical American diet was found to be 2.6 mg (12μmol)/d, but it was strongly dependent on diet (*79*). Pantothenate intake estimated for adolescents was significantly correlated with pantothenate in urine (*103*). Whole-blood pantothenate fell from 1.95–1.41 μg/ml (8.8–6.4 μmol/l)when six adult males were fed a pantothenate-free diet (*102*). Whole-blood content corresponded to intake (*103*), and the range in whole blood was reported to be 1.57–2.66 μg/ml (7.2– 12.1μmol/l (*104*). There is an excellent correlation of whole-blood concentrations of pantothenate with the erythrocyte concentration, with an average value being 334 ng/ml (1.5 μmol/l) (*103*). The lack of sufficient population data, however, suggests the current use of an adequate intake rather than a recommended intake as a suitable basis for recommendations.

Factors affecting requirements

A measurement of urinary excretion of pantothenate after feeding a formula diet containing both bound and free vitamin indicates that approximately 50 percent of the pantothenate present in natural foods may be bio-available (*79*).

Findings by age and life stage

Infant requirements are based on an estimation of pantothenic acid content of human milk, which according to reported values is approximately 2 mg/l (*20, 105*). For a reported average human-milk intake of 0.75 l/day (*106-108*) these values suggest that 1.6 mg/day is an adequate intake by the younger (0–6 months) infants. Taking into consideration growth and body size, 1.9 mg/day may be extrapolated for the older (6–12 months) infant (105).

The studies of Eissenstat *et al.* (*103*) of adolescents suggest that intakes of less than 4 mg/day were sufficient to maintain blood and urinary pantothenate. Kathman and Kies (*109*) found a range of pantothenate intake of 4 to approximately 8 mg/day in 12 adolescents who were 11–16 years old. The usual pantothenate intake for American adults has been reported to be 4–7 mg/day (*102, 109-111*). Hence, around 5 mg/day is apparently adequate.

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For pregnancy there is only one relatively recent study that found lower blood pantothenate levels but no difference in urinary excretion in pregnant women compared with non-pregnant controls (*112*).

For lactation blood pantothenate concentrations were found significantly lower at 3 months post-partum (*112*). With a loss of 1.7 mg/day (7.8 μmol/d) from a lactating woman and lower maternal blood concentrations found with intakes of about 5–6 mg/d, a recommended intake may be 7 mg/d.

Recommendations

The recommendations for pantothenate are given in ***Table 10***.

Table 10

Recommended nutrient intakes for pantothenate

Group	Recommended nutrient intake, mg/day
Infants and children	
0–6 months	1.7
7–12 months	1.8
1–3 years	2.0
4–6 years	3.0
7–9 years	4.0
Adolescents, 10–18 years	5.0
Adults	
Females, 19+	5.0
Males, 19+	5.0
Pregnancy	6.0
Lactation	7.0

Biotin

Background with requisite function in human metabolic processes

Deficiency

Biotin deficiency in humans has been clearly documented with prolonged consumption of raw egg whites, which contain biotin-binding avidin. Biotin deficiency was also observed in cases of parenteral nutrition with solutions lacking biotin given to patients with short-gut syndrome and other causes of malabsorption (*9, 113, 114*). Some cases of biotin deficiency were noted in infants with intractable diaper dermatitis and in those fed special formulas. Dietary deficiency in otherwise normal people is probably rare. Some patients have multiple carboxylase deficiencies and there are occasional biotinidase deficiencies. Clinical signs of deficiency include dermatitis of an erythematous and seborrheic type; conjunctivitis; alopecia; and central nervous system abnormalities such as hypotonia, lethargy, and developmental delay in infants and depression, hallucinations, and paresthesia of the extremities in adults.

Toxicity

Toxicity is not a problem because of limited intestinal absorption of biotin.

Functions

Biotin functions as a co-enzyme within several carboxylases after the carboxyl function of the vitamin becomes amide linked to the ε-amino of specific lysyl residues of the apoenzymes (*10, 11*). In humans and other mammals, biotin operates within four carboxylases. Three of

the four biotin-dependent carboxylases are mitochondrial (pyruvate carboxylase, methylcrotonyl-CoA carboxylase, and propionyl-CoA carboxylase) whereas the fourth (acetyl-CoA carboxylase) is found both in mitochondria and the cytosol. In all these cases biotin serves as carrier for the transfer of active bicarbonate into a substrate to generate a carboxyl product.

Biochemical indicators

Indicators used to estimate biotin requirements are urinary excretion and 3-hydroxyisovalerate excretion. The excretion rate of vitamin and metabolites in urine is assessed by avidin-based radioimmunoassay with HPLC. 3-Hydroxyisovalerate excretion inversely reflects the activity of β-methyl-crotonyl-CoA carboxylase, which is involved in leucine metabolism.

The present indicators for biotin status are its urinary excretion, as assessed with an avidin-based radioimmunoassay with HPLC, and 3-hydroxyisovalerate excretion (*115*). The isolation and chemical identification of more than a dozen metabolites of biotin established the main features of its use in microbes and mammals (*116, 117*). Quantification of the major biotin metabolites was done by Zempleni *et al*. (*118*). Both biotin and bisnorbiotin excretions decline in parallel in individuals on a diet containing raw egg whites (*115*). In these individuals the levels of urinary 3-hydroxyisovalerate, which increase as a result of decreased activity of β-methylcrotonyl-CoA carboxylase and altered leucine metabolism, rose from a normal mean of 112 to 272 μmol/24 hours. Decreased excretion of biotin, abnormally increased excretion of 3-hydroxyisovalerate, or both have been reported for overt cases of biotin deficiency (*119-124*). The lack of sufficient population data, however, suggests the current use of an adequate intake rather than a recommended intake as a suitable basis for recommendations.

Findings by age and life stage

The biotin content of human milk is estimated to be approximately 6 μg (24nmol)/l based on several studies (*125-127*) that report values ranging from near 4–7 μg (16.4–28.9 nmol) /l. Hence, the estimated intake of biotin for an infant consuming 0.75 l is 5 μg/day during the first half year and for older infants is perhaps 6 μg/day.

Requirements for children and adults have been extrapolated as follows (*6*):

Adequate intake for child or adult = (adequate intake young infant) (weight adult or child/weight infant) $^{0.75}$

For pregnancy there are at present insufficient data to justify an increase in the adequate intake, although Mock *et al.* (*128*) reported decreased urinary biotin and 3-hydroxyisovalerate in a large fraction of seemingly healthy pregnant women.

For lactation the intake may need to be increased by an additional 5 μg/day to cover the losses due to human-milk secretion.

Recommendations

The recommendations for biotin are given in ***Table 11***.

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Table 11

Recommended nutrient intakes for biotin

Group	Recommended nutrient intake μg/day
Infants and children	
0–6 months	5
7–12 months	6
1–3 years	8
4–6 years	12
7–9 years	20
Adolescents, 10–18 years	25
Adults	
Females, 19+	30
Males, 19+	30
Pregnancy	30
Lactation	35

General considerations for B-complex vitamins

Notes on suggested recommendations

The recommendations for infants are based largely on the composition and quantity of human milk consumed and are formally considered to be adequate intakes. Younger (0–6 months) infants are considered to derive adequate intake from milk alone; recommendations for older (7–12 months) infants are adjusted by metabolic scaling such that a factor – (weight of 7-12 mo/weight of 0-5 mo) $^{0.75}$ – is multiplied by the recommendation for the younger infant (*6*). Recommendations have been given to use the higher (6–11 months) level for the first year of life.

For most of the B vitamins, there is little or no direct information that can be used to estimate the amounts required by children and adolescents. Hence, an extrapolation from the adult level has been used where a factor – (weight child/weight adult) $^{0.75}$ × (1 + growth factor) – is multiplied by the adult recommendation (*6*).

For most of the B-complex vitamins covered here, data are not sufficient to justify altering recommendations for the elderly.

For pregnancy and lactation, increased maternal needs related to increases in energy and replacement of secretion losses were considered.

Dietary sources of B-complex vitamins

A listing of some better and usual food sources for the vitamins considered is given in ***Table 12.***

Table 12

Dietary sources of water-soluble vitamins

Vitamin	Good-to-moderate dietary sources[a]
Thiamin (B_1)	Pork, organ meats, whole grains, and legumes
Riboflavin (B_2)	Milk and dairy products, meats, and green vegetables
Niacin (nicotinic acid and nicotinamide)	Liver, lean meats, grains, and legumes; can be formed from tryptophan
Vitamin B_6 (pyridoxine, pyridoxamine, and pyridoxal)	Meats, vegetables, and whole-grain cereals
Pantothenic acid	Animal tissues, whole-grain cereals, and legumes; widely distributed
Biotin	Liver, yeast, egg, yolk, soy flour, and cereals

[a]Foods are listed according to the concentrations of vitamin which they contain.

Research suggestions

In view of the issues raised in this section on B-complex vitamins, the following suggestions are noted:

- Actual requirements are least certain for children, adolescents, pregnant and lactating women, and the elderly, and as such they deserve further study.
- Studies need to include graded levels of the vitamin above and below current recommendations and should consider or establish clearly defined cut-off values for clinical adequacy and inadequacy and be conducted for periods of time sufficient for ascertaining equilibrium dynamics.
- For status indicators, additional functional tests would be useful for riboflavin (e.g., the activity of FMN-dependent pyridoxine [pyridoxamine] 5'-phosphate oxidase in erythrocytes), niacin (e.g., sensitive blood measures, especially of NAD), and perhaps pantothenate.
- The food content and bio-availability of pantothenate and biotin need further investigation to establish the available and preferred food sources reasonable for different populations.

Primary efforts should now be in the arena of public health and nutrition education with emphasis on directing people and their governments to available and healthful foods; care necessary for their storage and preparation; and achievable means for adjusting intake with age, sex, and health status.

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Chapter 4
Folate and folic acid

Role of folate and folic acid in human metabolic processes

Folates accept one-carbon units from donor molecules and passes them on via various biosynthetic reactions (*1*). In their reduced form cellular folates function conjugated to a polyglutamate chain. These folates are a mixture of unsubstituted polyglutamyl tetrahydrofolates and various substituted one-carbon forms of tetrahydrofolate (e.g., 10-formyl, 5,10-methylene, and 5-methyl) (***Figure 6***). The reduced forms of the vitamin, particularly the unsubstituted dihydro and tetrahydro forms, are unstable chemically. They are easily split between the C-9 and N-10 bond to yield a substituted pteridine and *p*-aminobenzoylglutamate, which have no biologic activity (*2*). Substituting a carbon group at N-5 or N-10 decreases the tendency of the molecule to split; however, the substituted forms are also susceptible to oxidative chemical rearrangements and, consequently, loss of activity (*2*). The folates found in food consist of a mixture of reduced folate polyglutamates.

Although natural folates rapidly lose activity in foods over periods of days or weeks, folic acid (e.g., in fortified foods) is almost completely stable for months or even years. The chemical lability of all naturally occurring folates results in a significant loss of biochemical activity during harvesting, storage, processing, and preparation. Half or even three-quarters of initial folate activity may be lost during these processes. This is in contrast to the stability of the synthetic form of this vitamin, folic acid (*2*). In this form the pteridine (2-amino-4-hydroxypteridine) ring is not reduced (***Figure 6***), rendering it very resistant to chemical oxidation. However, folic acid is reduced in cells by the enzyme dihydrofolate reductase to the di- and tetrahydro forms (***Figure 7***). This takes place within the intestinal mucosal cells, and 5-methyltetrahydrofolate is released into the plasma.

Natural folates found in foods are all conjugated to a polyglutamyl chain containing different numbers of glutamic acids depending on the type of food. This polyglutamyl chain is removed in the brush border of the mucosal cells by the enzyme folate conjugase, and folate monoglutamate is subsequently absorbed (*1*). The primary form of folate entering human circulation from the intestinal cells is 5-methyltetrahydrofolate monoglutamate. This process is, however, limited in capacity. If enough folic acid is given orally, unaltered folic acid appears in the circulation (*3*), is taken up by cells, and is reduced by dihydrofolate reductase to tetrahydrofolate

The bio-availability of natural folates is affected by the removal of the polyglutamate chain by the intestinal conjugase. This process is apparently not complete (*4*), thereby reducing the bio-availability of natural folates by as much as 25–50 percent. In contrast, synthetic folic acid appears to have a bio-availability of close to 100 percent (*4, 5*). The low bio-availability and – more importantly – the poor chemical stability of the natural folates has a profound influence on the development of nutrient recommendations. This is particularly true if some of the dietary intake is in the synthetic form, folic acid, which is much more stable and bio-available. Food fortification of breakfast cereals, flour, etc. can add significant amounts of folic acid to the diet.

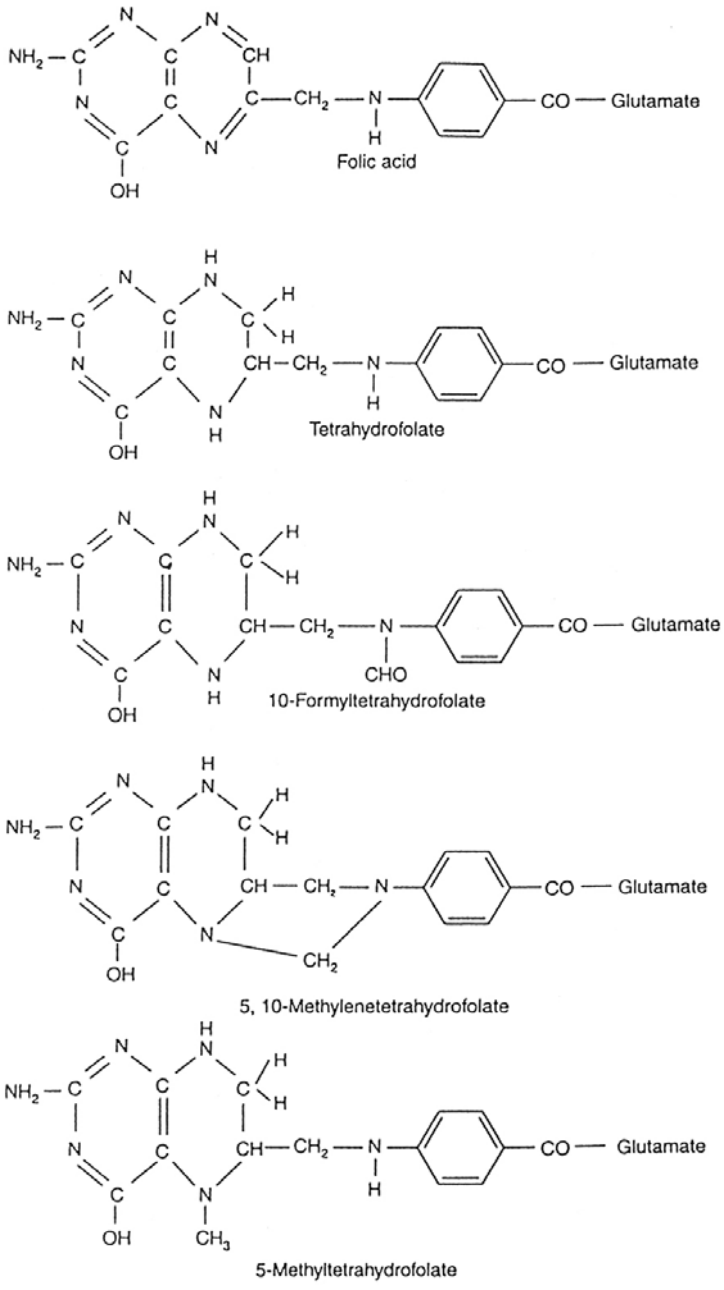
Functional folates have one-carbon groups derived from several metabolic precursors (e.g., serine, *N*-formino-L-glutamate, folate, etc.). With 10-formyltetrahydrofolate the formyl

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group is incorporated sequentially into C-2 and C-8 of the purine ring during its biosynthesis. Likewise the conversion of deoxyuridylate (a precursor to RNA) into thymidylate (a precursor to DNA) is catalysed by thymidylate synthase, which requires 5,10-methylenetetrahydrofofate. Thus, folate in its reduced and polyglutamylated forms is essential for the DNA biosynthesis cycle shown in ***Figure 6***.

Figure 6

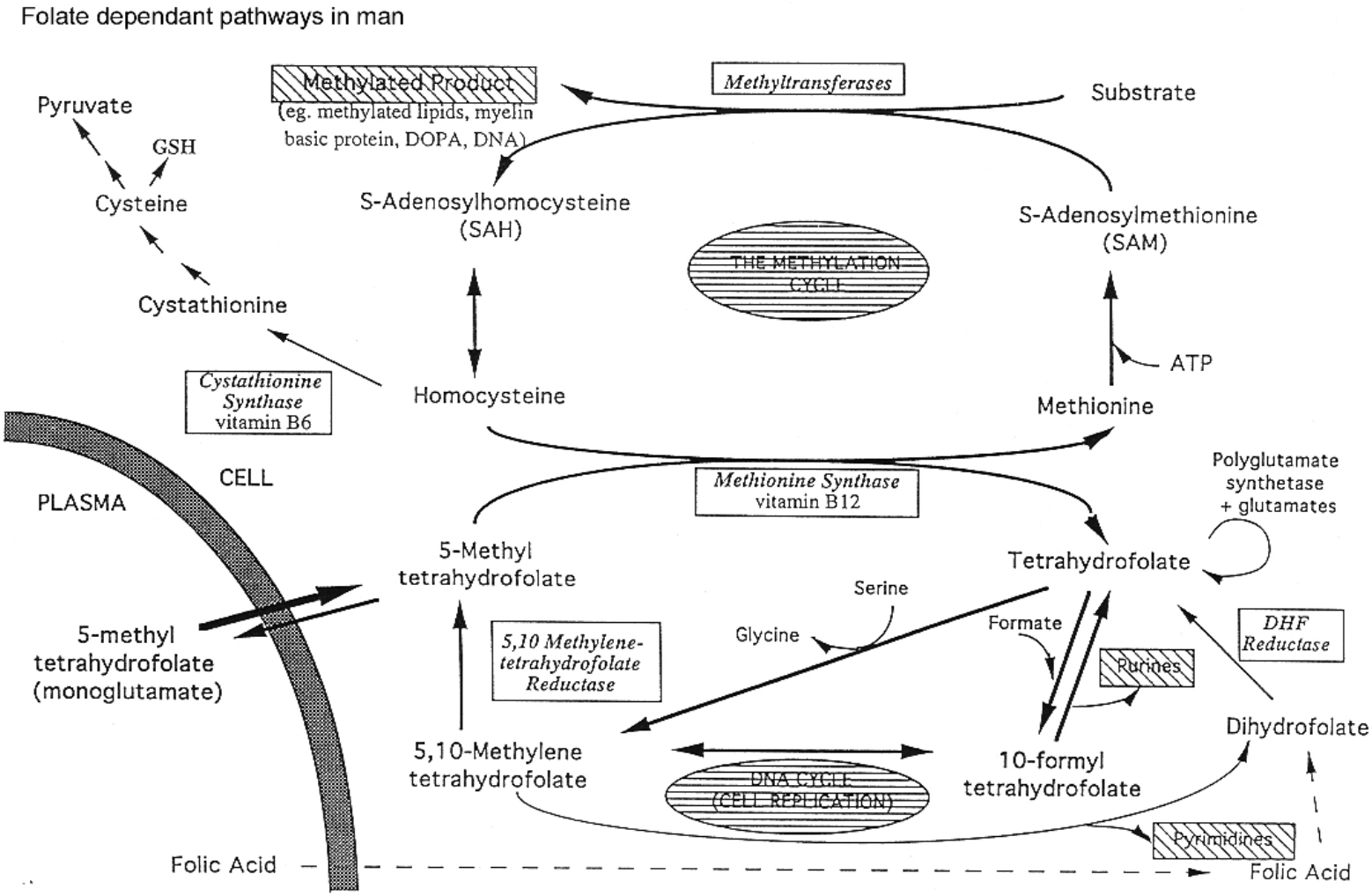
The chemical formula of folic acid (synthetic form) and the most important natural folates.



Note: In cells and thus in food the latter are conjugated to a polyglutamate tail.

Figure 7

The role of the folate co-factors in the DNA cycle and the methylation cycle.
The enzyme methionine synthase requires vitamin B_{12} as well as folate for activity.



Alternatively 5,10-methylenetetrahydrofolate can be channelled to the methylation cycle (*1*). This cycle has two functions. It ensures that the cell always has an adequate supply of *S*-adenosylmethionine, an activated form of methionine, which acts as a methyl donor to a wide range of methyltransferases. These enzymes methylate a wide range of substrates including lipids, hormones, DNA, proteins, etc. One such important methylation is that of myelin basic protein, which acts as insulation for nerves cells. When the methylation cycle is interrupted as it is during vitamin B_{12} deficiency, one of the clinical consequences is the demyelination of nerve resulting in a neuropathy which leads to ataxia, paralysis, and, if untreated, ultimately death. Other important methyltransferase enzymes down-regulate DNA and suppress cell division (*1*).

In the liver the methylation cycle also serves to degrade methionine. Methionine is an essential amino acid in Humans and is present in the diet of people in developed countries at about 60 percent over that required for protein synthesis and other uses. The excess methionine is degraded via the methylation cycle to homo-cysteine, which can either be catabolised to sulfate and pyruvate (with the latter being used for energy) or remethylated to methionine. The need to maintain intracellular *S*-adenosylmethionine levels is related to the amount of methionine metabolised via homo-cysteine.

The DNA and methylation cycles both regenerate tetrahydrofolate. However, there is a considerable amount of catabolism of folate (*6*) and a small loss of folate via excretion from the urine, skin, and bile. There is a need to replenish the body's folate content by uptake from the diet. If there is inadequate dietary folate, the activity of both the DNA and the methylation cycles will be reduced. A decrease in the former will reduce DNA biosynthesis and thereby reduce cell division. Although this will be seen in all dividing cells, the deficiency will be most obvious in cells that are rapidly dividing, for example, in a decrease in red cell production, producing anaemia. Other cells derived from bone marrow also decrease, leading to leucopenia and thrombocytopenia. Likewise there is a reduction in cell division in the lining of the gut. Taken together, this reduction in the DNA cycle results in an increased susceptibility to infection, a decrease in blood coagulation, and secondary malabsorption. In folate deficiency, the flux through the methylation cycle is decreased but the DNA cycle may be more sensitive. The most obvious expression of the decrease in the methylation cycle is an elevation in plasma homo-cysteine. This is due to a decreased availability of new methyl groups provided as 5-methyltetrahydrofolate, necessary for the remethylation of plasma homo-cysteine. Previously it was believed that a rise in plasma homo-cysteine was nothing more than a biochemical marker of possible folate deficiency. However, there is increasing evidence that elevations in plasma homo-cysteine are implicated in the aetiology of cardiovascular disease (*7*). This moderate elevation of plasma homo-cysteine occurs in subjects with a folate status previously considered adequate (*8*).

Interruption of the methylation cycle resulting from impaired folate status or deceased vitamin B_{12} or vitamin B_6 status may have serious long-term risks. Such interruption, as seen in vitamin B_{12} deficiency (e.g., pernicious anaemia), causes a very characteristic demyelination and neuropathy known as subacute combined degeneration of the spinal cord and peripheral nerves. If untreated, this leads to ataxia, paralysis, and ultimately death. Such neuropathy is not usually associated with folate deficiency but is seen if folate deficiency is very severe and prolonged (*9*). The explanation may lie in the well-established ability of nerve tissue to concentrate folate to a level of about five times that in the plasma. This may ensure that nerve tissue has an adequate level of folate when folate being provided to the rapidly dividing cells of the marrow has been severely compromised for a prolonged period. The resultant anaemia will thus inevitably present clinically earlier than the neuropathy.

Definition of populations at risk for folate deficiency

Nutritional deficiency of folate is common in people consuming a limited diet (*10*). This can be exacerbated by malabsorption conditions, including coeliac disease and tropical sprue. Pregnant women are at risk of folate deficiency because pregnancy significantly increases the folate requirement, especially during periods of rapid foetal growth (i.e., in the second and third trimester) (*6*). During lactation losses of folate in milk also increase the folate requirement.

During pregnancy there is an increased risk of foetal neural tube defects (NTDs), with risk increasing 10-fold as folate status goes from adequate to poor (*11*). Between days 21 and 27 post-conception, the neural plate closes to form what will eventually be the spinal cord and cranium. Spina bifida, anencephaly, and other similar conditions are collectively called NTDs. They result from improper closure of the spinal cord and cranium, respectively, and are the most common congenital abnormalities (*12*).

Delineation of dietary sources

Although folate is found in a wide variety of foods, it is present in a relatively low density (*10*) except in liver. Diets that contain adequate amounts of fresh green vegetables (i.e., in excess of three servings per day) will be good folate sources. Folate losses during harvesting, storage, distribution, and cooking can be considerable. Likewise, folate derived from animal products is subject to loss during cooking. Some staples, such as white rice and unfortified corn, are low in folate (see ***Chapter 2***).

In view of the increased requirement for folate during pregnancy and lactation and by select population groups and in view of its low bio-availability, it may be necessary to consider fortification of foods or selected supplementation of women of child-bearing years.

Evidence on which to base a recommended intake

The 1988 Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) Expert Consultation report (*13*) indicated that there were three states of folate nutrition: folate adequacy, impending folate deficiency, and overt folate deficiency. This represented the thinking at the time with respect to folate status – that a status necessary to prevent clinical deficiency was clearly present with folate adequacy and that further improvement in folate status would have no benefit. It would thus be appropriate to increase intake in those with impending folate deficiency or more importantly in those with overt folate deficiency but that nothing was to be gained by increasing the status of those who had adequate status. In the 1988 FAO/WHO report (*13)* it was suggested that adequate folate status is reflected in a red cell folate level of greater than 150 μg/L. Of less relevance was a liver folate level of greater than 7.5 μg/g, because such values are only available in rare circumstances. A normal *N*-formino-L-glutamate test was also cited as evidence of sufficiency, but this test has largely been discredited and abandoned as not having any useful function (*10*). In more recent literature red cell folate continues to be used as an important index of folate status (*14*). Plasma folate is also used but is subject to greater fluctuation. Indicators of haematologic status such as raised mean corpuscular volume, hypersegmentation of neutrophils, and, eventually, the first stages of anaemia remain important indicators of reduced folate status (*15*).

The biomarker plasma homo-cysteine is a very sensitive indicator of folate status and must be added to the indicators of folate adequacy. This applies not only to the deficient range of red blood cell folate but includes normal and even above-normal levels of red cell folate (*14*). There is also very strong evidence that plasma homo-cysteine is an independent risk

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factor for cardiovascular disease (*8, 16*). Any elevation in homo-cysteine, even at levels where overt folate deficiency is not an issue, may be undesirable because it is a risk factor for chronic disease. Thus, newer thinking would require consideration of a folate intake that would reduce plasma homo-cysteine to a minimum level of less than 7.0 μmol/l. Formerly acceptable levels of red cell folate may therefore be associated with an increased rise of cardiovascular disease and stroke (*17,18*). The possible benefit of lowering plasma homo-cysteine through increased folate intake can be proven only by an intervention trial with folic acid supplementation in large populations. Using plasma homo-cysteine as a biomarker for folate adequacy can only be done on an individual basis after the possibility of a genetic mutation or an inadequate supply of vitamin B_6 or vitamin B_{12} has been eliminated.

There is now conclusive evidence that most NTDs can be prevented by the ingestion of folic acid near the time of conception (*11,12*). Lower red cell folate, including what was previously considered an adequate or normal range, is associated with an increased risk of spina bifida and other NTDs (*19*). Red cell folate levels greater than 150 μg/L, which are completely adequate to prevent anaemia, are associated with increase risk of NTDs (*11*).

Low folate status, including red cell levels in the normal range, increases the risk of colorectal cancer (*20, 21*).

In 1998, the US National Academy of Sciences (*22*) exhaustively reviewed the evidence of folate intake, status, and health for all age groups and also reviewed the literature on the extra requirements during pregnancy and lactation. This review led to calculations of an estimated average requirement (EAR) and a subsequent estimation of the recommended dietary allowances (RDAs) to be the EAR plus 2 standard deviations. This definition of the RDA agrees with the definition of the FAO/WHO recommended nutrient intake (RNI), and members of this FAO/WHO expert group agreed that the values published by the US National Academy of Sciences were the best estimates of folate requirements based on the current literature. Therefore, the FAO/WHO expert group adopted the same approach, and the RNIs suggested in ***Table 13*** were based on the RDAs of the US National Academy of Sciences. These recommendations apply to healthy individuals and populations.

Table 13

Estimated average requirement (EAR) and recommended nutrient intake (RNI) for folic acid expressed as dietary folate equivalents, by age group

Group	EAR (μg/day)	RNI (μg/day)
Infants and children		
0–6 months [a]	65	80
7–12 months	65	80
1–3 years	120	160
4–6 years	160	200
7–9 years	250	300
Adolescents, 10–18 years	300	400
Adults		
19–65 years	320	400
65+ years	320	400
Pregnancy	520	600
Lactation	450	500

Adapted from the US National Academy of Sciences (*22*).

[a] Based on a human milk intake of 0.75 l/day.

Differences in bio-availability of folic acid and food folate

The RNIs suggested for groups in ***Table 13*** used food folate as the source of dietary folate because most societies in developing countries consume folate from naturally occurring sources. As discussed in the introduction, natural folates are found in a conjugated form in food, which reduces its bio-availability by perhaps as much as 50 percent (*4*). In addition, natural folates are much less stable. If chemically pure folic acid (pteroyl monoglutamate) is used to provide part of the RNI, by way of fortification or supplementation, the total dietary folate, which contains conjugated forms (pteroyl polyglutamates), could be reduced by an appropriate amount. On average the conjugated folate in natural foods is considered to be only half as available as synthetic folic acid. For example, the recommendation of usual mixed forms of folate in the diet is 400 μg/day, but 100 μg of this given as pure folic acid would be considered to be equivalent to 200 μg of dietary mixed folate. Hence, only an additional 200 μg of dietary folate would be needed.

The FAO/WHO expert group agreed with the findings of the Food and Nutrition Board of the US National Academy of Sciences (*22*):

> Since folic acid taken with food is 85 percent bio-available but food folate is only about 50 percent bio-available, folic acid taken with food is 85/50 (i.e., 1.7) times more available. Thus, if a mixture of synthetic folic acid plus food folate has been fed, dietary folate equivalents (DFEs) are calculated as follows to determine the EAR:
>
> μg of DFE provided = [μg of food folate + (1.7 x μg of synthetic folic acid)]
>
> To be comparable to food folate, only half as much folic acid is needed if taken on an empty stomach, i.e., 1 μg of DFE = 1 μg of food folate = 0.5 μg of folic acid taken on an empty stomach = 0.6 μg of folic acid with meals (*22*).

The experts from the National Academy of Sciences went on to say that the required estimates for the dietary folate equivalents could be lowered if future research indicates that food folate is more than 50 percent bio-available.

Neural tube defects

It is now agreed that a supplement of 400 μg of folic acid taken near the time of conception will prevent most neural tube defects (NTDs) (*23, 24*). The recommendation to prevent recurrence in women with a previous NTD birth remains 4.0 mg/day because of the high increase in risk in such cases and because that was the amount used in the most definitive trial (*25*). Because of the poorer bio-availability and stability of food folate, a diet based on food folate will not be optimum in prevention. One study determined that risk of NTD is 10-fold higher in people with poor folate status than in those with high normal folate status (*11*). A further study suggests that an extra 200 μg/day or possibly 100 μg/day if taken habitually in fortified food would prevent most if not all of folate-preventable NTDs (*26*). Ideally, an extra 400 μg/day should be provided because this is the amount used in various intervention trials (*12*) and can be achieved by supplementation. This amount could not be introduced by way of fortification, because exposure to high intakes of folic acid by people consuming a large intake of flour would run the risk of preventing the diagnosis of pernicious anaemia in the elderly. It is likely that depending on the staple chosen it would be possible to increase intake in most women by 100 μg/day without causing too high an exposure in other groups. It is suggested that this amount, although not optimal, will prevent most NTDs.

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Cardiovascular disease

Plasma homo-cysteine concentration, if only moderately elevated, is an independent risk factor for cardiovascular disease (*7, 8, 16*) and stroke (*18*). Increased risk was associated with values higher than 11 μmol/l (*8*), which is well within what is the normal range (5–15 μmol/l) of plasma homo-cysteine levels (*27*). In addition, even in populations that are apparently normal and consuming diets adequate in folate, there is a range of elevation of plasma homo-cysteine (*14*) that could be lowered by an extra 100 or 200 μg/day of folic acid (*8, 27*). Large-scale intervention trials regarding the significance of interrelationships among folate levels, plasma homo-cysteine levels, and cardiovascular disease have not been completed and therefore it would be premature to introduce public health measures in this area.

Colorectal cancer

Evidence suggests a link between colorectal cancer and dietary folate intake and folate status (20*, 21*). One study reported that women who take multivitamin supplements containing folic acid for prolonged periods have a significantly reduced risk of colorectal cancer (*28*). However, the scientific evidence is not sufficiently clear for recommending increased folate intake in populations at risk for colorectal cancer.

Upper limit

There is no evidence that it is possible to consume sufficient natural folate to pose a risk of toxicity (*22*). However, this clearly does not apply to folic acid given in supplements or fortified foods. The main concern is the masking of the diagnosis of pernicious anaemia, because high levels of folic acid correct the anaemia, allowing the neuropathy to progress undiagnosed to where it may become irreversible even upon treatment with vitamin B_{12} (*1, 29*). Consumption of large amounts of folic acid might also pose other less well-defined risks. Certainly, consumption of milligram amounts of folic acid would be undesirable. Savage and Lindenbaum (*30*) suggest that even at levels of the RNI there is a decreased opportunity to diagnose pernicious anaemia through its presentation via the anaemia.

The US National Academy of Sciences (*22*), after reviewing literature, has suggested an upper level of 1000 μg. Thus, 400 μg/day of folic acid, in addition to dietary folate, would seem safe. There is probably no great risk of toxicity at a range between 400 and 1000 μg of folic acid per day with the exception of some increased difficulty in diagnosing pernicious anaemia resulting from the masking of the anaemia.

Future research

There are many areas for future research:

- Folate status may be related to birth weight. Therefore it is important to study the relationship between folate status and birth weight, especially in populations where low birth weight is prevalent.
- Folate status probably differs widely in different developing countries. Red cell folate levels are an excellent determinant of status. Such estimates in representative populations would determine whether some communities are at risk from poor folate status.
- Some evidence indicates that elevated plasma homo-cysteine is a risk factor for cardiovascular disease and stroke. Elevated plasma homo-cysteine is largely related to poor folate status, with poor vitamin B_6 status, poor vitamin B_{12} status, or both also contributing. There are also genetic differences (*31*). The prevalence of elevated

plasma homo-cysteine and its relationship to cardiovascular disease should be established in different developing countries.

- More data should be generated on the bio-availability of natural folate from diets consumed in developing countries.
- Because the absorption of folate may be more efficient in humans with folate deficiency, folate absorption in these populations requires additional research.
- The relationship between folate deficiency and the incidence of NTDs in developing countries needs further investigation.
- Quantitation of the folate content of foods typically consumed in developing countries should be established for the different regions of the world.

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Chapter 5
Vitamin B_{12}

Role of vitamin B_{12} in human metabolic processes

Although the nutritional literature still uses the term vitamin B_{12}, a more specific name for vitamin B_{12} is cobalamin. Vitamin B_{12} is the largest of the B complex vitamins, with a molecular weight of over 1000. It consists of a corrin ring made up of four pyrroles with cobalt at the center of the ring (*1, 2*).

There are several vitamin B_{12}–dependent enzymes in bacteria and algae, but no species of plants have the enzymes necessary for vitamin B_{12} synthesis. This fact has significant implications for the dietary sources and availability of vitamin B_{12}. In mammalian cells there are only two vitamin B_{12}–dependent enzymes (*3*). One of these enzymes, methionine synthase, uses the chemical form of the vitamin which has a methyl group attached to the cobalt and is called methylcobalamin (see ***Figure 7*** in Chapter 4.). The other enzyme, methylmalonyl CoA mutase, uses vitamin B_{12} with a 5'-adeoxyadenosyl moiety attached to the cobalt and is called 5'-deoxyaldenosylcobalamin, or coenzyme B_{12}. In nature there are two other forms of vitamin B_{12}: hydroxycobalamin and aquacobalamin, where hydroxyl and water groups, respectively, are attached to the cobalt. The synthetic form of vitamin B_{12} found in supplements and fortified foods is cyanocobalamin, which has cyanide attached to the cobalt. These three forms of B_{12} are enzymatically activated to the methyl- or deoxyadenosylcobalamins in all mammalian cells.

Dietary sources and availability

Most microorganisms, including bacteria and algae, synthesise vitamin B_{12}, and they constitute the only source of the vitamin (*4*). The vitamin B_{12} synthesised in microorganisms enters the human food chain through incorporation into food of animal origin. In many animals gastrointestinal fermentation supports the growth of these vitamin B_{12}–synthesising microorganisms, and subsequently the vitamin is absorbed and incorporated into the animal tissues. This is particularly true for the liver, where vitamin B_{12} is stored in large concentrations. Products from these herbivorous animals, such as milk, meat, and eggs, constitute important dietary sources of the vitamin unless the animal is subsisting in one of the many regions known to be geochemically deficient in cobalt (*5*). Milk from cows and humans contains binders with very high affinity for vitamin B_{12}, whether they hinder or promote intestinal absorption is not entirely clear. Omnivores and carnivores, including humans, derive dietary vitamin B_{12} from animal tissues or products (i.e., milk, butter, cheese, eggs, meat, poultry, etc.). It appears that no significant amount of the required vitamin B_{12} by humans is derived from microflora, although vegetable fermentation preparations have also been reported as being possible sources of vitamin B_{12} (6).

Absorption

The absorption of vitamin B_{12} in humans is complex (*1, 2*). Vitamin B_{12} in food is bound to proteins and is released from the proteins by the action of a high concentration of hydrochloric acid present in the stomach. This process results in the free form of the vitamin, which is immediately bound to a mixture of glycoproteins secreted by the stomach and salivary glands. These glycoproteins, called R-binders (or haptocorrins), protect vitamin B_{12}

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from chemical denaturation in the stomach. The stomach's parietal cells, which secrete hydrochloric acid, also secrete a glycoprotein called intrinsic factor. Intrinsic factor binds vitamin B_{12} and ultimately enables its active absorption. Although the formation of the vitamin B_{12} – intrinsic factor complex was initially thought to happen in the stomach, it is now clear that this is not the case. At an acidic pH the affinity of the intrinsic factor for vitamin B_{12} is low whereas its affinity for the R-binders is high. When the contents of the stomach enter the duodenum, the R-binders become partly digested by the pancreatic proteases, which causes them to release their vitamin B_{12}. Because the pH in the duodenum is more neutral than that in the stomach, the intrinsic factor has a high binding affinity to vitamin B_{12}, and it quickly binds the vitamin as it is released from the R-binders. The vitamin B_{12}–intrinsic factor complex then proceeds to the lower end of the small intestine, where it is absorbed by phagocytosis by specific ileal receptors (*1, 2*).

Populations at risk for and consequences of vitamin B_{12} deficiency

Vegetarians

Because plants do not synthesise vitamin B_{12}, individuals who consume diets completely free of animal products (vegan diets) are at risk of vitamin B_{12} deficiency. This is not true of lacto-ovo-vegetarians, who consume the vitamin in eggs, milk, and other dairy products.

Pernicious anaemia

Malabsorption of vitamin B_{12} can occur at several points during digestion (*1, 4*). By far the most important condition resulting in vitamin B_{12} malabsorption is the auto-immune disease called pernicious anaemia (PA). In most cases of PA, antibodies are produced against the parietal cells causing them to atrophy, lose their ability to produce intrinsic factor, and secrete hydrochloric acid. In some forms of PA the parietal cells remain intact but auto-antiobodies are produced against the intrinsic factor itself and attach to it, thus preventing it from binding vitamin B_{12}. In another less common form of PA, the antibodies allow vitamin B_{12} to bind to the intrinsic factor but prevent the absorption of the intrinsic factor–vitamin B_{12} complex by the ileal receptors. As is the case with most auto-immune diseases, the incidence of PA increases markedly with age. In most ethnic groups it is virtually unknown to occur before the age of 50, with a progressive rise in incidence thereafter (*4*). However, African American populations are known to have an earlier age of presentation *(4)*. In addition to causing malabsorption of dietary vitamin B_{12}, PA also results in an inability to reabsorb the vitamin B_{12} which is secreted in the bile. Biliary secretion of vitamin B_{12} is estimated to be between 0.3 and 0.5 μg/day. Interruption of this so-called enterohepatic circulation of vitamin B_{12} causes the body to go into a significant negative balance for the vitamin. Although the body typically has sufficient vitamin B_{12} stores to last 3–5 years, once PA has been established the lack of absorption of new vitamin B_{12} is compounded by the loss of the vitamin because of negative balance. When the stores have been depleted, the final stages of deficiency are often quite rapid, resulting in death in a period of months if left untreated.

Atrophic gastritis

Historically, PA was considered to be the major cause of vitamin B_{12} deficiency, but it was a fairly rare condition, perhaps affecting 1 percent to a few percent of elderly populations. More recently it has been suggested that a far more common problem is that of hypochlorhydria associated with atrophic gastritis, where there is a progressive reduction with age of the ability of the parietal cells to secrete hydrochloric acid (*7*). It is claimed that perhaps up to one-quarter of elderly subjects could have various degrees of hypochlorhydria as a result of atrophic gastritis. It has also been suggested that bacterial overgrowth in the stomach and intestine in individuals suffering from atrophic gastritis may also reduce vitamin B_{12}

absorption. This absence of acid is postulated to prevent the release of protein-bound vitamin B_{12} contained in food but not to interfere with the absorption of the free vitamin B_{12} found in fortified foods or supplements. Atrophic gastritis does not prevent the reabsorption of bilary vitamin B_{12} and therefore does not result in the negative balance seen in individuals with PA. However, it is agreed that with time, a reduction in the amount of vitamin B_{12} absorbed from the diet will eventually deplete even the usually adequate vitamin B_{12} stores, resulting in overt deficiency.

When considering recommended nutrient intakes (RNIs) for vitamin B_{12} for the elderly, it is important to take into account the absorption of vitamin B_{12} from sources such as fortified foods or supplements as compared with dietary vitamin B_{12}. In the latter instances, it is clear that absorption of intakes of less than 1.5–2.0 μg/day is complete – that is, for intakes of less than 1.5–2.0 μg of free vitamin B_{12}, the intrinsic factor – mediated system absorbs all of that amount. It is probable that this is also true of vitamin B_{12} in fortified foods, although this has not specifically been examined. However, absorption of food-bound vitamin B_{12} has been reported to vary from 9 percent to 60 percent depending on the study and the source of the vitamin, which is perhaps related to its incomplete release from food (8). This has led many to estimate absorption as being up to 50 percent to correct for bio-availability of absorption from food.

Vitamin B_{12} interaction with folate or folic acid

One of the vitamin B_{12} – dependent enzymes, methionine synthase, functions in one of the two folate cycles (see ***Chapter 4***) – the methylation cycle. This cycle is necessary to maintain availability of the methyl donor *S*-adenosylmethionine; interruption reduces the wide range of methylated products. One such important methylation is that of myelin basic protein. Reductions in the level of *S*-adenosylmethionine seen in PA and other causes of vitamin B_{12} deficiency produce demyelination of the peripheral nerves and the spinal column, called sub-acute combined degeneration (*1, 2*). This neuropathy is one of the main presenting conditions in PA. The other principal presenting condition in PA is a megaloblastic anaemia morphologically identical to that seen in folate deficiency. Disruption of the methylation cycle should cause a lack of DNA biosynthesis and anaemia.

The methyl trap hypothesis is based on the fact that once the cofactor 5,10-methylenetetrahydrofolate is reduced by its reductase to form 5-methyltetrahydrofolate, the reverse reaction cannot occur. This suggests that the only way for the methyltetrahydrofolate to be recycled to tetrahydrofolate, and thus to participate in DNA biosynthesis and cell division, is through the vitamin B_{12} – dependent enzyme methionine synthase. When the activity of this synthase is compromised, as it would be in PA, the cellular folate will become progressively trapped as 5-methyltetrahydrofolate. This will result in a cellular pseudo folate deficiency where despite adequate amounts of folate an anaemia will develop that is identical to that seen in true folate deficiency. Clinical symptoms of PA, therefore, include neuropathy, anaemia, or both. Treatment with vitamin B_{12}, if given intramuscularly, will reactivate methionine synthase, allowing myelination to restart. The trapped folate will be released and DNA synthesis and generation of red cells will cure the anaemia. Treatment with high concentrations of folic acid will treat the anaemia but not the neuropathy of PA. It should be stressed that the so-called masking of the anaemia of PA is generally agreed not to occur at concentrations of folate found in food or at intakes of the synthetic form of folic acid found at usual RNI levels of 200 or 400 μg/day (*1*). However, there is some evidence that amounts less than 400 μg may cause a haematologic response and thus potentially treat the anaemia (*9*). The masking of the anaemia definitely occurs at high concentrations of folic acid (>1000 μg/day). This becomes a concern when considering fortification with synthetic folic acid of a dietary staple such as flour (see ***Chapter 4***).

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In humans the vitamin B_{12} – dependent enzyme methylmalonyl coenzyme A (CoA) mutase functions in the metabolism of propionate and certain of the amino acids, converting them into succinyl CoA, and in their subsequent metabolism via the citric acid cycle. It is clear that in vitamin B_{12} deficiency the activity of the mutase is compromised, resulting in high plasma or urine concentrations of methylmalonic acid (MMA), a degradation product of methylmalonyl CoA. In adults this mutase does not appear to have any vital function, but it clearly has an important role during embryonic life and in early development. Children deficient in this enzyme, through rare genetic mutations, suffer from mental retardation and other developmental defects.

Assessment of vitamin B_{12} status

Traditionally it was thought that low vitamin B_{12} status was accompanied by a low serum or plasma vitamin B_{12} level (*4*). Recently this has been challenged by Lindenbaum *et al*.(*10*), who suggested that a proportion of people with normal vitamin B_{12} levels are in fact vitamin B_{12} deficient. They also suggested that elevation of plasma homo-cysteine and plasma MMA are more sensitive indicators of vitamin B_{12} status. Although plasma homo-cysteine may also be elevated because of folate or vitamin B_6 deficiency, elevation of MMA apparently always occurs with poor vitamin B_{12} status. There may be other reasons why MMA is elevated, such as renal insufficiency, so the elevation of itself is not diagnostic. Many would feel that low or decreased plasma vitamin B_{12} levels should be the first indication of poor status and that this could be confirmed by an elevated MMA if this assay was available.

Evidence on which to base a recommended intake

Recommendations for nutrient intake

The Food and Nutrition Board of the National Academy of Sciences (NAS) Institute of Medicine (*8*) has recently exhaustively reviewed the evidence of intake, status, and health for all age groups and during pregnancy and lactation. This review has lead to calculations of what they have called an estimated average requirement (EAR). The EAR is defined by NAS as "the daily intake value that is estimated to meet the requirement, as defined by the specific indicator of adequacy, in half of the individuals in a life-stage or gender group" (*8)*. They have estimated the recommended dietary allowances to be this figure plus 2 standard deviations (SDs). Some members of the Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) Expert Consultation were involved in the preparation and review of the NAS recommendations and judge them to have been the best estimates based on available scientific literature. The FAO/WHO expert group felt it appropriate to adopt the same approach used by the NAS in deriving the RNIs. Therefore the RNIs suggested in ***Table 14*** are based on the NAS EARs plus 2 SDs.

Adults

Several lines of evidence point to an adult average requirement of about 2.0 µg/day. The amount of intramuscular vitamin B_{12} needed to maintain remission in people with PA suggests a requirement of about 1.5 µg/day (*10*), but they would also be losing 0.3–0.5µg/day through interruption of their enterohepatic circulation, which is not typical. This might suggest a requirement of 0.7–1.0 µg/day. Because vitamin B_{12} is not completely absorbed from food, an adjustment of 50 percent has to be added giving a range of 1.4–2.0 µg/day (*4*). Therapeutic response to ingested food vitamin B_{12} suggests a minimum requirement of something less than 1.0 µg/day (*8*). Diets containing 1.8 µg/day seemed to maintain adequate status but lower intakes showed some signs of deficiency. (*8*). Dietary intakes of less than 1.5 µg/day were reported to be inadequate in some subjects (*11*).

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In summary, the average requirement could be said to be 2 µg/day (*8*). The variability of the requirements for vitamin B_{12} is accounted for by adding two SDs, that is, 2.4 µg/day as an RNI for adults, including the elderly.

Table 14

Estimated average requirement (EAR) and recommended nutrient intake (RNI) for vitamin B_{12}, by age group

Adapted from the US National Academy of Sciences (8).

Group	EAR µg/day	RNI µg/day
Infants and children		
0–6 months	0.32	0.4
7–12 months	0.32	0.5
1–3 years	0.7	0.9
4–6 years	1.0	1.2
7–9 years	1.5	1.8
Adolescents, 10–18 years	2.0	2.4
Adults		
19–65 years	2.0	2.4
65+ years	2.0	2.4
Pregnancy	2.2	2.6
Lactation	2.4	2.8

Children

The Food and Nutrition Board of the NAS Institute of Medicine (*8*) suggested the same intakes for adolescents with progressive reduction of intake for younger groups.

Pregnancy

The previous FAO/WHO (12) Expert Consultation suggested that 0.1–0.2 µg/day of vitamin B_{12} is transferred to the foetus (*13*) during the last two trimesters of pregnancy. On the basis of on foetal liver content from post-mortem samples (*14*, *15, 16*), there is further evidence that the foetus accumulates on average 0.1–0.2 µg/day during pregnancies of women with diets which have adequate vitamin B_{12}. It has been reported that children born to vegetarians or other women with a low vitamin B_{12} intake subsequently develop signs of clinical vitamin B_{12} deficiency such as neuropathy (*17*). Therefore, when calculating the EAR for pregnant women, 0.2 µg/day of vitamin B_{12} is added to the EAR for adults to result in an EAR of 2.2 µg/day and an RNI of 2.6 µg /day during pregnancy.

Lactation

It is estimated that 0.4 µg/day of vitamin B_{12} is found in the human milk of women with adequate vitamin B_{12} status (*8)*. Therefore an extra 0.4 µg/day of vitamin B_{12} is needed during lactation in addition to the normal adult requirement of 2.0 µg/day, giving a total EAR of 2.4 µg/day during lactation and an RNI of 2.8 µg/day.

Infants

As with other nutrients, the principal way to determine requirements of infants is to examine the levels in milk from mothers on adequate diets. There is a wide difference in the vitamin

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B_{12} values reported in human milk because of differences in methodology. The previous FAO/WHO report (12) used milk vitamin B_{12} values of normal women of about 0.4 µg/l. For an average milk production of 0.75 l/day, the vitamin B_{12} intake by infants would be 0.3 µg/day (*18)*. Other studies have reported ranges of vitamin B_{12} in human milk to be 0.4–0.8 µg/L (*17, 19, 20, 21, 22*). Although daily intakes ranging from 0.02 to 0.05 µg/day have been found to prevent deficiency (*23, 24*), these intakes are totally inadequate for long-term health. An EAR of 0.3–0.6 µg/day would result in an RNI of 0.36–0.72 µg/d. It might be prudent to use the lower figure of 0.4 µg/day for the first 6 months of pregnancy and 0.7 µg/day for last trimester.

Upper limits

The absorption of vitamin B_{12} mediated by intrinsic factor is limited to 1.5–2.0 µg per meal because of the limited capacity of the receptors. In addition, between 1 percent and 3 percent of any particular oral administration of vitamin B_{12} is absorbed by passive diffusion. Thus, if 1000 µg vitamin B_{12} (sometimes used to treat those with PA) is taken orally, the amount absorbed would be 2.0 µg by active absorption plus about 30 µg by passive diffusion. This amount has never been reported to have any side effects (*8*). Similar large amounts have been used in some preparations of nutritional supplements without apparent ill effects. However, there are no established benefits for such amounts. Such high intakes thus represent no benefit in those without malabsorption and should probably be avoided.

Future research

Because they do not consume any animal products, vegans are at risk of vitamin B_{12} deficiency. It is generally agreed that in some communities the only source of vitamin B_{12} is from contamination of food by microorganisms. When vegans move to countries where standards of hygiene are more stringent, there is good evidence that risk of vitamin B_{12} deficiency increases in adults and, particularly, in children born to and breast-fed by women who are strict vegans.

- As standards of hygiene improve in developing countries, there is a concern that the prevalence of vitamin B_{12} deficiency might increase. This should be ascertained by estimating plasma vitamin B_{12} levels, preferably in conjunction with plasma MMA levels in representative adult populations and in infants.
- The contribution which fermented vegetable foods make to vitamin B_{12} status of vegan communities should be investigated.
- The prevalence of atrophic gastritis should be investigated in developing countries.

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Chapter 6
Vitamin C

Vitamin C (chemical names: ascorbic acid and ascorbate) is a six-carbon lactone which is synthesised from glucose by many animals. Vitamin C is synthesised in the liver in some mammals and in the kidney in birds and reptiles. However, several species – including humans, non-human primates, guinea pigs, Indian fruit bats, and Nepalese red-vented bulbuls – are unable to synthesise vitamin C. When there is insufficient vitamin C in the diet, humans suffer from the potentially lethal deficiency disease scurvy (*1*). Humans and primates lack the terminal enzyme in the biosynthetic pathway of ascorbic acid, l-gulonolactone oxidase, because the gene encoding for the enzyme has undergone substantial mutation so that no protein is produced (*2*).

Role in human metabolic processes

Background biochemistry

Vitamin C is an electron donor (reducing agent or antioxidant), and probably all of its biochemical and molecular functions can be accounted for by this function. The potentially protective role of vitamin C as an antioxidant is discussed in the antioxidants chapter of this report.

Enzymatic functions

Vitamin C acts as an electron donor for 11 enzymes (*3, 4*). Three of those enzymes are found in fungi but not in humans or other mammals (*5, 6*). They are involved in reutilisation pathways for pyrimidines and the deoxyribose moiety of deoxynucleosides. Of the 8 remaining human enzymes, three participate in collagen hydroxylation (*7-9*) and two in carnitine biosynthesis (*10, 11*); of the three enzymes which participate in collagen hydroxylation, one is necessary for biosynthesis of the catecholamine norepinephrine (*12, 13*), one is necessary for amidation of peptide hormones (*14, 15*), and one is involved in tyrosine metabolism (*4, 16*).

Ascorbate interacts with enzymes having either monooxygenase or dioxygenase activity. The monooxygenases dopamine β-monooxygenase and peptidyl-glycine α-monooxygenase incorporate a single oxygen atom into a substrate, either a dopamine or a glycine-terminating peptide. The remaining enzymes are dioxygenases which incorporate two oxygen atoms in two different ways. The enzyme 4-hydroxyphenylpyruvate dioxygenase incorporates two oxygen atoms into one product. The other dioxygenase incorporates one oxygen atom into succinate and one into the enzyme-specific substrate.

Miscellaneous functions

The concentrations of vitamin C in gastric juice were several fold higher (median, 249 μmol/l; range, 43–909 μmol/l) than those found in the plasma of the same normal subjects (39 μmol/l, 14–101 μmol/l) (*17*). Gastric juice vitamin C may prevent the formation of *N*-nitroso compounds, which are potentially mutagenic (*18*). High intakes of vitamin C correlate with reduced gastric cancer risk (*19*), but a cause-and-effect relationship has not been established.

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Vitamin C protects low-density lipoproteins *ex vivo* against oxidation and may function similarly in the blood (*20;* see ***Chapter 17***).

A common feature of vitamin C deficiency is anaemia. The antioxidant properties of vitamin C may stabilise folate in food and in plasma, and increased excretion of oxidized folate derivatives in human scurvy was reported (*21*). Vitamin C promotes absorption of soluble non-haem iron possibly by chelation or simply by maintaining the iron in the reduced (ferrous, Fe^{2+}) form (*22, 23*). The effect can be achieved with the amounts of vitamin C obtained in foods. However, the amount of dietary vitamin C required to increase iron absorption ranges from 25 mg upwards and depends largely on the amount of inhibitors, such as phytates and polyphenols, present in the meal (*24*). (See ***Chapter 13*** on iron for further discussion.)

Overview of significant scientific information

From the 15th century, scurvy was dreaded by seamen and explorers forced to subsist for months on diets of dried beef and biscuits. Scurvy was described by the Crusaders, during the sieges of numerous European cities, and as a result of the famine in 19th century Ireland. Three important manifestations of scurvy – gingival changes, pain in the extremities, and haemorrhagic manifestations – preceded oedema, ulcerations, and ultimately death. Skeletal and vascular lesions in scurvy probably arise from a failure of osteoid formation. In infantile scurvy the changes are mainly at the sites of most active bone growth; characteristic signs are a pseudoparalysis of the limbs caused by extreme pain on movement and caused by haemorrhages under the periosteum, as well as swelling and haemorrhages in areas of the gums surrounding erupting teeth (*25*). In adults one of the early, principle adverse effects of the collagen-related pathology may be impaired wound healing (*26*). Vitamin C deficiency can be detected from early signs of clinical deficiency, such as the follicular hyperkeratosis, petechial haemorrhages, swollen or bleeding gums, and joint pain, or from the very low concentrations of ascorbate in plasma, blood, or leukocytes. The Sheffield studies (*26, 27*) and later studies in Iowa (*28, 29*) were the first major attempts made to quantify vitamin C requirements. The studies indicated that the amount of vitamin C required to prevent or cure early signs of deficiency was between 6.5 and 10 mg/day. This range represents the lowest physiologic requirement. The Iowa studies (*28, 29*) and Kallner et al (*30*) established that at tissue saturation, whole body vitamin C content is approximately 20 mg/kg, or 1500 mg, and that during depletion vitamin C is lost at 3 percent of whole body content per day.

Clinical signs of scurvy appear in men at intakes lower than 10 mg/day (*27*) or when the whole body content falls below 300 mg (*28*). Such intakes are associated with plasma ascorbate concentrations below 11 μmol/l or leukocyte levels less than 2 nmol/10^8 cells. However, the plasma concentrations fall to around 11 μmol/l when dietary vitamin C is between 10 and 20 mg/day. At intakes greater than 25–35 mg/day, plasma concentrations start to rise steeply, indicating a greater availability of vitamin C for metabolic needs. In general, plasma ascorbate closely reflects the dietary intake and ranges between 20 and 80 μmol/l. Note that during infection or physical trauma, an increase in the number of circulating leukocytes occurs and these take up vitamin C from the plasma (*31, 32*). Therefore, both plasma and leukocyte levels may not be very precise indicators of body content or status at such times. However, leukocyte ascorbate remains a better indicator of vitamin C status than plasma ascorbate most of the time and only in the period immediately after the onset of an infection are both values unreliable.

Intestinal absorption of vitamin C is by an active, sodium-dependent, energy-requiring, carrier-mediated transport mechanism (*33*) and as intakes increase, the tissues progressively become more saturated**.** The physiologically efficient, renal-tubular reabsorption mechanism retains vitamin C in the tissues up to a whole body content of

ascorbate of about 20 mg/kg body weight (*30*). However, under steady state conditions, as intakes rise from around 100 mg/day there is an increase in urinary output in so that at 1000 mg/day almost all absorbed vitamin C is excreted (*34, 35*).

Definition of population at risk

The populations at risk of vitamin C deficiency are those for whom the fruit and vegetable supply is minimal. Epidemics of scurvy are associated with famine and war, when people are forced to become refugees and food supply is small and irregular. Persons in whom the total body vitamin C content is saturated can subsist without vitamin C for approximately 2 months before the appearance of clinical signs, and as little as 6.5–10 mg/day vitamin C will prevent the appearance of scurvy. In general, vitamin C status will reflect the regularity of fruit and vegetable consumption but also socio-economic conditions, because intake is determined not just by availability, but by cultural preferences and cost.

In Europe and the United States an adequate intake of vitamin C is indicated by the results of various national surveys (*36-38*). In the United Kingdom and Germany, the mean dietary intakes of vitamin C in adult men and women were 87 and 76 (*37*) and 75 and 72 mg/day (*36*), respectively. In addition, a recent survey of elderly men and women in the United Kingdom reported vitamin C intakes of 72 (SD 61) and 68 (SD 60) mg/day, respectively (*39*). In the United States, in the National Health and Nutrition Examination Survey (*38*), the median consumption of vitamin C from foods during the years 1988–91 was 73 and 84 mg/day in men and women, respectively. In all these studies there was a wide variation in vitamin C intake and 25–30 percent of the US population consumed less than 2.5 servings of fruit and vegetables daily. Likewise a survey of Latin American children in the United States suggested that less than 15 percent consumed the recommended intake of fruits and vegetables (*40*). It is not possible to relate servings of fruits and vegetables to an exact amount of vitamin C, but the World Health Organization (WHO) dietary goal of 400 g (*41*) aimed to provide sufficient vitamin C to meet the 1970 Food and Agriculture Organization of the United Nations (FAO)/WHO guidelines – that is, approximately 20–30 mg/day – and lower the risk of chronic disease. The WHO goal has been roughly translated into the recommendation of five portions per day (*42*).

Reports from India show that the available supply of vitamin C is 43 mg/capita/day, and in the different states of India it ranges from 27 to 66 mg/day. In one study, low-income children consumed as little as 8.2 mg/day of vitamin C in contrast to a well-to-do group of children where the intake was 35.4 mg/day (*43*). Other studies done in developing countries found plasma vitamin C concentrations lower than those reported for developed countries, for example, 20–27 μmol/l for apparently healthy adolescent boys and girls in China and 3–54 μmol/l (median 14 μmol/l) for similarly aged Gambian nurses (*44, 45*), although values obtained in a group of adults from a rural district in Northern Thailand were quite acceptable (17–118 μmol/l, median 44 μmol/l) (*46*). However, it is difficult to assess the extent to which sub-clinical infections are lowering the plasma vitamin C concentrations seen in such countries.

Data describing a positive association between vitamin C consumption and health status are frequently reported, but intervention studies do not support the observations. Low plasma concentrations are reported in patients with diabetes (*47*) and infections (*48*) and in smokers (*49*), but the relative contribution of diet and stress to these situations is uncertain (see ***Chapter 17***). Epidemiologic studies indicate that diets with a high vitamin C content have been associated with lower cancer risk, especially for cancers of the oral cavity, oesophagus, stomach, colon, and lung (*39, 50-52*). However, there appears to be no effect of consumption of vitamin C supplements on the development of colorectal adenoma and

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stomach cancer (*52-54*), and data on the effect of vitamin C supplementation on coronary heart disease and cataract development are conflicting (*55-74*). Currently there is no consistent evidence from population studies that heart disease, cancers, or cataract development are specifically associated with vitamin C status. This of course does not preclude the possibility that other components in vitamin C – rich fruits and vegetables provide health benefits, but it is not yet possible to separate such an effect from other factors such as lifestyle patterns of people who have a high vitamin C intake.

Dietary sources of vitamin C and limitations to vitamin C

Ascorbate is found in many fruits and vegetables (*75*). Citrus fruits and juices are particularly rich sources of vitamin C but other fruits including cantaloupe, honeydew melon, cherries, kiwi fruits, mangoes, papaya, strawberries, tangelo, watermelon, and tomatoes also contain variable amounts of vitamin C. Vegetables such as cabbage, broccoli, Brussels sprouts, bean sprouts, cauliflower, kale, mustard greens, red and green peppers, peas, tomatoes, and potatoes may be more important sources of vitamin C than fruits. This is particularly true because the vegetable supply often extends for longer periods during the year than does the fruit supply.

In many developing countries, limitations in the supply of vitamin C are often determined by seasonal factors (i.e., the availability of water, time, and labour for the management of household gardens and the short harvesting season of many fruits). For example, mean monthly ascorbate intakes ranged from 0 to 115 mg/day in one Gambian community in which peak intakes coincided with the seasonal duration of the mango crop and to a lesser extent with orange and grapefruit harvests. These fluctuations in dietary ascorbate intake were closely reflected by corresponding variations in plasma ascorbate (11.4–68.4 μmol/L) and human milk ascorbate (143–342 μmol/L) (*76*).

Vitamin C is also very labile, and the loss of vitamin C on boiling milk provides one dramatic example of a cause of infantile scurvy. The vitamin C content of food is strongly influenced by season, transport to market, shelf life, time of storage, cooking practices, and chlorination of water. Cutting or bruising of produce releases ascorbate oxidase. Blanching techniques inactivate the oxidase enzyme and help to preserve ascorbate as also will low pH, as in the preparation of sauerkraut (pickled cabbage). In contrast, heating and exposure to copper or iron or to mildly alkaline conditions destroys the vitamin, and too much water can leach it from the tissues during cooking.

The use of citrus fruits by the British navy in the 18th century gave rise to the term ‘limey’, a colloquial term for British sailors. However, it is important to realise that the amount of vitamin C in a food is usually not the major determinant of a food’s importance for supply, but rather regularity of intake. For example, in countries where the potato is an important staple food and refrigeration facilities are limited, seasonal variations in plasma ascorbate are due to the considerable deterioration in the potato’s vitamin C content during storage; the content can decrease from 30 to 8 mg/100 g over 8–9 months (*77*). Such data can indicate the important contribution the potato can make to human vitamin C requirements even though the potato vitamin C concentration is low.

An extensive study has been made of losses of vitamin C during the packaging, storage, and cooking of blended foods (maize and soya-based relief foods). Data from a US Agency for Internation Development programme show that vitamin C losses from packaging and storage in polythene bags of such relief foods are much less significant than the 52–82 percent losses attributable to conventional cooking procedures (*78*).

Information used to derive dietary requirement of vitamin C

Calculating the dietary intake from the physiologic requirements

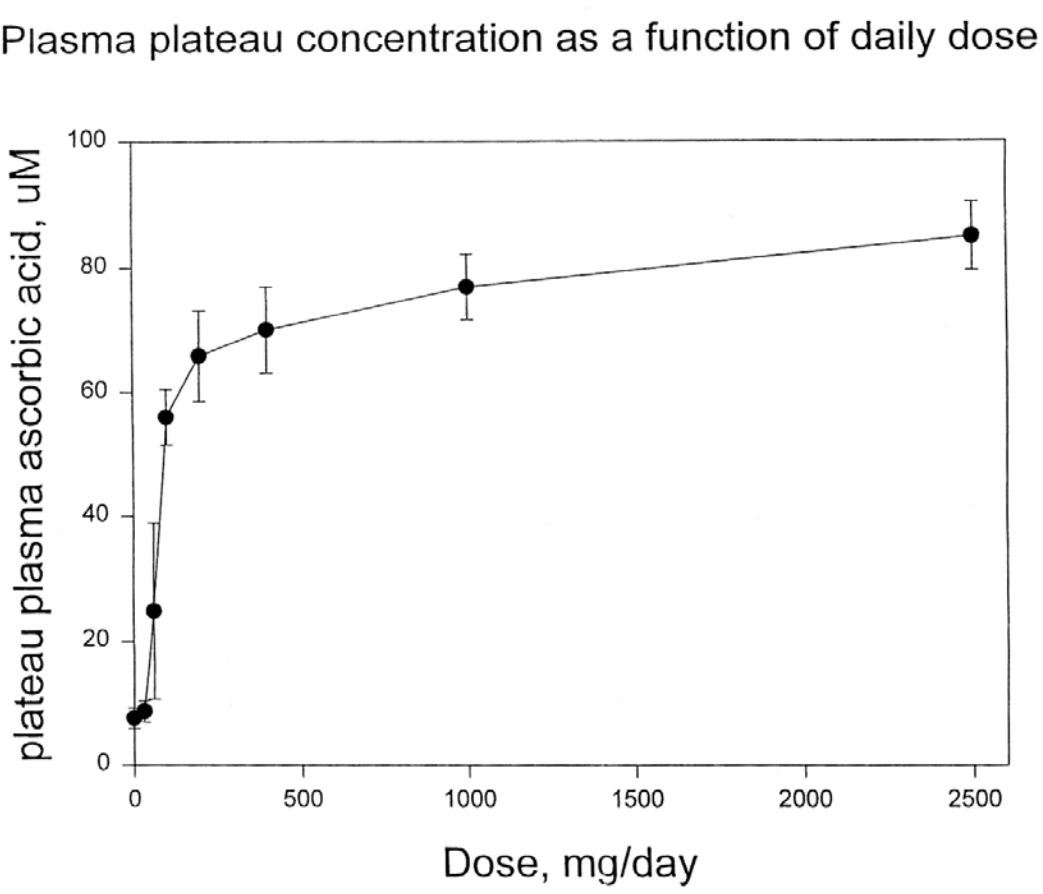
Adults

At saturation the whole body content of ascorbate in adult males is approximately 20 mg/kg, or 1500 mg. Clinical signs of scurvy appear when the whole body content falls below 300–400 mg, and the last signs disappear when the body content reaches about 1000 mg (*28, 30*). In these experiments, ascorbate in the whole body was catabolised at an approximate rate of 3 percent/day (2.9 percent/day, SD 0.6) (*29*).

There is a sigmoidal relationship between intake and plasma concentrations of vitamin C (*79*). Below 30 mg/day, plasma concentrations are around 11 μmol/l. Above this intake, plasma concentrations increase steeply to 60 μmol/l and plateau at around 80 μmol/l, which represents the renal threshold. Under near steady state conditions, plateau concentrations of vitamin C are achieved by intakes in excess of 200 mg/day (***Figure 8***) (*34*). At low doses dietary vitamin C is almost completely absorbed, but over the range of usual dietary intakes (30–180 mg/day), absorption may decrease to 75 percent because of competing factors in the food (35, *80*).

Figure 8

Relationship between intake and plasma concentrations of vitamin C



A body content of 900 mg falls half way between tissue saturation and the point at which clinical signs of scurvy appear. Assuming an absorption efficiency of 85 percent, and a catabolic rate of 2.9, the average intake of vitamin C can be calculated as:

900 x 2.9/100 x 100/85 = 30.7 mg/day, which can be rounded off to 30 mg/day.

The recommended nutrient intake (RNI) would therefore be:

900 x (2.9 + 1.2)/100 x 100/85 = 43.4 mg/day, which can be rounded off to 45 mg/day.

An RNI of 45 mg would achieve 50 percent saturation in the tissues in 97.5 percent of adult males. An intake of 45 mg vitamin C will produce a plasma ascorbate concentrations near the base of the steep slope of the diet-plasma dose response curve (***Figure 8***). No turnover studies have been done in women, but from the smaller body size and whole body content of women, requirements might be expected to be lower. However, in depletion studies plasma concentrations fell more rapidly in women than in men (*81*). It would seem prudent, therefore, to make the same recommendation for non-pregnant, non-lactating women as for men. Thus, an intake of 45 mg/day will ensure that measurable amounts of ascorbate will be present in the plasma of most people and will be available to supply tissue requirements for metabolism or repair at sites of depletion or damage. A whole body content of around 900 mg of vitamin C would provide at least 1 month's safety interval, even for a zero intake, before the body content falls to 300 mg (*82*).

The Sheffield (*27*) and Iowa studies (*28*) indicated that the minimum amount of vitamin C needed to cure scurvy in men was less than 10 mg/day. This level however, is not sufficient to provide measurable amounts of ascorbate in plasma and leukocyte cells, which will remain low. As indicated above, no studies have been done on women and minimum requirements to protect non-pregnant and non-lactating women against scurvy might be slightly lower than in men. Although 10 mg/day will protect against scurvy, this amount provides no safety margin against further losses. The mean requirement is therefore calculated by interpolation between 10 and 45 mg/day, at an intake of 25–30 mg/day.

Pregnancy and lactation

During pregnancy there is a moderate extra drain on vitamin C, particularly during the last trimester, and 8 mg/day of vitamin C is reported to be sufficient to prevent scorbutic signs in infants aged 4–17 months (*83*). Therefore, the additional needs during pregnancy are unlikely to be more, particularly during the last trimester. An extra 10 mg/day throughout pregnancy should enable reserves to accumulate to meet the extra needs of the growing foetus in the last trimester.

During lactation, however, 20 mg/day of vitamin C is secreted in milk. For an assumed absorption efficiency of 85 percent, an extra 25 mg will be needed by the mother. It is therefore recommended that the RNI should be set at 70 mg to fulfil the needs of both the mother and infant during lactation.

Children

As mentioned earlier, 8 mg/day of vitamin C is sufficient to prevent scorbutic signs in infants (*83*). The vitamin C concentration in mature human milk is estimated to be 40 mg/l (mean, SD 10) (*84*), but the amount of vitamin C in human milk appears to reflect maternal dietary intake and not the infants needs (*82, 83*, *85*). RNIs for infants aged 0–6 months are therefore set, somewhat arbitrarily, at 25 mg/day, and the RNI is gradually increased as children got older.

Elderly

Elderly people frequently have low plasma ascorbate values and intakes lower than those in younger people, often because of problems of poor dentition or mobility (*86*). Elderly people are also more likely to have underlying sub-clinical diseases, which can also influence plasma ascorbate concentrations (see ***Chapter 17***). It has been suggested, however, that the requirements of elderly people do not differ substantially from those of younger people in the absence of pathology, which may influence absorption or renal functioning (*82*). The RNIs for the elderly are therefore the same as those for adults (45 mg/day).

Smokers

Kallner *et al.* (*87*) reported that the turnover of vitamin C in smokers was 50 percent greater than that in non-smokers. However, there is no evidence that the health of smokers would be influenced in any way by increasing their RNI. The panel therefore found no justification in making a separate RNI for smokers.

Recommended nutrient intakes for vitamin C

Table 15 presents a summary of the above discussed RNIs for vitamin C by age groups.

Table 15

Recommended nutrient intakes (RNIs) for vitamin C

Group	RNI mg/day [a]
Infants and children	
0–6 months	25
7–12 months	30 [b]
1–3 years	30 [b]
4–6 years	30 [b]
7–9 years	35 [b]
Adolescents, 10–18 years	40 [b]
Adults	
19–65 years	45
65+ years	45
Pregnancy	55
Lactation	70

[a] Amount required to half saturate body tissues with vitamin C in 97.5 percent of the population. Larger amounts may often be required to ensure an adequate absorption of non-haem iron.

[b] Arbitrary values.

Vitamin C toxicity

The potential toxicity of excessive doses of supplemental vitamin C relates to intra-intestinal events and to the effects of metabolites in the urinary system. Intakes of 2–3 g/day of vitamin C produce unpleasant diarrhoea from the osmotic effects of the unabsorbed vitamin in the intestinal lumen in most people (*88*). Gastrointestinal disturbances can occur after ingestion of as little as 1 g because approximately half of the amount would not be absorbed at this dose (*35*).

Oxalate is an end product of ascorbate catabolism and plays an important role in kidney stone formation. Excessive daily amounts of vitamin C produce hyperoxaluria. In four volunteers who received vitamin C in the range of 5–10 g/day, this amounted to

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approximately a doubling of urinary oxalate excretion, from 50 to 87 mg/day (range 60–126 mg/day) (*89*). However, the risk of oxalate stones formation may become significant at high intakes of vitamin C (>1 g) (*90*), particularly in subjects with high amounts of urinary calcium (*89*).

Vitamin C may precipitate haemolysis in some people, including those with glucose-6-phosphate dehydrogenase deficiency (*91*), paroxysmal nocturnal haemaglobinuria (*92*), or other conditions where increased risk of red cell haemolysis may occur or where protection against the removal of the products of iron metabolism may be impaired, as in people with the haptoglobin Hp2-2 phenotype (*93*). Of these conditions, only the haptoglobin Hp 2-2 condition was associated with abnormal vitamin C metabolism (lower plasma ascorbate than expected) under conditions where intake of vitamin C was provided mainly from dietary sources. Therefore, 1 g vitamin C appears to be the advisable upper limit of dietary intake.

Future research

Research is needed to gain a better understanding of the following:

- functions of endogenous gastric ascorbate and its effect on iron absorption;
- functional measurements of vitamin C status which reflect the whole body content of vitamin C and are not influenced by infection; and
- reasons for the vitamin C uptake by granulocytes, which is associated with infection.

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Chapter 7
Vitamin A

Role of vitamin A in human metabolic processes

Vitamin A (retinol) is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function, and reproduction. These dietary needs for vitamin A are normally provided for as preformed retinol (mainly as retinyl ester) and pro-vitamin A carotenoids.

Overview of vitamin A metabolism

Preformed vitamin A in animal foods occurs as retinyl esters of fatty acids in association with membrane-bound cellular lipid and fat-containing storage cells. Pro-vitamin A carotenoids in foods of vegetable origin are also associated with cellular lipids but are embedded in complex cellular structures such as the cellulose-containing matrix of chloroplasts or the pigment-containing portion of chromoplasts. Normal digestive processes free vitamin A and carotenoids from embedding food matrices, a more efficient process from animal than from vegetable tissues. Retinyl esters are hydrolysed and the retinol and freed carotenoids are incorporated into lipid-containing, water-miscible micellar solutions. Products of fat digestion (e.g., fatty acids, monoglycerides, cholesterol, and phospholipids) and secretions in bile (e.g., bile salts and hydrolytic enzymes) are essential for the efficient solubilisation of retinol and especially for solubilisation of the very lipophilic carotenoids (e.g., α- and β-carotene, β-cryptoxanthin, and lycopene) in the aqueous intestinal milieu. Micellar solubilisation is a prerequisite to their efficient passage into the lipid-rich membrane of intestinal mucosal cells (i.e., enterocytes) (*1-3*). Diets critically low in dietary fat (under about 5–10 g daily) (*4*) or disease conditions that interfere with normal digestion and absorption leading to steatorrhea (e.g., pancreatic and liver diseases and frequent gastroenteritis) can therefore impede the efficient absorption of retinol and carotenoids. Retinol and some carotenoids enter the intestinal mucosal brush border by diffusion in accord with the concentration gradient between the micelle and plasma membrane of enterocytes. Some carotenoids pass into the enterocyte and are solubilized into chylomicrons without further change whereas some of the pro-vitamin A carotenoids are converted to retinol by a cleavage enzyme in the brush border (*3*). Retinol is trapped intracellularly by re-esterification or binding to specific intracellular binding proteins. Retinyl esters and unconverted carotenoids together with other lipids are incorporated into chylomicrons, excreted into intestinal lymphatic channels, and delivered to the blood through the thoracic duct (*2*).

Tissues extract most lipids and some carotenoids from circulating chylomicrons, but most retinyl esters are stripped from the chylomicron remnant, hydrolysed, and taken up primarily by parenchymal liver cells. If not immediately needed, retinol is re-esterified and retained in the fat-storing cells of the liver (variously called adipocytes, stellate cells, or Ito cells). The liver parenchymal cells also take in substantial amounts of carotenoids. Whereas most of the body's vitamin A reserve remains in the liver, carotenoids are also deposited elsewhere in fatty tissues throughout the body (*1*). Usually, turnover of carotenoids in tissues is relatively slow, but in times of low dietary carotenoid intake, stored carotenoids are mobilised. A recent study in one subject using stable isotopes suggests that retinol can be

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derived not only from conversion of dietary pro-vitamin carotenoids in enterocytes – the major site of bioconversion – but also from hepatic conversion of circulating pro-vitamin carotenoids (*5*). The quantitative contribution to vitamin A requirements of carotenoid converted to retinoids beyond the enterocyte is unknown.

Following hydrolysis of stored retinyl esters, retinol combines with a plasma-specific transport protein, retinol-binding protein (RBP). This process, including synthesis of the unoccupied RBP (apo-RBP), occurs to the greatest extent within liver cells but it may also occur in some peripheral tissues. The RBP-retinol complex (holo-RBP) is secreted into the blood where it associates with another hepatically synthesised and excreted larger protein, transthyretin. The transthyretin-RBP-retinol complex circulates in the blood, delivering the lipophilic retinol to tissues; its large size prevents its loss through kidney filtration (*1*). Dietary restriction in energy, proteins, and some micronutrients can limit hepatic synthesis of proteins specific to mobilisation and transport of vitamin A. Altered kidney functions or fever associated with infections (e.g., respiratory infections [*6*] or diarrhoea [*7*]) can increase urinary vitamin A loss.

Holo-RBP transiently associates with target-tissue membranes, and specific intracellular binding proteins then extract the retinol. Some of the transiently sequestered retinol is released into the blood unchanged and is recycled (i.e., conserved) (*1,8*). A limited reserve of intracellular retinyl esters is formed, that subsequently can provide functionally active retinol and its oxidation products (i.e., isomers of retinoic acid) as needed intracellularly. These biologically active forms of vitamin A are associated with specific cellular proteins which bind with retinoids within cells during metabolism and with nuclear receptors that mediate retinoid action on the genome (*9*). Retinoids modulate the transcription of several hundreds of genes (*10-12*). In addition to the latter role of retinoic acid, retinol is the form required for functions in the visual (*13*) and reproductive systems (*14*) and during embryonic development (*15*).

Holo-RBP is filtered into the glomerulus but recovered from the kidney tubule and recycled. Normally vitamin A leaves the body in urine only as inactive metabolites which result from tissue utilisation and as potentially recyclable active glucuronide conjugates of retinol in bile secretions (*8*). No single urinary metabolite has been identified which accurately reflects tissue levels of vitamin A or its rate of utilisation. Hence, at this time urine is not a useful biologic fluid for assessment of vitamin A nutriture.

Biochemical mechanisms for vitamin A functions

Vitamin A functions at two levels in the body. The first is in the visual cycle in the retina of the eye; the second is in all body tissues systemically to maintain growth and the soundness of cells. In the visual system, carrier-bound retinol is transported to ocular tissue and to the retina by intracellular binding and transport proteins. Rhodopsin, the visual pigment critical to dim-light vision, is formed in rod cells after conversion of all-*trans* retinol to retinaldehyde, isomerization to the 11-*cis*-form, and binding to opsin. Alteration of rhodopsin through a cascade of photochemical reactions results in ability to see objects in dim light (*13*). The speed at which rhodopsin is regenerated relates to the availability of retinol. Night blindness is usually an indicator of inadequate available retinol, but it can also be due to a deficit of other nutrients, which are critical to the regeneration of rhodopsin, such as protein and zinc, and to some inherited diseases, such as retinitis pigmentosa.

The growth and differentiation of epithelial cells throughout the body are especially affected by vitamin A deficiency (VAD). Goblet cell numbers are reduced in epithelial tissues. The consequence is that mucous secretions with their antimicrobial components diminish. Cells lining protective tissue surfaces fail to regenerate and differentiate, hence

flatten and accumulate keratin. Both factors – the decline in mucous secretions and loss of cellular integrity – diminish resistance to invasion by potentially pathogenic organisms. The immune system is also compromised by direct interference with production of some types of protective secretions and cells (*11*). Classical symptoms of xerosis (drying or nonwetability) and desquamation of dead surface cells as seen in ocular tissue (i.e., xerophthalmia) are the external evidence of the changes also occurring to various degrees in internal epithelial tissues.

Current understanding of the mechanism of vitamin A action within cells outside the visual cycle is that cellular functions are mediated through specific nuclear receptors. These receptors are activated by binding with specific isomers of retinoic acid (i.e., all-*trans* and 9-*cis* retinoic acid). Activated receptors bind to DNA response elements located upstream of specific genes to regulate the level of expression of those genes (*12*). The synthesis of a large number of proteins vital to maintaining normal physiologic functions is regulated by these retinoid-activated genes. There also may be other mechanisms of action that are as yet undiscovered (*10*).

Population at risk and consequences of vitamin A deficiency

Definition of vitamin A deficiency

VAD is not simply defined. WHO defines it as tissue concentrations of vitamin A low enough to have adverse health consequences even if there is no evidence of clinical xerophthalmia (*16*). In addition to the specific signs and symptoms of xerophthalmia and the risk of irreversible blindness, non-specific symptoms include increased morbidity and mortality, poor reproductive health, increased risk of anaemia, and contributions to slowed growth and development. Because these non-specific adverse consequences may occur from other nutrient deficits as well, it is difficult to attribute non-ocular symptoms specifically to VAD in the absence of biochemical measurements reflective of vitamin A status.

Geographic distribution and magnitude

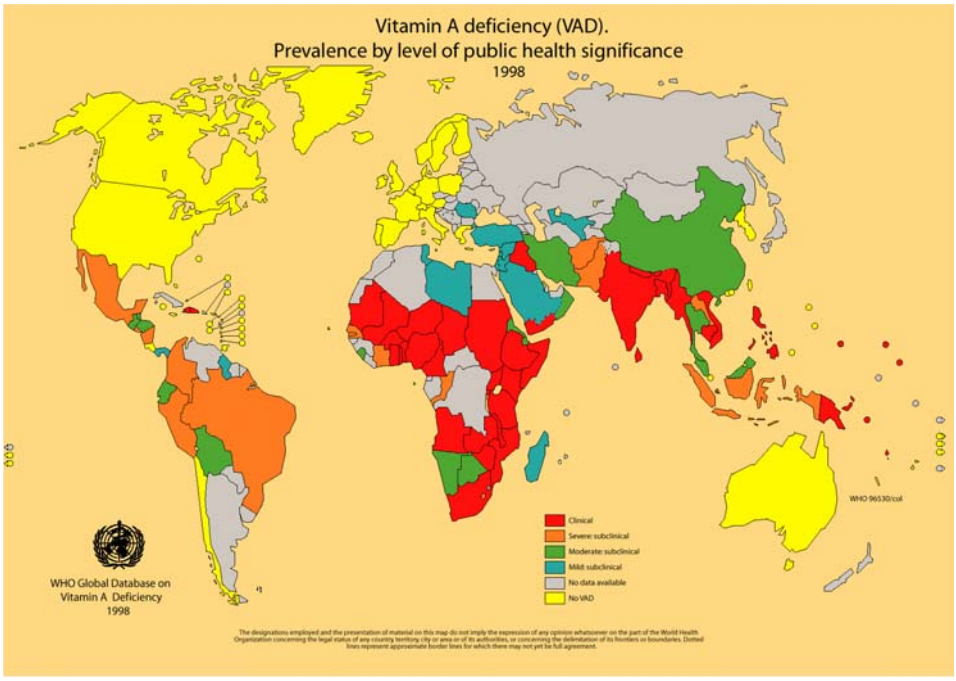
In 1996 WHO mapped the global distribution of VAD (***Figure 9***) and categorised countries by degree of significance as a public health problem on the basis of both clinical and moderate and severe sub-clinical (prevalence of low blood levels of retinol) indicators of deficiency (*16,18*). In the early 1990s, WHO estimated that about 3 million children had some form of xerophthalmia annually and, on the basis of blood levels, another 250 million were sub-clinically deficient (*18*). The magnitude of the sub-clinical estimate is currently being re-evaluated to quantitatively establish a benchmark for measuring prevalence trends. The actual number of sub-clinical deficiencies based on the prevalence of low serum levels of retinol, however, remains uncertain because of the confounding and poorly quantitative role of infections (see later discussion).

Epidemiologic studies repeatedly report clustering of VAD, presumably resulting from concurrent occurrence of several risk factors. This clustering may occur among both neighbourhoods and households (*19*).

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Figure 9

Prevalence of VAD in the world (17)



Age and gender

VAD can occur in individuals of any age. However, it is a disabling and potentially fatal public health problem for children under 6 years of age. VAD-related blindness is most prevalent in children under 3 years of age (*20*). This period is characterised by high requirements for vitamin A to support early rapid growth, the transition from breast-feeding to dependence on other dietary sources of the vitamin, and increased frequency of respiratory and gastrointestinal infections. The increased mortality risk from concurrent infections extends at least to 6 years of age and is associated with both clinical and sub-clinical VAD (*21*). There is little information regarding the health consequences of VAD in school-age children. The prevalence of Bitot's spots (i.e., white foamy patches on the conjunctiva) may be highest in this age group but their occurrence may reflect past more than current history of VAD (*22*). Women of reproductive age are thought also to be vulnerable to VAD during pregnancy and lactation because they often report night blindness (*23,24*) and because their breast-milk is frequently low in vitamin A (*25,26*). Not all night blindness in pregnant women, however, responds to vitamin A (*24*).

There is no consistent, clear indication in humans of a gender differential in vitamin A requirements during childhood. Growth rates and presumably the need for vitamin A from birth to 10 years for boys are consistently higher than those for girls (*27*). In the context of varied cultural and community settings, however, variations in gender-specific child-feeding and care practices are likely to subsume a small gender differential in requirements to account for reported gender differences and prevalence of xerophthalmia. Pregnant and lactating women require additional vitamin A to support maternal and foetal tissue growth and lactation losses, additional vitamin A which is not needed by other post-adolescent adults (*28*).

Risk factors

VAD is most common in populations consuming most of their vitamin A needs from pro-vitamin carotenoid sources and where minimal dietary fat is available (*29*). About 90 percent of ingested preformed vitamin A is absorbed, whereas the absorption efficiency of pro-vitamin A carotenoids varies widely depending on the type of plant source and the fat content of the accompanying meal (*30*). Where it is possible to increase dietary fat, this will likely improve the absorption of vitamin A activity from the diet.

In areas with endemic VAD, fluctuations in the incidence of VAD throughout the year reflect the balance between intake and need. Periods of general food shortage (and specific shortages in vitamin A–rich foods), peak incidence of common childhood infectious diseases (diarrhoea, respiratory infections, and measles), and periodic seasonal growth spurts affect the balance. Seasonal food availability can influence VAD prevalence by directly influencing access to pro-vitamin A sources; for example, the scarcity of mangoes in hot arid months followed by the glutting of the market with mangoes during harvest seasons (*31*). Seasonal growth spurts in children frequently follow seasonal postharvest increases in energy and macronutrient intakes. These increases are usually obtained from staple grains (e.g., rice) and tubers (e.g., light-coloured yams) which are not good sources of some micronutrients (e.g., vitamin A to support the growth spurt) (*32*).

Food habits and taboos often restrict consumption of potentially good food sources of vitamin A (e.g., mangoes and green leafy vegetables). Culture-specific factors for feeding children, adolescents, and pregnant and lactating women are common (*29,33-35*). Illness- and childbirth-related proscription of the use of specific foods pervade in many traditional cultures (*36*). Such influences alter short- and long-term food distribution within families. However, some cultural practices can be protective of vitamin A status and they need to be identified and reinforced.

Morbidity and mortality

The consequences of VAD are manifested differently in different tissues. In the eye, the symptoms and signs, together referred to as xerophthalmia, have a long, well-recognised history and have until recently been the basis for estimating the global burden from the disease (*20*). Although ocular symptoms and signs are the most specific indicators of VAD, they occur only after other tissues have impaired functions that are less specific and less easily assessed.

The prevalence of ocular manifestations (i.e., xerophthalmia or clinical VAD) is now recognised to far underestimate the magnitude of the problem of functionally significant VAD. Many more preschool-age children and perhaps older children and women who are pregnant or lactating have their health compromised when they are sub-clinically deficient. In young children, sub-clinical deficiency, like clinical deficiency, increases the severity of some infections, particularly diarrhoea and measles, and the risk of dying (*21,37*). The incidence (*38*) and prevalence (*39*) of diarrhoea may also increase with sub-clinical VAD. Meta-analyses conducted by three independent groups using data from several randomised trials provide convincing evidence that community-based improvement of the vitamin A status of deficient children 6 months to 6 years of age reduces their risk of dying by 20–30 percent on average (*21,40,41*). Mortality in children who are blind from keratomalacia or who have corneal disease is reported to be from 50 percent to 90 percent (*20,42*) and measles mortality associated with VAD is increased up to 50 percent (*43*). Limited data are available from controlled studies of the possible link between morbidity history and vitamin A status of pregnant and lactating women (*44*).

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There are discrepancies in the linkage between incidence and severity of infectious morbidity of various aetiologies and vitamin A status. The weight of evidence supports an association of VAD with severity of an infection once acquired, except for respiratory diseases, which are nonresponsive (*16,37-39,45*). The severity of the pneumonia associated with measles, however, is an exception because it decreases with treatment with vitamin A supplements (*43,46*).

Infectious diseases depress circulating retinol and contribute to vitamin A depletion. Enteric infections may alter absorptive-surface area, compete for absorption-binding sites, and increase urinary loss (*7,47,48*). Febrile systemic infections also increase urinary loss (*6,49*) and metabolic utilisation rates and may reduce apparent retinol stores if fever occurs frequently (*50*). In the presence of latent deficiency, disease occurrence is often associated with precipitating ocular signs (*51,52*). Measles virus infection is especially devastating to vitamin A metabolism, adversely interfering with both efficiencies of utilisation and conservation (*43,52,53*). Severe protein-energy malnutrition affects many aspects of vitamin A metabolism, and even when some retinyl ester stores are still present, malnutrition – often coupled with infection – can prevent transport-protein synthesis, that results in immobilisation of existing vitamin A stores (*54*).

The compromised integrity of the epithelium, together with the possible alteration in hormonal balance at severe levels of deficiency, impairs normal reproductive functions in animals (*9,14,15,25,55,56*). Controlled human studies are, of course, lacking. In animals and Humans, congenital anomalies can result if the foetus is exposed to severe deficiency or large excesses of vitamin A at critical periods early in gestation (first trimester) when foetal organs are being formed (*25,57*). Reproductive performance measured by infant outcomes in one community-based clinical intervention trial, however, was not influenced by vitamin A status (*44*).

The growth of children may be impaired by VAD. Interventions with vitamin A only have not consistently demonstrated improved growth in community studies because VAD seldom occurs in isolation of other nutrient deficiencies that also affect growth and may be more limiting (*58*).

A lack of vitamin A can affect iron metabolism when deficiencies of both nutrients coexist and particularly in environments that favour frequent infections (*59*). Maximum haemoglobin response occurs when iron and vitamin A deficiencies are corrected together (*60*). VAD appears to influence the availability of storage iron for use by haematopoietic tissue (*60,61*). However, additional research is needed to clarify the mechanisms of the apparent interaction.

Units of expression

In blood, tissues, and human milk, vitamin A levels are conventionally expressed in µg/dL or µmol/l of all-*trans* retinol. Except for postprandial conditions, most of the circulating vitamin A is retinol whereas in most tissues (such as the liver), secretions (such as human milk), and other animal food sources it exists mainly as retinyl esters, that are usually hydrolysed before analytical detection.

To express the vitamin A activity of carotenoids in diets on a common basis, a joint FAO/WHO Expert Group (*62*) in 1967 introduced the concept of the retinol equivalent (RE) and established the following relationships among food sources of vitamin A:

1 µg retinol	=	1 RE
1 µg β-carotene	=	0.167 µg RE
1 µg other pro-vitamin A carotenoids	=	0.084 µg RE

These equivalencies were derived from balance studies to account for the less-efficient absorption of carotenoids (thought to be about one-third that of retinol) and their bioconversion to vitamin A (one-half for β-carotene and one-fourth for other pro-vitamin carotenoids). It was recognised at the time that the recommended conversion factors (i.e., 1:6 for vitamin A:β-carotene and 1:12 for vitamin A: all other pro-vitamin carotenoids) were only average estimates for a mixed diet. Recently there has been renewed interest in examining bio-availability factors by using more quantitative stable isotope techniques for measuring whole-body stores in response to controlled intakes (*63-65*) and by following postabsorption carotenoids in the triacylglycerol-rich lipoprotein fraction (*66-68*). The data are inconsistent but in general suggests that revision toward lower bio-availability estimates is likely. Until additional definitive data are available, however, the above conversion factors will be used *(67, 68)*.

Retinol equivalents in a diet are calculated as the sum of the weight of the retinol portion of preformed vitamin A with the weight of β-carotene divided by its conversion factor and with the weight of other carotenoids divided by their conversion factor (*69*). Most recent food composition tables report β-carotene and sometimes other pro-vitamin A carotenoids as µg/g edible portion. However, older food composition tables frequently report vitamin A as international units (IUs). The following applies to determining comparable values as µg:

1 IU retinol	=	0.3 µg retinol
1 IU β-carotene	=	0.6 µg β-carotene
1 IU retinol	=	3 IU β-carotene

It is strongly recommended that weight or molar units replace the use of IU to decrease confusion and overcome limitations in the non-equivalence of the IU values for retinol and beta-carotene.

Dietary sources

Preformed vitamin A is found almost exclusively in animal products, such as human milk, glandular meats, liver and fish liver oils (especially), egg yolk, and whole milk and dairy products. Preformed vitamin A is also used to fortify processed foods, that may include sugar, cereals, condiments, fats, and oils (*70*). Pro-vitamin A carotenoids are found in green leafy vegetables (e.g., spinach, amaranth, and young leaves from various sources), yellow vegetables (e.g., pumpkins, squash, and carrots), and yellow and orange noncitrus fruits (e.g., mangoes, apricots, and papaya). Red palm oil produced in several countries worldwide is especially rich in pro-vitamin A (*71*). Some other indigenous plants also may be unusually rich sources of pro-vitamin A. Such examples are the palm fruit known in Brazil as *burití*, that is found in areas along the Amazon (as well as elsewhere in Latin America) (*72*), and the fruit known as *gac* in Vietnam, that is used to colour rice, particularly on ceremonial occasions (*73*). Foods containing pro-vitamin A carotenoids tend to be less biologically available but more affordable than animal products. It is mainly for this reason that carotenoids provide most of the vitamin A activity in the diets of economically deprived populations.

Dietary intake and patterns

Vitamin A status cannot be assessed from dietary intake alone, but dietary intake assessment can provide evidence of risk of an inadequate status. Quantitative collection of dietary information is fraught with measurement problems. These problems arise both from obtaining representative quantitative dietary histories from individuals, communities, or both and from interpreting these data while accounting for differences in bio-availability, preparation losses, and variations in food composition data among population groups (*70*). This is especially

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difficult in populations consuming most of their dietary vitamin A from pro-vitamin carotenoid sources. Simplified guidelines have been developed recently in an effort to improve the obtaining of reliable dietary intake information from individuals and communities (*68*,*74*).

World and regional supply and patterns

In theory the world's food supply is sufficient to meet global requirements. Great differences exist, however, in the available sources (animal and vegetable) and in per capita consumption of the vitamin among different countries, age categories, and socio-economic groups. VAD as a global public health problem, therefore, is largely due to inequitable food distribution among and within countries and households in relation to need for ample bio-available vitamin A sources (*75,76*).

Earlier FAO global estimates in 1984 indicated that preformed vitamin A constituted about one-third of total dietary vitamin A activity (*69*). World availability of vitamin A for human consumption at that time was approximately 220 μg of preformed retinol per capita daily and 560 μg RE from pro-vitamin carotenoids (about 3400 μg carotenoids for a 6:1 conversion factor) per person per day, for a total of about 790 μg RE. These values are based on supply estimates and not consumption estimates. Losses commonly occur during food storage and processing, both industrially and in the home (*70*).

The estimated available regional supply of vitamin A from a more recent global evaluation (*76*) shown in ***Table 16*** illustrates the variability in amounts and sources of vitamin A. The variability is further complicated by access to the available supply, that varies with household income, poverty being a yardstick for risk of VAD. VAD is most prevalent in Southeast Asia, Africa, and the Western Pacific, where vegetable sources contribute nearly 80 percent or more of the available supply of retinol equivalents. Furthermore, in Southeast Asia the total available supply is about half of that of most other regions and is particularly low in animal sources. In contrast, the Americas, Europe, and Eastern Mediterranean regions have a supply ranging from 800 to 1000 μg RE/day, one-third of which comes from animal sources. Recent national data from the USA Continuing Survey of Food Consumption (*77*) and the National Health and Nutrition Examination Survey (*78*) included mean dietary intakes of children 0–6 years of age of 864 ± 497 and 921 ± 444 μg RE daily. In the Dietary and Nutritional Survey of British Adults (*79*), the median intake of men and women 35-49 years old was 1118 μg RE and 926 μg RE, respectively, which corresponded to serum retinol concentrations of 2.3 μmol/l and 1.8 μmol/l, respectively. In another selected survey in the United Kingdom, median intakes for nonpregnant women who did not consume liver or liver products during the survey week were reported to be 686 μg RE daily (*80*).

The available world supply figures in ***Table 16*** were recently reassessed based on a bio-availability ratio of 1:30 for retinol to other pro-vitamin A carotenoids (*81*). This conversion factor was justified on the basis of one published controlled intervention study conducted in Indonesia (*82*) and a limited number of other studies not yet published in full. Applying the unconfirmed conversion factor to the values in ***Table 16*** would lead to the conclusion that regional and country needs for vitamin A could not be met from predominantly vegetarian diets. This is inconsistent with the preponderance of epidemiologic evidence. Most studies report a positive response when vegetable sources of pro-vitamin A are given under controlled conditions to deficient subjects freed of confounding parasite loads and provided with sufficient dietary fat (*83,84*). Emerging data are likely to justify a lower biologic activity for pro-vitamin A carotenoids because of the mix of total carotenoids found in food sources in a usual meal (*67,68*). This Consultation concluded that the 1:6

bioconversion factor originally derived on the basis of balance studies should be retained until there is firm confirmation from ongoing studies that use more precise methodologies.

Table 16

Available supply of vitamin A by WHO region

Region	Total μg RE/day	Animal sources μg RE/day	Vegetable sources μg RE/day
Africa	775	122	654 (84)[a]
Americas	814	295	519 (64)
Southeast Asia	431	53	378 (90)
Europe	738	271	467 (63)
Eastern Mediterranean	936	345	591 (63)
Western Pacific	997	216	781 (78)
Total	782	212	565 (72)

[a]Numbers in parentheses indicate the percent of total retinol equivalents from carotenoid food sources.
Source: ACC/SCN, 1993 (*76*).

Evidence for making recommendations

Indicators of vitamin A deficiency

Ocular signs of VAD are measured by clinical examination and history and are quite specific in preschool-age children. However, these are rare occurrences that require examination of large populations to obtain incidence and prevalence data. Sub-clinical VAD is more prevalent, requiring smaller sample sizes to obtain valid prevalence estimates (*16*).

A full description of clinical indicators with coloured illustrations for each is found in the WHO Field Guide (*20*). The most frequently occurring is night blindness, which is the earliest manifestation of xerophthalmia. In mild form it is generally noticeable after stress from a bright light that bleaches the rhodopsin (visual purple) found in the retina. VAD prolongs the time to regenerate rhodopsin, thus delays adaptation time in dark environments. Night-blind young children tend to stumble when going from bright to dimly lighted areas and they, as well as night-blind mothers, tend to remain inactive at dusk and at night (*85*). No field-applicable objective tool is currently available for measuring night blindness in children under about 3 years of age. It can be measured by history in certain cultures (*86*). When night blindness is prevalent, many cultures coin a word descriptive of the characteristic symptom that they can reliably recall on questioning, making this a useful tool for assessing the prevalence of VAD (*87*). Questioning for night blindness is not consistently a reliable assessment measure where a local term is absent. In addition, there is no clearly defined blood retinol level that is directly associated with occurrence of the symptom. Vitamin A – related night blindness, however, responds rapidly, usually within 1–2 days, to administration of vitamin A.

Sub-clinical vitamin A deficiency

Direct measurement of concentrations of vitamin A in the liver where it is stored or in the total body pool relative to known specific vitamin A–related functions (e.g., night blindness) would be the indicator of choice for determining requirements. This cannot be done with the methodology now available for population use. There are several practical biochemical methods for estimating sub-clinical vitamin A status but all have limitations (*16,86,88,89*). Each method is useful to identify deficient populations, but no one of these indicators is definitive or directly related quantitatively to disease occurrence. The indicators of choice are

listed in ***Table 17***. These indicators are less specific to VAD than clinical eye signs and less sensitive for measuring sub-clinical vitamin A status. WHO recommends that where feasible at least two sub-clinical biochemical indicators, or one biochemical and a composite of non-biochemical risk factors, should be measured and that both types of indicators should point to deficiency in order to identify populations at high risk of VAD (*16*). Cut-off points given in ***Table 17*** represent the consensus gained from practical experience comparing populations with some evidence of VAD with those without VAD. There are no field studies that quantitatively relate the prevalence of adverse health symptoms (e.g., incidence or prevalence of severe diarrheal disease) and relative levels of biologic indicator cut-off values. Furthermore, each of the biochemical indicators listed is subject to confounding factors, which may be unrelated to vitamin A status (e.g., infections).

Table 17

Biochemical indicators of sub-clinical VAD in mothers and in children 6–71 months of age

Indicator	Cut-off to indicate deficiency
Night blindness (24–71 mo)	≥ 1% report a history of night blindness
Biochemical	
Breast milk retinol	≤1.05 μmol/l (≤8 μg/g milk fat)
Serum retinol	≤0.70 μmol/l
Relative dose response	≥20%
Modified relative dose response	ratio ≥0.06

Source: Adapted from World Health Organization, Geneva, 1996 (*16*).

Although all biochemical indicators currently available have limitations, the biochemical indicator of choice for population assessment is the distribution of serum levels of vitamin A (serum retinol). Only at very low blood levels (<0.35 μmol/l) is there an association with corneal disease prevalence (*90*). Blood levels between 0.35 and 0.70 μmol/l are likely to characterise sub-clinical deficiency (*91*), but sub-clinical deficiency may still be present at levels between 0.70 and 1.05 μmol/l and occasionally above 1.05 μmol/l (*92*). The prevalence of values below 0.70 μmol/l is a generally accepted population cut-off for preschool-age children to indicate risk of inadequate vitamin A status (*16*) and above 1.05 μ mol/l to indicate an adequate status (*93,94*). As noted elsewhere, clinical and sub-clinical infections can lower serum levels of vitamin A on average as much as 25 percent independently of vitamin A intake (*95,96*). Therefore, at levels between about 0.5 and 1.05 μ mol/l, the relative dose response or modified relative dose response test on a subsample of the population can be useful for identifying the prevalence of critically depleted body stores when interpreting the left portion of serum retinol distribution curves.

Determining mean requirements and setting safe levels of intake

Requirement and safe level of intake for vitamin A in this report do not differ significantly from those of the 1988 FAO/WHO Expert Consultation (*69*) except for adapting to the age categories defined in this consultation and during pregnancy. The term safe level of intake used in the 1988 report is retained in this report because the levels in ***Table 18*** do not strictly correspond to the definition of a recommended nutrient intake.

The mean requirement for an individual is defined as the minimum daily intake of vitamin A as presented in μg retinol equivalents (μg RE) to prevent xerophthalmia in the absence of clinical or sub-clinical infection. This intake should account for proportionate bio-availability of preformed vitamin (about 90 percent) and pro-vitamin A carotenoids from a diet that contains sufficient fat (e.g., at least 5–10 g). Bio-availability of carotenoids varies widely by source (e.g., fibrous green leafy vegetables or soft-tissue fruits). The required level of intake is set to prevent clinical signs of deficiency, allow for normal growth, and reduce the risk of vitamin A – related severe morbidity and mortality on a population basis. It does not allow for frequent or prolonged periods of infections or other stresses.

The safe level of intake for an individual is defined as the average continuing intake of vitamin A required to permit adequate growth and other vitamin A–dependent functions and to maintain an acceptable total body reserve of the vitamin. This reserve helps offset periods of low intake or increased need resulting from infections and other stresses. Useful indicators include a plasma retinol concentration above 0.70 μmol/l, that is associated with a relative dose response below 20 percent, or a modified relative dose response below 0.06. For lactating women, breast-milk retinol levels above 1.05 μmol/l (or above 8 μg/g milk fat) are considered to reflect minimal maternal stores because levels above 1.75 μmol/l are common to populations known to be healthy and without evidence of insufficient dietary vitamin A (*25,26*).

Infants and children

Vitamin A requirements for infants are calculated from the vitamin A provided in human milk. During at least the first 6 months of life, exclusive breast-feeding can provide sufficient vitamin A to maintain health, permit normal growth, and maintain sufficient stores in the liver (*97*).

Reported retinol concentrations in human milk varies widely from country to country (0.70–2.45 μmol/l). In some developing countries the vitamin A intake of breast-fed infants who grow well and do not show signs of deficiency is from 120 to 170 μg RE/day (*26,97*). Such intakes are considered adequate to cover infant requirements if the infant's weight is assumed to be at least at the 10th percentile according to WHO standards (*69*). However, this intake is unlikely to build adequate body stores because xerophthalmia is common in preschool-age children in the same communities with somewhat lower intakes. Because of the need for vitamin A to support the growth rate of infancy, which can vary considerably, a requirement estimate of 180 μg RE/day seems appropriate.

The safe level for infants up to 6 months of age is based on observations of breast-fed infants in communities in which good nutrition is the norm. Average consumption of human milk by such infants is about 750 ml/day during the first 6 months (*97*). Assuming an average concentration of vitamin A in human milk of about 1.75 μmol/l, the mean daily intake would be about 375 μg RE, which is therefore the recommended safe level. From 7-12 months, human milk intake averages 650 ml, which would provide 325 μg vitamin A daily. Because breast-fed infants in endemic vitamin A–deficient populations are at increased risk of death

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from 6 months onward, the requirement and recommended safe intake are increased to 190 μg and 400 μg, respectively.

The requirement (with allowance for variability) and the recommended intake for older children may be estimated from those derived for late infancy (i.e., 20 and 39 μg RE/kg body weight/day) (*69*). On this basis, including allowances for storage requirements and variability, requirements for preschool children would be in the range of 200–400 μg RE daily. In poor communities where children 1–6 years old are reported to have intakes of about 100–200 μg RE/day, signs of VAD do occur; in southern India these signs were relieved and risk of mortality was reduced when the equivalent of 350–400 μg RE/day was given to children weekly (*98*). In the United States most preschool-age children maintain serum retinol levels of 0.70 μmol/l or higher while consuming diets providing 300–400 μg RE/day (from the databank for the third National Health and Nutrition Examination Survey).

Adults

Estimates for the requirements and recommended safe intakes for adults are also estimated from those derived for late infancy, i.e. 4.8 and 9.3 μg RE/kg body weight/day (*69*). Detailed accounts for arriving at the requirement for vitamin A is provided in the FAO/WHO report of 1988 (*69*) and will not be repeated here because there are no new published studies to indicate a need to revise the assumptions on which the calculations were based. The safe intakes recommended are consistent with the per capita vitamin A content in the food supply of countries that show adequate vitamin A status in all sectors of the population. Additional evidence that the existing safe level of intake is adequate for adults on a population basis is provided by an analysis of dietary data from the 1990 survey of British adults in whom there was no evidence of VAD (*79*). The median intake for another survey of nonpregnant UK women who did not consume liver or liver products during the survey week was 686 μg/day (80). This value is substantially above the estimated mean requirement for pregnant women and falls quite short of the amount in which teratology risk is reported (*99-101*). About one-third of the calculated retinol equivalents consumed by the British women came from pro-vitamin A sources (20 percent from carrots).

Pregnancy

During pregnancy additional vitamin A is needed for the growth and maintenance of the foetus for providing a limited reserve in the foetal liver and for maternal tissue growth. There are no reliable figures available for the specific vitamin A requirements for these processes (*28*).

Newborn infants need around 100 μg retinol daily to meet their needs for growth. During the third trimester the foetus grows rapidly and, although obviously smaller in size than the infant born full term, the foetus presumably has similar needs. Incremental maternal needs associated with pregnancy are assumed to be provided from maternal reserves in populations of adequately nourished healthy mothers. In populations consuming at the basal requirement, an increment of 100 μg/day during the full gestation period should enhance maternal storage during early pregnancy and allow adequate amounts of vitamin A for the rapidly growing foetus in late pregnancy. However, this increment may be minimal for women who normally ingest only the basal requirement level of vitamin A inasmuch as the needs and growth rate of the foetus will not be affected by the mother's initial vitamin A reserves.

A recent study in Nepal (*44*), where night blindness is prevalent in pregnant women, provided 7000 μg RE (about 23,300 IU) weekly to pregnant and lactating women (equivalent to 1000 μg RE/day). This level of intake normalised serum levels of vitamin A and was

associated with a decrease in prevalence of night blindness and a decrease in maternal mortality. The findings of this study need to be confirmed. In the interim period, however, it seems prudent, recognising that a large portion of the world's population of pregnant women lives under conditions of deprivation, to increase by 200 μg the recommended safe level to ensure adequacy of intake during pregnancy. Because therapeutic levels of vitamin A are generally higher than preventive levels, the safe intake level recommended during pregnancy is 800 μg RE/day. Women who are or who might become pregnant should carefully limit their total daily vitamin A intake to a maximum of 3000 μg RE (10 000 IU) to minimise risk of foetal toxicity (*102*).

Lactation

If the amounts of vitamin A recommended for infants are supplied by human milk, mothers should absorb at least as much in their diets to replace maternal losses. Thus, the increments in basal and recommended intakes during lactation are 180 μg RE and 350 μg RE, respectively, for the safe recommended intake per day. After the infant reaches the age of 6 months or when solid foods are introduced, the mother's need for additional amounts of vitamin A lessens.

Elderly

There is no indication that the vitamin A requirements of healthy elderly individuals differs from those of other adults. It should be remembered, however, that diseases that impede vitamin A absorption, storage, and transport might be more common in the elderly than in other age groups.

Recommended safe intakes

Table 18 provides the estimated mean requirements for vitamin A and the recommended safe intakes, taking into account the age and gender differences in mean body weights. For most values the true mean and variance are not known. Values in the table have been rounded. It should be noted that there are no adequate data available to derive mean requirements for any group and, therefore, a recommended nutrient intake cannot be calculated. However, information is available on cures achieved in a few vitamin A–deficient adult men and on the vitamin A status of groups receiving intakes that are low but nevertheless adequate to prevent the appearance of deficiency-related syndromes. The figures for mean dietary requirements are derived from these, with the understanding that the curative dose is higher than the preventive dose. They are at the upper limits of the range so as to cover the mean dietary requirements of 97.5 percent of the population (*69*).

In calculating the safe intake, a normative storage requirement was calculated as a mean for adults equivalent to 434 μg RE/day, and the recommended safe intake was derived in part by using this value + 2 standard deviations. It is doubtful that this value can be applied to deal with growing children. The safe intake for children was compared with the distribution of intakes and comparable serum vitamin A levels reported for children 0–6 years of age from the United States and with distributions of serum levels of vitamin A of children 9–62 months in Australia (*103*), where evidence of VAD is rare.

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Table 18

Estimated mean requirement and safe level of intake for vitamin A

Age group	Mean requirement μg RE/day	Recommended safe intake μg RE/day
Infants and children		
0–6 months	180	375
7–12 months	190	400
1–3 years	200	400
4–6 years	200	450
7–years	250	500
Adolescents, 10–18 years	330–400	600
Adults		
Females,19–65 years	270	500
Males, 19–65 years	300	600
65+	300	600
Pregnant women	370	800
Lactating women	450	850

Source: Adapted from FAO/WHO, Rome 1988 (*69*).

Toxicity

Because vitamin A is fat soluble and can be stored, primarily in the liver, routine consumption of large amounts of vitamin A over a period of time can result in toxic symptoms, including liver damage, bone abnormalities and joint pain, alopecia, headaches and vomiting, and skin desquamation. Hypervitaminosis A appears to be due to abnormal transport and distribution of vitamin A and retinoids caused by overloading of the plasma transport mechanisms *(104)*.

The smallest daily supplement associated with liver cirrhosis that has been reported is 7500 μg taken for 6 years (*100,101*). Very high single doses can also cause transient acute toxic symptoms that may include bulging fontanels in infants; headaches in older children and adults; and vomiting, diarrhoea, loss of appetite, and irritability in all age groups. Rarely does toxicity occur from ingestion of food sources of preformed vitamin A. When this occurs, it usually results from very frequent consumption of liver products. Toxicity from food sources of pro-vitamin A carotenoids is not reported except for the cosmetic yellowing of skin.

Infants, including newborns (*105*), administered single doses equivalent to 15 000–30 000 μg retinol (50 000–100 000 IU) in oil generally show no adverse symptoms. However, daily prophylactic or therapeutic doses should not exceed 900 μg, that is well above the mean requirement of about 200 μg daily for infants. An excess of bulging fontanels occurred in infants under 6 months of age in one endemically deficient population given two or more doses of 7500 μg or 15 000 μg preformed vitamin A in oil (*106,107*), but other large-scale controlled clinical trials have not reported excess bulging after three doses of 7500 μg given with diptheria-pertussis-tetanus immunisations at about 6, 10, and 14 weeks of age (*108*). No effects were detected at 3 years of age that related to transient vitamin A–induced bulging that had occurred before 6 months of age (*105,109*).

Most children 1–6 years of age tolerate single oral doses of 60 000 μg (200 000 IU) vitamin A in oil at intervals of 4–6 months without adverse symptoms (*100*). Occasionally diarrhoea or vomiting is reported but is transient with no lasting sequelae. Older children seldom experience toxic symptoms unless they habitually ingest vitamin A in excess of 7500 μg (25 000 IU) for prolonged periods of time (*100*).

When taken by women at early stages of gestation at daily levels of more than 7500 μg (25 000 IU), foetal anomalies and poor reproductive outcomes are reported (*101*). One report suggests an increased risk of teratogenicity at intakes as low as 3000 μg (10 000 IU) but this is not confirmed by other studies (*102*). Women who are pregnant or might become pregnant should avoid taking excessive amounts of vitamin A. A careful review of the latest available information by a WHO Expert Group recommended that daily intakes in excess of 3000 μg (10 000 IU) or weekly intakes in excess of 7500 μg (25 000 IU), should not be taken at any period during gestation (*102*). High doses of vitamin A (60 000 μg, or 200 000 IU) can be safely given to breast-feeding mothers for up to 2 months postpartum and for 6 weeks for women who are not breast-feeding.

Future research

Further research is needed:

- on the interaction of vitamin A and iron with infections, as they relate to serum levels and disease incidence and prevalence;
- on the relation among vitamin A, iron, and zinc and their role in the severity of infections;
- on the nutritional role of 9-*cis* retinoic acid and the mechanism which regulates its endogenous production;
- on the bio-availability of pro-vitamin A carotenoids from different classes of leafy and other green and orange vegetables, tubers, and fruits as typically provided in diets (e.g., relative to level of fat in the diet or meal); and
- to identify a reliable indicator of vitamin A status for use in direct quantification of mean requirements and for relating status to functions.

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Chapter 8
Vitamin D

Summary of the role of vitamin D in human metabolic processes

Vitamin D is required to maintain normal blood levels of calcium and phosphate, that are in turn needed for the normal mineralisation of bone, muscle contraction, nerve conduction, and general cellular function in all cells of the body. Vitamin D achieves this after its conversion to the active form 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], or calcitriol. This active form regulates the transcription of a number of vitamin D–dependent genes coding for calcium-transporting proteins and bone matrix proteins.

Vitamin D also modulates the transcription of cell cycle proteins, that decrease cell proliferation and increase cell differentiation of a number of specialised cells of the body (e.g., osteoclastic precursors, enterocytes, keratinocytes, etc.). This property may explain the actions of vitamin D in bone resorption, intestinal calcium transport, and skin. Vitamin D also possesses immuno-modulatory properties that may alter responses to infections *in vivo*. The cell differentiating and immuno-modulatory properties underlie the reason why vitamin D derivatives are now used successfully in the treatment of psoriasis and other skin disorders.

Clinical assays measure 1,25-$(OH)_2D_2$ and 1,25-$(OH)_2D_3$, collectively called 1,25-$(OH)_2$D. Similarly, calcidiol is measured as 25-OH-D but it is a mixture of 25-OH-D_2 and 25-OH-D_3. For the purposes of this document, 1,25-$(OH)_2$D and 25-OH-D will be used to refer to calcitriol and calcidiol, respectively.

Overview of the role of vitamin D

Vitamin D, a seco-steroid, can either be made in the skin from a cholesterol-like precursor (7-dehydrocholesterol) by exposure to sunlight or can be provided pre-formed in the diet (*1*). The version made in the skin is referred to as vitamin D_3 whereas the dietary form can be vitamin D_3 or a closely related molecule of plant origin known as vitamin D_2 . Because vitamin D can be made in the skin, it should not strictly be called a vitamin, and some nutritional texts refer to the substance as a prohormone and to the two forms as cholecalciferol (D_3) or ergocalciferol (D_2).

From a nutritional perspective, the two forms are metabolised similarly in humans, are equal in potency, and can be considered equivalent. It is now firmly established that vitamin D_3 is metabolised first in the liver to 25-hydroxyvitamin-D (25-OH-D or calcidiol) (*2*) and subsequently in the kidneys to 1,25-$(OH)_2$D (*3*) to produce a biologically active hormone. The 1,25-$(OH)_2$D, like all vitamin D metabolites, is present in the blood complexed to vitamin D binding protein, a specific α-globulin. The 1,25-$(OH)_2$D is believed to act on target cells similarly to the way a steroid hormone would act. Free hormone crosses the plasma membrane and interacts with a specific nuclear receptor known as the vitamin D receptor, a DNA-binding, zinc-finger protein with a molecular weight of 55,000 (*4*). This ligand-receptor complex binds to a specific vitamin D–responsive element and, with associated transcription factors (e.g., retinoid X receptor), enhances transcription of mRNAs which code for calcium-transporting proteins, bone matrix proteins, or cell cycle–regulating proteins (*5*). As a result of these processes ,1,25-$(OH)_2$D stimulates intestinal absorption of calcium and phosphate and mobilises calcium and phosphate by stimulating bone resorption (*6*). These functions serve

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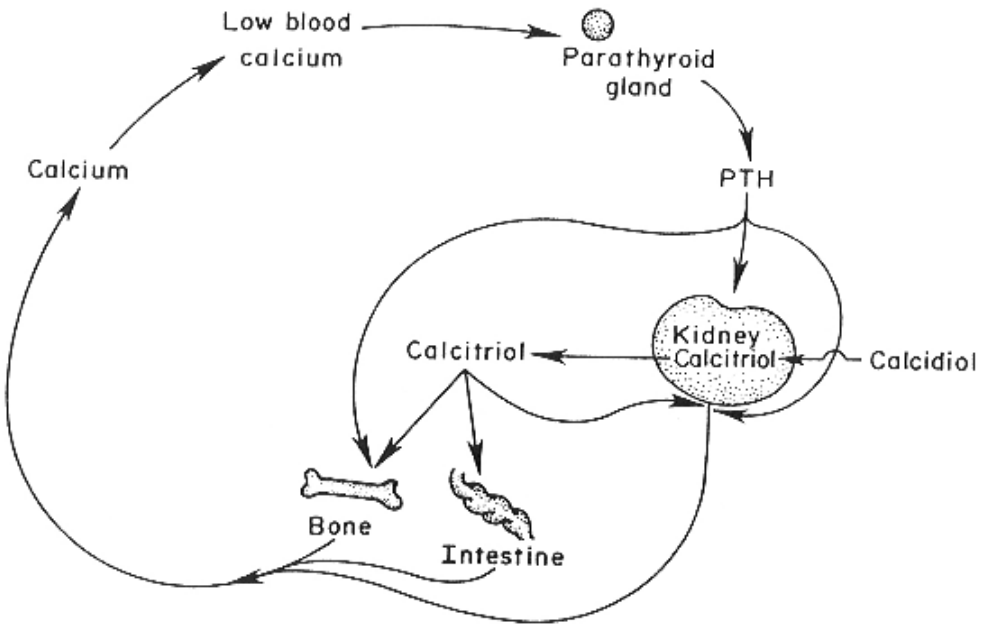
the common purpose of restoring blood levels of calcium and phosphate to normal when concentrations of the two ions are low.

Lately, interest has focused on other cellular actions of 1,25-$(OH)_2$D. With the discovery of 1,25-$(OH)_2$D receptors in many classical non-target tissues such as brain, various bone marrow–derived cells, skin, thymus, etc. (*7*), the view has been expressed that 1,25-$(OH)_2$D induces fusion and differentiation of macrophages (*8, 9*). This effect has been widely interpreted to mean that the natural role of 1,25-$(OH)_2$D is to induce osteoclastogenesis from colony forming units–granulatory monocytes in the bone marrow. The 1,25-$(OH)_2$D also suppresses interleukin 2 production in activated T lymphocytes (*10, 11*), an effect which suggests the hormone might play a role in immuno-modulation *in vivo*. Other tissues (e.g., skin) are directly affected by exogenous administration of vitamin D, though the physiologic significance of these effects is poorly understood. The pharmacologic effects of 1,25-$(OH)_2$D are profound and have resulted in the development of vitamin D analogues, that are approved for use in hyper-proliferative conditions such as psoriasis (*12*).

In calcium homeostasis 1,25-$(OH)_2$D works in conjunction with parathyroid hormone (PTH) to produce its beneficial effects on the plasma levels of ionised calcium and phosphate (*5, 13*). The physiologic loop (***Figure 10***) starts with calcium sensing by the calcium receptor of the parathyroid gland (*14*). When the level of ionised calcium in plasma falls, PTH is secreted by the parathyroid gland and stimulates the tightly regulated renal enzyme 25-OH-D-1-α-hydroxylase to make more 1,25-$(OH)_2$D from the large circulating pool of 25-OH-D. The resulting increase in 1,25-$(OH)_2$D (with the rise in PTH) causes an increase in calcium transport within the intestine, bone, and kidney. All these events raise plasma calcium levels back to normal, that in turn is sensed by the calcium receptor of the parathyroid gland. The further secretion of PTH is turned off not only by the feedback action of calcium, but also by a short feedback loop involving 1,25-$(OH)_2$D directly suppressing PTH synthesis in the parathyroid gland (not shown in figure).

Figure 10

Calcium homeostasis



Source: Adapted from Jones *et al*. (*13*).

Although this model oversimplifies the events involved in calcium homeostasis it is easy to see from it that sufficient 25-OH-D must be available to provide adequate 1,25-$(OH)_2$D synthesis and hence an adequate level of plasma calcium and that vitamin D deficiency will result in inadequate 25-OH-D and 1,25-$(OH)_2$D synthesis, inadequate calcium homeostasis, and a constantly elevated PTH level (termed: secondary hyperparathyroidism).

It becomes evident from this method of presentation of the role of vitamin D that the nutritionist can focus on the plasma levels of 25-OH-D and PTH to gain an insight into vitamin D status. Not shown but also important is the endpoint of the physiologic action of vitamin D, namely adequate plasma calcium and phosphate ions, that provide the raw materials for bone mineralisation.

Populations at risk for vitamin D deficiency

Infants

Infants constitute a population at risk for vitamin D deficiency because of relatively large vitamin D needs brought about by their high rate of skeletal growth. At birth, infants have acquired *in utero* the vitamin D stores that must carry them through the first months of life. A recent survey of French neonates revealed that 64 percent had 25-OH-D values below 30 nmol/l, the lower limit of the normal range (*15*). Breast-fed infants are particularly at risk because of the low concentrations of vitamin D in human milk (*16*). This problem is further compounded in some infants fed human milk by a restriction in exposure to ultraviolet (UV) light for seasonal, latitudinal, cultural, or social reasons. Infants born in the autumn months at extremes of latitude are particularly at risk because they spend the first 6 months of their life indoors and therefore have little opportunity to synthesise vitamin D in their skin during this period. Consequently, although vitamin D deficiency is rare in developed countries, sporadic cases of rickets are still being reported in many northern cities but almost always in infants fed human milk (*17-20*).

Infant formulas are supplemented with vitamin D at levels ranging from 40 international units (IUs) or 1 µg /418.4 kJ to 100 IU or 2.5 µg /418.4 kJ, that provide approximately between 6 µg and 15 µg of vitamin D, respectively. These amounts of dietary vitamin D are sufficient to prevent rickets.

Adolescents

Another period of rapid growth of the skeleton occurs at puberty and increases the need not for the vitamin D itself, but for the active form 1,25-$(OH)_2$D. This need results from the increased conversion of 25-OH-D to 1,25-$(OH)_2$D in adolescents (*21*). Furthermore, unlike infants, adolescents are usually outdoors and therefore usually are exposed to UV light sufficient for synthesising vitamin D for their needs. Excess production of vitamin D in the summer and early fall months is stored mainly in the adipose tissue (*22*) and is available to sustain high growth rates in the winter months that follow. Insufficient vitamin D stores during these periods of increased growth can lead to vitamin D insufficiency (*23*).

Elderly

Over the past 20 years, clinical research studies of the basic biochemical machinery handling vitamin D have suggested an age-related decline in many key steps of vitamin D action (*24*) including rate of skin synthesis, rate of hydroxylation leading to activation to the hormonal form, and response of target tissues (e.g., bone) as well as reduced skin exposure (*25*). Not surprisingly a number of independent studies from around the world have shown that there appears to be vitamin D deficiency in a subset of the elderly population, as characterised by low blood levels of 25-OH-D coupled with elevations of plasma PTH and alkaline

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phosphatase (*26*). There is evidence that this vitamin D deficiency contributes to declining bone mass and increases the incidence of hip fractures (*27*). Although some of these studies may exaggerate the extent of the problem by focusing on institutionalised individuals or in-patients with decreased sun exposures, in general they have forced health professionals to re-address the intakes of this segment of society and look at potential solutions to correct the problem. Several groups have found that modest increases in vitamin D intakes (between 10 and 20 μg/day) reduce the rate of bone loss and the fracture rate (*25-29*).

These findings have led agencies and researchers to suggest an increase in recommended vitamin D intakes for the elderly from the suggested 2.5–5 μg /day to a value that is able to maintain normal 25-OH-D levels in the elderly, such as 10–15 μg/day. This vitamin D intake results in lower rates of bone loss and is suggested for the middle-aged (50–70 years) and old-aged (>70 years) populations (*33*). The increased requirements are justified mainly on the grounds of the reduction in skin synthesis of vitamin D, a linear reduction occurring in both men and women, that begins with the thinning of the skin at age 20 years (*24*).

Pregnancy and lactation

Elucidation of the changes in calciotropic hormones occurring during pregnancy and lactation has revealed a role for vitamin D in the former but probably not the latter. Even in pregnancy, the changes in vitamin D metabolism which occur, namely an increase in the maternal plasma levels of 1,25-$(OH)_2$D (*34*) due to a putative placental synthesis of the hormone (*35*), do not seem to impinge greatly on the maternal vitamin D requirements. The concern that modest vitamin D supplementation might be deleterious to the foetus is not justified. Furthermore, because transfer of vitamin D from mother to foetus is important for establishing the newborn's growth rate, the goal of ensuring adequate vitamin D status with conventional prenatal vitamin D supplements probably should not be discouraged.

In lactating women there appears to be no direct role for vitamin D because increased calcium needs are regulated by PTH-related peptide (*36, 37*), and recent studies have failed to show any change in vitamin D metabolites during lactation (*38, 39*). As stated above, the vitamin D content of human milk is low (*16*). Consequently, there is no great drain on maternal vitamin D reserves either to regulate calcium homeostasis or to supply the need of human milk. Because human milk is a poor source of vitamin D, rare cases of nutritional rickets are still found, but these are almost always in breast-fed babies deprived of sunlight exposure (*17-20*). Furthermore, there is little evidence that increasing calcium or vitamin D supplements to lactating mothers results in an increased transfer of calcium or vitamin D in milk (*38*). Thus, the current thinking, based on a clearer understanding of the role of vitamin D in lactation, is that there is little purpose in recommending additional vitamin D for lactating women. The goal for mothers who breast-feed their infants seems to be merely to ensure good nutrition and sunshine exposure in order to ensure normal vitamin D status during the perinatal period.

Table 19

Randomised, controlled trials with dietary vitamin D supplements

Reference	Study Group	n [b]	Age (years) Mean	Age (years) SD	Regimen	Duration (years)	Results[a]
Dawson-Hughes *et al.*, 1991 (*28*)	Healthy, post-menopausal women living independently	249	62	0.5	10 µg vitamin D + 400 mg calcium	1.0	Reduced late wintertime bone loss from vertebrae; net spine BMD; no change in whole-body BMD.
Chapuy *et al.,* 1992 (*29*)	Healthy, elderly women living in nursing homes or in apartments for the elderly	3270	84	6	20 µg vitamin D + 1200 mg calcium	1.5	Hip fractures 43% ; non-vertebral fractures 32%; in subset (*n*=56), BMD of proximal femur 2.7% in vitamin D group and 4.6% in placebo group.
Chapuy *et al.,* 1994 (*30*) [c]						3.0	Hip fractures 29%; non-vertebral fractures 24%.
Dawson-Hughes *et al.,* 1995 (*31*)	Healthy post-menopausal women living independently	261	64	5	2.5 µg or 17.5 µg vitamin D + 500 mg calcium	2.0	Loss of BMD from femoral neck lower in 17.5µg group (-1.06%) than in 2.5 µg group (-2.54%); no difference in BMD at spine.
Lips *et al.,* 1996 (*32*)	Healthy elderly living independently, in nursing homes, or in apartments for the elderly	2578 (1916-Fem.) (662-Male)	80	6	10 µg vitamin D		No difference in fracture incidence; in subset (*n*=248) of women from nursing homes, BMD 2.3% after 2 years.

[a] ↑ Increase; ↓ decrease.
[b] Number of subjects enrolled in the study.
[c] Same study as Chapuy *et al.* (*29*) after further 1.5 years of treatment.
Source: Adapted with permission from Shearer (*25*).

Evidence used for estimating recommended vitamin D intake

Lack of accuracy in estimating dietary intake and skin synthesis

The unique problem of estimating total intake of a substance that can be provided in the diet or made in the skin by exposure to sunlight makes it difficult to estimate adequate total intakes of vitamin D for the general population. Accurate food composition data are not available for vitamin D, accentuating the difficulty for estimating dietary intakes. Whereas this has led two recent US national surveys to avoid attempting this task, the second National Health and Nutrition Examination Survey (NHANES II) estimated vitamin D intakes to be 2.9 µg/day and 2.3 µg/day for younger and older women, respectively. A recent study of elderly women by Kinyamu *et al.* (*40*) concurred with this assessment, finding an intake of 3.53 µ g/day.

Skin synthesis is equally difficult to estimate, being affected by such imponderables as age, season, latitude, time of day, skin exposure, sun screen use, etc. In vitamin D – replete individuals, estimates of skin synthesis are put at around 10 µg /day (*24, 41*), with total intakes estimated at 15 µg/day (*24*).

Use of plasma 25-OH-D as a measure of vitamin D status

Numerous recent studies have used plasma 25-OH-D as a measure of vitamin D status, and there is a strong presumptive relationship of this variable with bone status. Thus, it is not surprising that several nutritional committees (e.g., the Food and Nutrition Board of the US National Academy of Sciences' Institute of Medicine in conjunction with Health Canada) have chosen to use a biochemical basis for estimating required intakes and used these estimates to derive recommended intakes (*33*). The method used involved the estimation of the mean group dietary intake of vitamin D required to maintain the plasma 25-OH-D levels above 27 nmol/l, that is necessary to ensure normal bone health. Previously, many studies had established 27 nmol/l as the lower limit of the normal range (e.g., NHANES III [*41*]). This dietary intake of vitamin D for each population group was rounded to the nearest 50 IU (1.25 µg) and then doubled to cover the needs of all individuals within that group irrespective of sunlight exposure. They termed this amount *adequate intake* (AI) and used it in place of recommended dietary allowance (RDA), that had been used by US agencies since 1941. We are recommending the use of those figures here as recommended nutrient intakes (RNIs) because it is an entirely logical approach to estimating the vitamin D needs for the whole population.

Table 20

Preliminary unweighted results from NHANES III data[a]

Percentile	25-OH-D[b] ng/ml
1st	7.6
5th	10.9
10th	13.2
50th	24.4
90th	40.1
95th	45.9
99th	59.0

[a]Total number of samples used in data analysis: 18,323; mean 25-OH-D value for United States: 25.89 ng/ml (+/- 11.08). Frequency distribution of serum or plasma 25-OH-D. Values for all ages, ethnicity groups, both sexes.[b]High values: four values 90-98 ng/ml, one value of 160.3 ng/ml. Values < 5 ng/ml (lowest standard) entered arbitrarily in the database as "3". Source: NHANES III (*42*).

Because study after study had been recommending increases in vitamin D intakes for the elderly, it might have been expected that the proposed increases in suggested intakes from 5 µg/day (RDAs in the United States [*43*], RNIs in Canada [*44*]) to 10 µg/day or 15 µg/day (AI) would be welcomed. However, a recent editorial in a prominent medical journal attacked the recommendations as being too conservative (*45*). This came on the heels of an article in the same journal (*46*) reporting the level of hypovitaminosis D to be as high as 57 percent in a population of ageing (mean 62 years) medical in-patients in the Boston area.

Of course, such in-patients are by definition sick and should not be used to calculate normal intakes. Indeed, the new NHANES III study (*42)* of 18 323 normal individuals from all regions of the United States suggests that approximately 5 percent had values of 25-OH-D below 27 nmol/l (*47*) (***Table 20***). Although the data are skewed by sampling biases that favour sample collection in the southern states in winter months and northern states in the summer months, even subsets of data collected in northern states in September give the incidence of low 25-OH-D in the elderly in the 6–18 percent range (*47*) as compared with 57 percent in the institutionalized in-patient population (*43*). Ideally, such measurements of the normal population should be made at the end of the winter months and before UV irradiation has reached a strength sufficient to allow skin synthesis of vitamin D. Thus, the NHANES III study may still underestimate the incidence of hypovitaminosis D in a northern elderly population in winter. Nevertheless, in lieu of additional studies of selected human populations, it would seem that the recommendations of the Food and Nutrition Board are reasonable guidelines for vitamin D intakes, at least for the near future. This considered approach allows for a period of time to monitor the potential shortfalls of the new recommendations as well as to assess whether the suggested guidelines can be attained, a point that was repeatedly stated about the RDAs.

Considerations in viewing recommended intakes for vitamin D

In recommending intakes for vitamin D, it must be recognised that in most locations in the world in a broad band around the equator (between latitudes 42°N and 42°S), the most physiologically relevant and efficient way of acquiring vitamin D is to synthesise it endogenously in the skin from 7-dehydrocholesterol by sun (UV) light exposure. In most situations, approximately 30 minutes of skin exposure (without sunscreen) of the arms and face to sunlight can provide all the daily vitamin D needs of the body (*24*). However, skin synthesis of vitamin D is negatively influenced by factors which may reduce the ability of the skin to provide the total needs of the individual (*24*):

- latitude and season – both influence the amount of UV light reaching the skin;
- the ageing process – thinning of the skin reduces the efficiency of this synthetic process;
- skin pigmentation: the presence in the skin of darker pigments interferes with UV light reaching the appropriate layer of the skin;
- clothing – virtually complete covering of the skin for medical, social, cultural, or religious reasons leaves insufficient skin exposed to sunlight; and
- sunscreen use – widespread and liberal use of sun-blockers reduces skin damage by the sun but also deleteriously affects synthesis of vitamin D.

Because not all of these problems can be solved in all geographic locations, particularly during winter at latitudes higher than 42° where synthesis is virtually zero, it is

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recommended that individuals not synthesising vitamin D should correct their vitamin D status by consuming the amounts of vitamin D appropriate for their age group (***Table 21***).

Summary of the RNIs for vitamin D by age group

Table 21

RNIs for vitamin D according to age groups

Age group	RNI µg/day
Infants	
0–6 months	5
7–12 months	5
1–3 years	5
4–6 years	5
7–9 years	5
Adolescents, 10–18 years	5
Adults	
19–50 years	5
Older adults, 51–65 years	10
Elderly adults, 65+ years	15
Pregnant women	5
Lactating women	5

[a]Units: for vitamin D, 1 IU = 25 ng, 40 IU = 1 µg, 200 IU = 5 µg, 400 IU = 10 µg, 600 IU = 15 µg, 800 IU = 20 µg; for 25-OH-D, 1 ng/ml = 2.5 nmol/l, 10 ng/ml = 25 nmol/l, 11 ng/ml = 28.5 nmol/l (low limit), 30 ng/ml = 75 nmol/l (normal), 60 ng/ml = 150 nmol/l (upper limit).

Vitamin D toxicity

The adverse effects of high vitamin D intakes – hypercalciuria and hypercalcemia – do not occur at these new recommended intake levels. In fact, it is worth noting that the recommended intakes for all age groups are still well below the lowest observed adverse effect level of 50 µg/day and have not yet even reached the no observed adverse effect level of 20 µg/day (*33, 48*). Outbreaks of idiopathic infantile hypercalcemia in the United Kingdom in the post–World War II era led to the withdrawal of vitamin D fortification from all foods in that country because of concerns that they were due to hypervitaminosis D. There are some suggestions in the literature that these outbreaks of idiopathic infantile hypercalcemia may have been multifactorial with genetic and dietary components and were not just due to technical problems with over-fortification as was assumed (*49,50*). In retrospect, the termination of the vitamin D fortification may have been counter productive because it exposed segments of the UK community to vitamin deficiency and may have discouraged other nations from starting vitamin D fortification programmes (*50*). This is all the more cause for concern because hypovitaminosis D is still a problem worldwide, particularly in developing countries at high latitudes and in countries where skin exposure to sunlight is discouraged (*51*).

Future research

Further research is needed to determine:

- whether vitamin D supplements during pregnancy have any positive effects later in life;
- whether vitamin D has a role in lactation;
- the long-term effects of higher vitamin D intakes;
- whether dietary vitamin D supplements are as good as exposure to UV light; and
- whether vitamin D is only needed for regulation of calcium and phosphate.

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Chapter 9
Vitamin E

Summary of the role of vitamin E in human metabolic processes

A large body of scientific evidence indicates that reactive free radicals are involved in many diseases, including heart disease and cancers (*1*). Cells contain many potentially oxidizable substrates such as polyunsaturated fatty acids (PUFAs), proteins, and DNA. Therefore, a complex antioxidant defence system normally protects cells from the injurious effects of endogenously produced free radicals as well as from species of exogenous origin such as cigarette smoke and pollutants. Should our exposure to free radicals exceed the protective capacity of the antioxidant defence system, a phenomenon often referred to as oxidative stress (*2*), then damage to biologic molecules may occur. There is considerable evidence that disease causes an increase in oxidative stress; therefore, consumption of foods rich in antioxidants, which are potentially able to quench or neutralise excess radicals, may play an important role in modifying the development of such diseases.

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defence system and is exclusively obtained from the diet. The term "vitamin E" refers to a family of eight naturally occurring homologues that are synthesised by plants from homogentisic acid. All are derivatives of 6-chromanol and differ in the number and position of methyl groups on the ring structure. The four tocopherol homologues (*d*-α-, *d*-β-, *d*-γ-, and *d*-δ-) have a saturated 16-carbon phytyl side chain, whereas the tocotrienols (*d*-α-, *d*-β-, *d*-γ-, and *d*-δ-) have three double bonds on the side chain. There is also a widely available synthetic form, *dl*-α-tocopherol, prepared by coupling trimethylhydroquinone with isophytol. This consists of a mixture of eight stereoisomers in approximately equal amounts; these isomers are differentiated by rotations of the phytyl chain in various directions that do not occur naturally. For dietary purposes, vitamin E activity is expressed as α-tocopherol equivalents (α-TEs). One α-TE is the activity of 1 mg *RRR*-α-tocopherol (*d*-α-tocopherol). To estimate the α-TE of mixed diet containing natural forms of vitamin E, the number of milligrams of β-tocopherol should be multiplied by 0.5, γ-tocopherol by 0.1, and α-tocotrienol by 0.3. Any of the synthetic all-*rac*-α-tocopherol (*dl*-α-tocopherol) should be multiplied by 0.74. One milligram of the latter compound in the acetate form is equivalent to 1 IU of vitamin E.

Vitamin E is an example of a phenolic antioxidant. Such molecules readily donate the hydrogen from the hydroxyl (-OH) group on the ring structure to free radicals, which then become unreactive. On donating the hydrogen, the phenolic compound itself becomes a relatively unreactive free radical because the unpaired electron on the oxygen atom is usually delocalised into the aromatic ring structure thereby increasing its stability (*3*).

The major biologic role of vitamin E is to protect PUFAs and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals. Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation, a series of chemical reactions involving the oxidative deterioration of PUFAs. Elevated levels of lipid peroxidation products are associated with numerous diseases and clinical conditions (*4*). Although vitamin E is primarily located in cell and organelle membranes where it can exert its maximum protective effect, its concentration may only be one molecule for every 2000 phospholipid molecules.

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This suggests that after its reaction with free radicals it is rapidly regenerated, possibly by other antioxidants (5).

Absorption of vitamin E from the intestine depends on adequate pancreatic function, biliary secretion, and micelle formation. Conditions for absorption are like those for dietary lipid, that is, efficient emulsification, solubilisation within mixed bile salt micelles, uptake by enterocytes, and secretion into the circulation via the lymphatic system (*6*). Emulsification takes place initially in the stomach and then in the small intestine in the presence of pancreatic and biliary secretions. The resulting mixed micelle aggregates the vitamin E molecules, solubilises the vitamin E, and then transports it to the brush border membrane of the enterocyte probably by passive diffusion. Within the enterocyte, tocopherol is incorporated into chylomicrons and secreted into the intracellular space and lymphatic system and subsequently into the blood stream. Tocopherol esters, present in processed foods and vitamin supplements, must be hydrolysed in the small intestine before absorption.

Vitamin E is transported in the blood by the plasma lipoproteins and erythrocytes. Chylomicrons carry tocopherol from the enterocyte to the liver, where they are incorporated into parenchymal cells as chylomicron remnants. The catabolism of chylomicrons takes place in the systemic circulation through the action of cellular lipoprotein lipase. During this process tocopherol can be transferred to high-density lipoproteins (HDLs). The tocopherol in HDLs can transfer to other circulating lipoproteins, such as LDLs and very low-density lipoproteins (VLDLs) (*7*). During the conversion of VLDL to LDL in the circulation, some α-tocopherol remains within the core lipids and thus is incorporated in LDL. Most α-tocopherol then enters the cells of peripheral tissues within the intact lipoprotein through the LDL receptor pathway, although some may be taken up by membrane binding sites recognising apolipoprotein A-I and A-II present on HDL (*8*).

Although the process of absorption of all the tocopherol homologues in our diet is similar, the α form predominates in blood and tissue. This is due to the action of binding proteins that preferentially select the α form over the others. In the first instance, a 30-kDa binding protein unique to the liver cytoplasm preferentially incorporates α-tocopherol in the nascent VLDL (*9*). This form also accumulates in non-hepatic tissues, particularly at sites where free radical production is greatest, such as in the membranes of mitochondria and endoplasmic reticulum in the heart and lungs (*10*).

Hepatic intracellular transport may be expedited by a 14.2-kDa binding protein that binds α-tocopherol in preference to the other homologues (*11*). Other proteinaceous sites with apparent tocopherol-binding abilities have been found on erythrocytes, adrenal membranes, and smooth muscle cells (*12*). These may serve as vitamin E receptors which orient the molecule within the membrane for optimum antioxidant function.

These selective mechanisms explain why vitamin E homologues have markedly differing antioxidant abilities in biologic systems and illustrates the important distinction between the *in vitro* antioxidant effectiveness of a substance in the stabilisation of, for example, a food product and its *in vivo* potency as an antioxidant. From a nutritional perspective, the most important form of vitamin E is α-tocopherol; this is corroborated in animal model tests of biopotency which assess the ability of the various homologues to prevent foetal absorption and muscular dystrophies (***Table 22***).

Plasma vitamin E concentrations vary little over a wide range of dietary intakes. Even daily supplements of the order of 1600 IU/day for 3 weeks only increased plasma levels 2–3 times and on cessation of treatment plasma levels returned to pretreatment levels in 5 days (*13*). Likewise, tissue concentrations only increased by a similar amount when patients undergoing heart surgery were given 300 mg/day of the natural stereoisomer for 2 weeks

preoperatively (*14*). Kinetic studies with deuterated tocopherol (*15*) suggest that there is rapid equilibration of new tocopherol in erythrocytes, liver, and spleen but that turnover in other tissues such as heart, muscle, and adipose tissue is much slower. The brain is markedly resistant to depletion and repletion with vitamin E (*16*). This presumably reflects an adaptive mechanism to avoid detrimental oxidative reactions in this key organ.

The primary oxidation product of α-tocopherol is a tocopheryl quinone that can be conjugated to yield the glucuronate after prior reduction to the hydroquinone. This is excreted in the bile or further degraded in the kidneys to α-tocopheronic acid and hence excreted in the bile. Those vitamin E homologues not preferentially selected by the hepatic binding proteins are eliminated during the process of nascent VLDL secretion in the liver and probably excreted via the bile (*17*). Some vitamin E may also be excreted via skin sebaceous glands (*18*).

Table 22

Approximate biological activity of naturally occurring tocopherols and tocotrienols compared with *d*-α-tocopherol

Common name	Biological activity compared with *d*-α-tocopherol, %
d-α-tocopherol	100
d-β-tocopherol	50
d-γ-tocopherol	10
d-δ-tocopherol	3
d-α-tocotrienol	30
d-β-tocotrienol	5
d-γ-tocotrienol	not known
d-δ-tocotrienol	not known

Defining populations at risk of vitamin E deficiency

There are many signs of vitamin E deficiency in animals most of which are related to damage to cell membranes and leakage of cell contents to external fluids. Disorders provoked, for example, by traces of peroxidized PUFAs in the diets of animals with low vitamin E status are cardiac or skeletal myopathies, neuropathies, and liver necrosis (*19*) (***Table 23***). Muscle and neurological problems are also a consequence of human vitamin E deficiency (*20*). Early diagnostic signs of deficiency include leakage of muscle enzymes such as creatine kinase and pyruvate kinase into plasma, increased levels of lipid peroxidation products in plasma, and increased erythrocyte haemolysis.

The assessment of the vitamin E requirement for humans is confounded by the infrequent occurrence of clinical signs of deficiency because these usually only develop in adults with fat-malabsorption syndromes or liver disease, in individuals with genetic anomalies in transport or binding proteins, and possibly in premature infants (*19, 21*). This suggests that diets contain sufficient vitamin E to satisfy nutritional needs.

Several animal models (*22*) suggest that increasing intakes of vitamin E inhibit the progression of vascular disease by preventing the oxidation of LDL. Evidence suggests that oxidized lipoprotein is a key event in the development of the atheromatous plaque which may ultimately occlude the blood vessel (*23*).

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Table 23

Diseases and syndromes in animals associated with vitamin E deficiency and excess intakes of polyunsaturated fatty acids

Syndrome	Affected organ or tissue	Species
Encephalomalacia	Cerebellum	Chick
Exudative diathesis	Vascular	Turkey
Microcytic anaemia	Blood, bone marrow	Chick
Macrocytic anaemia	Blood, bone marrow	Monkey
Pancreatic fibrosis	Pancreas	Chick, mouse
Liver necrosis	Liver	Pig, rat
Muscular degeneration	Skeletal muscle	Pig, rat, mouse
Microangiopathy	Heart muscle	Pig, lamb, calf
Kidney degeneration	Kidney tubules	Monkey, rat
Steatitis	Adipose tissue	Pig, chick
Testicular degeneration	Testes	Pig, calf, chick
Malignant hyperthermia	Skeletal muscle	Pig

Human studies, however, have been less consistent in providing evidence for a role of vitamin E in preventing heart disease. Vitamin E supplements reduce *ex vivo* oxidizability of plasma LDLs but there is no correlation between *ex vivo* lipoprotein oxidizability and endogenous vitamin E levels in an unsupplemented population (*24*). Likewise, the few randomised double-blind, placebo-controlled intervention trials with human volunteers which focused on the relationship between vitamin E and cardiovascular disease have given inconsistent results. There was a marked reduction in non-fatal myocardial infarction in patients with coronary artery disease (as defined by angiogram) who were randomly assigned to take pharmacologic doses of vitamin E (400 and 800 mg/day) or placebo in the Cambridge Heart Antioxidant Study involving 2000 men and women (*25*). However, the incidence of major coronary events in male smokers who received 20 mg/day of vitamin E for approximately 6 years was not reduced in the Alpha-Tocopherol, Beta-Carotene study (*26*).

Epidemiologic studies suggest that dietary vitamin E influences the risk of cardiovascular disease. Gey *et al*. (*27*) reported that lipid-standardized plasma vitamin E concentrations in middle-aged men across 16 European countries predicted 62 percent of the variance in the mortality from ischaemic heart disease. In the United States both the Nurses Health Study (*28*) involving 87000 females in an 8-year follow-up and the Health Professionals Follow-up Study in 40000 men (*29*) concluded that persons taking supplements of 100 mg/day or more of vitamin E for at least 2 years had approximately a 40 percent lower incidence of myocardial infarction and cardiovascular mortality than did those who did not use supplements. However, in US studies there was no influence of dietary vitamin E alone on incidence of cardiovascular disease when those taking supplements were removed from the analyses. A possible explanation for the significant relationship between dietary vitamin E and cardiovascular disease in European countries but not in the United States may be found in the widely differing sources of vitamin E in European countries. It is reported that sunflower seed oil, which is rich in α-tocopherol, tends to be consumed more widely in the southern European countries with the lower cardiovascular disease risk than in northern European countries where soybean oil, which contains more of the γ form, is preferred (*30*) (***Table 24***). However, a study carried out which compared plasma α- and γ-tocopherol concentrations in middle-aged men and women in Toulouse (southern France) with Belfast (Northern Ireland) found that the concentrations of γ-tocopherol in Belfast were twice as high as those in Toulouse; α-tocopherol concentrations were identical in men in both countries but higher in women in Belfast than in Toulouse ($P<0.001$) (*31*).

Table 24

Cross-country correlations between coronary heart disease mortality in men and the supply of vitamin E homologues across 24 European countries

Homologue	Correlation coefficient, *r*
Total vitamin E	-0.386
d-α-tocopherol	-0.753
d-β-tocopherol	-0.345
d-γ-tocopherol	-0.001
d-δ-tocopherol	0.098
d-α-tocotrienol	-0.072
d-β-tocotrienol	-0.329
d-γ-tocotrienol	-0.210

The correlation with *d*-α-tocopherol is highly significant ($P<0.001$) whereas all other correlations do not achieve statistical significance.
Source: Based on reference *30*.

It has also been suggested vitamin E supplementation (200–400 mg/day) may be appropriate therapeutically to moderate some aspects of degenerative diseases such as Parkinson's disease, reduce the severity of neurologic disorders such as tardive dyskinesia, prevent periventricular haemorrhage in pre-term babies, reduce tissue injury arising from ischaemia and reperfusion during surgery, delay cataract development, and improve mobility in arthritis sufferers (*32*). However, very high doses may also induce adverse pro-oxidant effects (*33*), and the long-term advantages of such treatments have not been proven.

Delineation of dietary sources and possible limitations to its availability worldwide

Because vitamin E is naturally present in plant-based diets and animal products and is often added by manufacturers to vegetable oils and processed foods, intakes are probably adequate to avoid overt deficiency in most situations. Exceptions may be during ecologic disasters and cultural conflicts resulting in food deprivation and famine.

Analysis of the Food and Agriculture Organization of the United Nations country food balance sheets indicates that about half the α-tocopherol in a typical northern European diet such as in the United Kingdom is derived from vegetable oils (*30*). Animal fats, vegetables, and meats each contribute about 10 percent to the total *per capita* supply and fruit, nuts, cereals, and dairy products each contribute about 4 percent. Less than 2 percent is each obtained from eggs, fish and pulses.

There are marked differences in *per capita* α-tocopherol supply among different countries ranging from approximately 8-10 mg/head/day (e.g., Iceland, Finland, New Zealand, and Japan) to 20–25 mg/head/day (e.g., France, Greece, and Spain) (*30*). This variation can be ascribed mainly to the type and quantity of dietary oils used in different countries and the proportion of the different homologues in the oils (***Table 25***). For example, sunflower seed oil contains approximately 55 mg α-tocopherol/100 g in contrast to soybean oil that contains only 8 mg/100 ml (*34*). Consumption of these oils varies markedly among countries. Soybean, a rich source of the less biologically active γ form, is most commonly used in northern European countries whereas sunflower seed oils, which mainly contain the α form, are generally used in southern Europe (*30*).

Table 25

Vitamin E content (mg tocopherol/100g) in vegetable oils

Oil	α–tocopherol	γ–tocopherol	δ–tocopherol	α - tocotrienol
Coconut	0.5	0	0.6	0.5
Maize (Corn)	11.2	60.2	1.8	0
Palm	25.6	31.6	7.0	14.3
Olive	5.1	Trace	0	0
Peanut	13.0	21.4	2.1	0
Soybean	10.1	59.3	26.4	0
Wheatgerm	133.0	26.0	27.1	2.6
Sunflower	48.7	5.1	0.8	0

Source: Slover HT, 1971. (34)

Summary of evidence for determining recommended nutrient intakes

In the chapter on antioxidants, it was decided that there was insufficient evidence to enable a recommended nutrient intake (RNI) to be based on the additional health benefits obtainable from nutrient intakes above those usually found in the diet. Even for vitamin E with its important biologic antioxidant properties, there was no consistent evidence for protection against chronic disease from dietary supplements. Nevertheless, the main function of vitamin E appears to be that of preventing oxidation of PUFAs, and this has been used by those bodies proposing RNIs for vitamin E because there is considerable evidence in different animal species that low vitamin E and PUFAs excess gives rise to a wide variety of clinical signs.

There is very little clinical evidence of deficiency disease in humans except in certain inherited conditions where the metabolism of vitamin E is disturbed. Even biochemical evidence of poor vitamin E status in both adults and children is minimal. Meta-analysis of data collected within European countries indicates that optimum intakes may be implied when plasma concentrations of vitamin E exceed 25–30 μmol/L of lipid-standardized α-tocopherol (*35*). However, this approach should be treated with caution, as plasma vitamin E concentrations do not necessarily reflect intakes or tissue reserves because only 1 percent of the body tocopherol may be in the blood (*36*) and the amount in the circulation is strongly influenced by circulating lipid (*37*). Nevertheless, the lipid-standardized vitamin E concentration (e.g., tocopherol-cholesterol ratio) greater that 2.25 (calculated as μmol/mmol) is believed to represent satisfactory vitamin E status (*36, 37*). The erythrocytes of subjects with values below this concentration of vitamin E may show evidence of an increasing tendency to haemolyze when exposed to oxidizing agents and thus such values should be taken as an indication of biochemical deficiency (*38*). However, the development of clinical evidence of vitamin E deficiency (e.g., muscle damage or neurologic lesions) can take several years of exposure to extremely low vitamin E levels (*39*).

The main factor used to assess the adequacy of vitamin E intakes by the US and UK advisory bodies was the dietary intake of PUFAs. PUFAs are very susceptible to oxidation, and their increased intake without a concomitant increase in vitamin E can lead to a reduction in plasma vitamin E concentrations (*40*) and to elevations in some indexes of oxidative damage in human volunteers (*41*). Generally, however, diets high in PUFAs are also high in vitamin E, and to set a dietary recommendation based on extremes of PUFA intake would deviate considerably from median intakes of vitamin E in most Western populations. Hence 'safe' allowances for the United Kingdom (men 10 and women 7 mg/day) (*42*) and 'arbitrary' allowances for the United States (men 10 and women 8 mg/day) (*43*) for vitamin E intakes approximate the median intakes in those countries. It is worth noting that there were only 11 (0.7 percent) subjects out of 1629 adults in the 1986–1987 British Nutrition Survey who had

α-tocopherol – cholesterol ratios <2.25. Furthermore, although the high intake of soybean oil with its high content of γ-tocopherol substitutes for the intake of α-tocopherol in the British diet, a comparison of α-tocopherol-cholesterol ratios found almost identical results in two groups of randomly selected, middle-aged adults in Belfast (Northern Ireland) and Toulouse (France), two countries with very different intakes of α-tocopherol (*34*) and cardiovascular risk (*31*).

It is suggested that when the main PUFA in the diet is linoleic acid, a *d*-α-tocopherol-PUFA ratio of 0.4 (expressed as mg tocopherol per g PUFA) is adequate for adult humans (*44, 45*), and the ratio has been recommended in the United Kingdom for infant formulas (*46*). Use of this ratio to calculate the vitamin E requirements of men and women with energy intakes of 2550 and 1940 kcal/day containing PUFA at 6 percent of the energy intake (approximately 17 and 13 g, respectively) (*42*) produced values of 7 and 5 mg/day of α-TEs, respectively. In both the United States and the United Kingdom, median intakes of α-TE are in excess of these amounts and the α-tocopherol-PUFA ratio is approximately 0.6 (*47*), which is well above the 0.4 ratio which would be considered adequate. The Nutrition Working Group of the International Life Sciences Institute Europe (*48*) has suggested an intake of 12 mg α-tocopherol for a daily intake of 14 g PUFAs to compensate for the high consumption of soya oil in certain countries where over 50 percent of vitamin E intake is accounted for by the less biologically active γ form. As indicated above, however, plasma concentrations in France and Northern Ireland suggest that an increased amount of dietary vitamin E is not necessary to maintain satisfactory plasma concentrations (*31*).

At present, data are not sufficient to formulate recommendations for vitamin E intake for different age groups except for infancy. There is some indication that new-born infants, particularly if born prematurely, are vulnerable to oxidative stress because of low body stores of vitamin E, impaired absorption, and reduced transport capacity resulting from low concentrations at birth of circulating low-density lipoproteins (*49*). However, term infants almost achieve adult plasma vitamin E concentrations in the first week (*50*) and although the concentration of vitamin E in early human milk can be variable, after 12 days it remains fairly constant at 0.32 mg TE/100 ml milk (51). Thus a human-milk-fed infant consuming 850 ml would have an intake of 2.7 mg. It seems reasonable that formula milk should not contain less than 0.3 mg TE/100 ml of reconstituted feed and not less than 0.4 mg TE/g PUFA.

No specific recommendations concerning the vitamin E requirements in pregnancy and lactation have been made by other advisory bodies (*42, 43*) mainly because there is no evidence of vitamin E requirements different from those of other adults and presumably also as the increased energy intake would compensate for the increased needs for infant growth and milk synthesis.

Vitamin E appears to have very low toxicity, and amounts of 100–200 mg of the synthetic all-*rac*-α-tocopherol are consumed widely as supplements (*28, 29*). Evidence of pro-oxidant damage has been associated with the feeding of supplements but usually only at very high doses (e.g., >1000 mg/day) (*33*).

Future research

More investigation is required of the role of vitamin E in biologic processes which do not necessarily involve its antioxidant function. These processes include:

- structural roles in the maintenance of cell membrane integrity;
- anti-inflammatory effects by direct and regulatory interaction with the prostaglandin synthetase complex of enzymes, which participate in the metabolism of arachidonic acid;
- DNA synthesis;

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- stimulation of the immune response; and
- regulation of intercellular signalling and cell proliferation through modulation of protein kinase-C.

Similarly, more investigation is required of the growing evidence that inadequate vitamin E status may increase susceptibility to infection particularly by allowing the genomes of certain relatively benign viruses to convert to more virulent strains (*52*).

There is an important need to define optimum vitamin E intakes. Intervention trials with morbidity and mortality endpoints may take years to complete. One approach to circumvent this delay may be to assess the effects of different intakes of vitamin E on biomarkers of oxidative damage to lipids, proteins, and DNA because their occurrence *in vivo* is implicated in many diseases, including vascular disease and certain cancers.

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Chapter 10
Vitamin K

Vitamin K is an essential fat-soluble micronutrient which is needed for a unique post-translational chemical modification in a small group of proteins with calcium-binding properties, collectively known as vitamin K – dependent proteins or Gla-proteins. Thus far, the only unequivocal role of vitamin K in health is in the maintenance of normal coagulation. The vitamin K – dependent coagulation proteins are synthesised in the liver and comprise factors II, VII, IX, and X, which have a haemostatic role (i.e., they are procoagulants that arrest and prevent bleeding), and proteins C and S, which have an anticoagulant role (i.e., they inhibit the clotting process). Despite this duality of function, the overriding effect of nutritional vitamin K deficiency is to tip the balance in coagulation towards a bleeding tendency caused by the relative inactivity of the procoagulant proteins. Vitamin K – dependent proteins synthesised by other tissues include the bone protein osteocalcin and matrix Gla protein; their functions remain to be clarified.

Biological role of vitamin K

Vitamin K is the family name for a series of fat-soluble compounds, which have a common 2-methyl-1, 4-naphthoquinone nucleus but differ in the structures of a side chain at the 3-position. They are synthesised by plants and bacteria. In plants the only important molecular form is phylloquinone (vitamin K_1), which has a phytyl side chain. Bacteria synthesise a family of compounds called menaquinones (vitamin K_2), which have side chains based on repeating unsaturated 5-carbon (prenyl) units. These are designated menaquinone-n (MK-n) according to the number (n) of prenyl units. Some bacteria also synthesise menaquinones in which one or more of the double bonds is saturated. The compound 2-methyl-1,4-naphthoquinone (common name menadione) may be regarded as a provitamin because vertebrates can convert it to MK-4 by adding a 4-prenyl side chain at the 3-position.

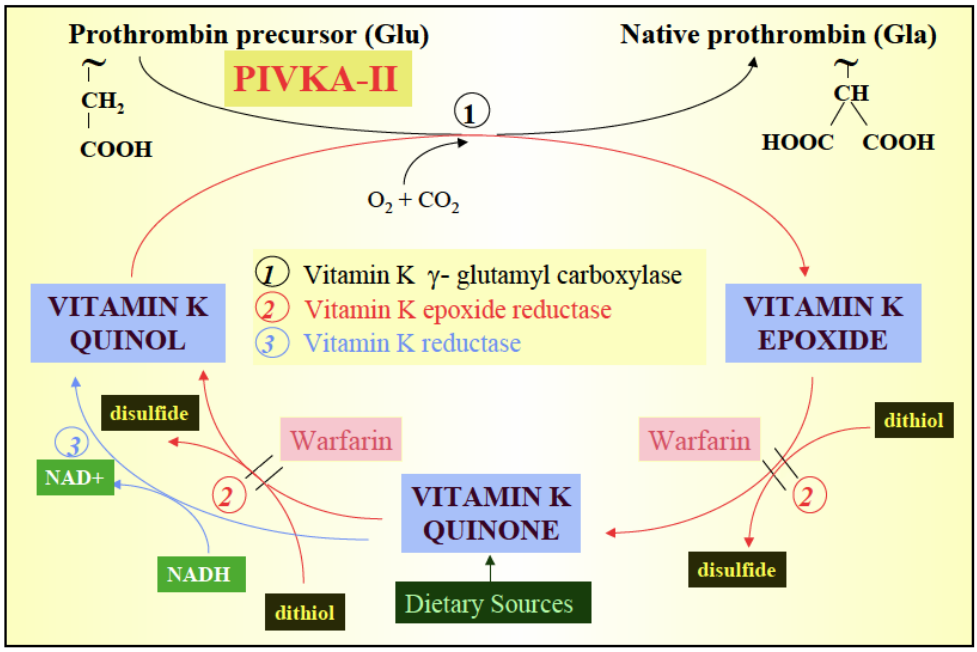
The biologic role of vitamin K is to act as a cofactor for a specific carboxylation reaction that transforms selective glutamate (Glu) residues to γ-carboxyglutamate (Gla) residues (*1,2*). The reaction is catalysed by a microsomal enzyme, γ-glutamyl, or vitamin K – dependent carboxylase, which in turn is linked to a cyclic salvage pathway known as the vitamin K epoxide cycle (***Figure 11***).

Scheme shows the cyclic metabolism of vitamin K in relation to the conversion of glutamate (Glu) to γ-carboxyglutamate (Gla) residues for the coagulation protein prothrombin. A general term for the glutamate precursors of vitamin K-dependent proteins is proteins induced by vitamin K absence, abbreviated PIVKA. For prothrombin (factor II) the glutamate precursor is known as PIVKA-II. The active form of vitamin K needed for carboxylation is the reduced form, vitamin K quinol. Known enzyme reactions are numbered 1, 2, and 3. The carboxylation reaction is driven by a vitamin K-dependent carboxylase activity (*reaction 1*) which simultaneously converts vitamin K quinol to vitamin K 2,3-epoxide. Vitamin K 2,3-epoxide is reduced back to the quinone and then to the quinol by vitamin K epoxide reductase (*reaction 2*). The reductase activity denoted 2 is dithiol dependent and is inhibited by coumarin anticoagulants such as warfarin. Dietary vitamin K may enter the cycle via an NAD(P)H-dependent vitamin K reductase activity (*reaction 3*), which is not inhibited by warfarin.

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Figure 11

The vitamin K epoxide cycle



The four vitamin K-dependent procoagulants (factor II or prothrombin, and factors VII, IX, and X) are serine proteases that are synthesised in the liver and then secreted into the circulation as inactive forms (zymogens). Their biologic activity depends on their normal complement of Gla residues, which are efficient chelators of calcium ions. In the presence of Gla and calcium ions these proteins bind to the surface membrane phospholipids of platelets and endothelial cells where, together with other cofactors, they form membrane-bound enzyme complexes. When coagulation is initiated, the zymogens of the four vitamin K-dependent clotting factors are cleaved to yield the active protease clotting factors (*1-3*). Two other vitamin K-dependent proteins called protein C and protein S play a regulatory role in the inhibition of coagulation. The function of protein C is to degrade phospholipid-bound activated factors V and VIII in the presence of calcium. Protein S acts as a synergistic cofactor to protein C by enhancing the binding of activated protein C to negatively charged phospholipids. Yet another vitamin K-dependent plasma protein (protein Z) is suspected to have a haemostatic role but its function is currently unknown.

Apart from the coagulation proteins, several other vitamin K-dependent proteins have been isolated from bone, cartilage, kidney, lungs, and other tissues (*4, 5*). Only two, osteocalcin and matrix Gla protein (MGP), have been well characterised. Both are found in bone but MGP also occurs in cartilage, blood vessel walls, and other soft tissues. There is evidence that protein S is synthesised by several tissues including the vessel wall and bone and may have other functions besides its well-established role as a coagulation inhibitor. It also seems likely that one function of MGP is to inhibit mineralisation (*6*). Thus far, no clear biologic role for osteocalcin has been established despite its being the major non-collagenous bone protein synthesised by osteoblasts (*7-9*). This failure to establish a biologic function for osteocalcin has hampered studies of the possible detrimental effects of vitamin K deficiency on bone health. Evidence of a possible association of a suboptimal vitamin K status with increased fracture risk remains to be confirmed (*7-9*).

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Overview of metabolism

Absorption and transport

Dietary vitamin K, mainly as phylloquinone, is absorbed chemically unchanged from the proximal intestine after solubilisation into mixed micelles composed of bile salts and the products of pancreatic lipolysis (*10*). In healthy adults the efficiency of absorption of phylloquinone in its free form is about 80 percent (*10, 11*). Within the intestinal mucosa the vitamin is incorporated into chylomicrons, is secreted into the lymph, and enters the blood via the lacteals (*11, 12*). Once in the circulation, phylloquinone is rapidly cleared (*10*) at a rate consistent with its continuing association with chylomicrons and the chylomicron remnants which are produced by lipoprotein lipase hydrolysis at the surface of capillary endothelial cells (*13*). After an overnight fast, more than half of the circulating phylloquinone is still associated with triglyceride-rich lipoproteins, with the remainder being equally distributed between low-density and high-density lipoproteins (*13*). Phylloquinone is the major circulating form of vitamin K but MK-7 is present in plasma at lower concentrations and has a lipoprotein distribution similar to phylloquinone (*13*). Although phylloquinone in blood must have been derived exclusively from the diet, it is not known whether circulating menaquinones such as MK-7 are derived from the diet, intestinal flora, or a combination of these sources.

Tissue stores and distribution

Until the 1970s, the liver was the only known site of synthesis of vitamin K-dependent proteins and hence was presumed to be the only significant storage site for the vitamin. However, the discovery of vitamin K-dependent processes and proteins in a number of extra-hepatic tissues suggests that this may not be the case.

Human liver stores normally comprise about 90 percent menaquinones and 10 percent phylloquinone (*14, 15*). There is evidence that the phylloquinone liver stores are very labile; under conditions of severe dietary depletion, liver concentrations were reduced to about 25 percent of initial levels after only 3 days (*15*). This high turnover of hepatic reserves of phylloquinone is in accord with the high losses of this vitamer through excretion (*10*). Knowledge of hepatic stores of phylloquinone in different population groups is limited. Adult hepatic stores in a UK study were about 11 pmol/g (*14*) whereas in a study from Japan they were about twofold higher(*15*). Such reserves are about 20 000–40 000-fold lower than those for retinol for relative daily intakes of phylloquinone that are only about 10-fold lower than those of vitamin A (*16*).

The relationship between hepatic and total-body stores of vitamin K is not known. Other sites of storage may be adipose tissue and bone; both are known to be sites where vitamin K-bearing chylomicrons and chylomicron remnants may be taken up. It has been reported that the predominant vitamer in human cortical and trabecular bone is phylloquinone; unlike the situation in liver, no menaquinones higher than MK-8 were detected (*17*).

In contrast to the hepatic preponderance of long-chain menaquinones, the major circulating form of vitamin K is invariably phylloquinone. The menaquinones MK-7 and possibly MK-8 are also present but the common hepatic forms MKs 9–13 are not detectable in blood plasma (*16, 18*). This might be a consequence of a different route of absorption (e.g., the possibility of a portal route for long-chain MKs *versus* the established lymphatic route for phylloquinone) but might suggest that once in the liver, the lipophilic long-chain menaquinones are not easily mobilised (*16, 18, 19*).

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Bio-activity

Very little information exists on the relative effectiveness of different hepatic forms of K vitamins for the coagulation function of vitamin K in humans. This information is important because of the preponderance of long-chain menaquinones in human liver. Early bioassay data from rats suggested that long-chain menaquinones (MKs-7, 9, and 10) were more efficient than phylloquinone in reversing vitamin K deficiency when single doses were give parenterally and that their sustained response may be due to their slower hepatic turnover (*18, 19*). A longer duration of the biologic response of MK-9 compared with phylloquinone in vitamin K-deficient rats was also observed by Groenen-van Dooren *et al.* (*20*). On the other hand Will and Suttie (*21*) showed that, when given orally, the dietary requirement of MK-9 for the maintenance of prothrombin synthesis in rats is higher than that for phylloquinone. They also reported that the initial hepatic turnover of MK-9 was two- to three-fold slower than that of phylloquinone.

Suttie (*18*) emphasised that the existence of a large pool of menaquinones in human liver does not necessarily mean that menaquinones make a proportionately greater contribution to the maintenance of vitamin K sufficiency. In humans the development of sub-clinical signs of vitamin K deficiency detected in dietary phylloquinone restriction studies argues against this, especially when placed alongside the lack of change of hepatic menaquinone stores (*15*). One explanation is that much of the hepatic menaquinones is not biologically available to the microsomal γ-glutamyl carboxylase because of a different sub-cellular location, especially location in the mitochondria and possibly other non-microsomal sites (*18*).

Excretion

Vitamin K is extensively metabolised in the liver and excreted in the urine and bile. In tracer experiments it was found that about 20 percent of an injected dose of phylloquinone was recovered in the urine whereas about 40–50 percent was excreted in the faeces via the bile (*10*); the proportion excreted was the same regardless of whether the injected dose was 1 mg or 45 μg. It seems likely, therefore, that about 60–70 percent of the amounts of phylloquinone absorbed from each meal will ultimately be lost to the body by excretion. These results suggest that the body stores of phylloquinone are being constantly replenished.

Two major human excretion products have been identified: carboxylic acids with 5 and 7-carbon sidechains that are excreted in the urine as glucuronide conjugates (*10*). The biliary metabolites have not been clearly identified but are initially excreted as water-soluble conjugates and become lipid soluble during their passage through the gut, probably through deconjugation by the gut flora. There is no evidence for body stores of vitamin K being conserved by an enterohepatic circulation. Vitamin K itself is too lipophilic to be excreted in the bile and the sidechain-shortened carboxylic acid metabolites are not biologically active.

Populations at risk

Vitamin K deficiency bleeding in infants

In infants up to around age 6 months, vitamin K deficiency, although rare, represents a significant public health problem throughout the world (*19, 22, 23*). The deficiency syndrome is traditionally known as haemorrhagic disease of the newborn or more recently, to give a better definition of the cause, vitamin K deficiency bleeding (VKDB).

The time of onset of VKDB is more unpredictable than previously supposed and it is now useful to recognise three syndromes: early, classic, and late (***Table 26***). Until the 1960s, VKDB was considered to be solely a problem of the first week of life. Then, in 1966, came the first reports from Thailand of a new vitamin K deficiency syndrome that typically

presented between 1 and 2 months of life and is now termed late VKDB. In 1977 Bhanchet and colleagues (*24*), who had first described this syndrome, summarised their studies of 93 affected Thai infants, establishing the idiopathic history, preponderance of breast-fed infants (98 percent), and high incidence of intracranial bleeding (63 percent). More reports from South East Asia and Australia followed, and in 1983 McNinch *et al*. (*25*) reported the return of VKDB in the United Kingdom. This increased incidence was ascribed to a decrease in the practice of vitamin K prophylaxis and to an increased trend towards exclusive human milk feeding (*25*). Human milk has lower concentrations of vitamin K than do infant milk formulas (*26*).

Without vitamin K prophylaxis, the incidence of late VKDB (per 100,000 births), based on acceptable surveillance data, has been estimated to be 4.4 in the United Kingdom, 7.2 in Germany, and as high as 72 in Thailand (*27*). Of real concern is that late VKDB, unlike the classic form, has a high incidence of death or severe and permanent brain damage resulting from intracranial haemorrhage (*19, 22, 23*).

Epidemiologic studies worldwide have identified two major risk factors for both classic and late VKDB: exclusive human milk feeding and the failure to give any vitamin K prophylaxis (*19, 22, 23*). The increased risk for infants fed human milk compared with formula milk is probably related to the relatively low concentrations of vitamin K (phylloquinone) in breast milk compared with formula milks (*26, 28, 29*). For classic VKDB, studies using the detection of under-carboxylated prothrombin or proteins induced by vitamin K absence (PIVKA)-II as a marker of sub-clinical vitamin K deficiency have suggested that it is the low cumulative intake of human milk in the first week of life rather than an abnormally low milk concentration *per se* that seems to be of greater relevance (*30, 31*). Thus, classic VKDB may be related, at least in part, to a failure to establish early breast-feeding.

Table 26

Classification of vitamin K deficiency bleeding of the newborn

Syndrome	Time of presentation	Common bleeding sites	Comments
Early VKDB	0–24 hours	Cephalohaematoma, intracranial, intrathoracic, intra-abdominal	Maternal drugs a frequent cause (e.g., Warfarin, anti-convulsants)
Classic VKDB	1–7 days	Gastrointestinal, skin, nasal, circumcision	Mainly idiopathic, maternal drugs
Late VKDB	1–12 weeks	Intracranial, skin, gastrointestinal	Mainly iodiopathic, may be presenting feature of underlying disease (e.g., cystic fibrosis, α-1-antitrypsin deficiency, biliary atresia); some degree of cholestasis often present

[a] VKDB, vitamin K deficiency bleeding.
Source: Shearer (19).

For late VKDB other factors seem to be important because the deficiency syndrome occurs when breast-feeding is well established and mothers of affected infants seem to have normal concentrations of vitamin K in their milk (*31*). Instead some (although not all) infants

who develop late haemorrhagic disease of the newborn are later found to have abnormalities of liver function that may affect their bile acid production and result in a degree of malabsorption of vitamin K. The degree of cholestasis may be mild and its course may be transient and self-correcting, but affected infants will have increased dietary requirements for vitamin K because of a reduced absorption efficiency.

Vitamin K prophylaxis in infants

Because bleeding can occur spontaneously and because no screening test is available, it is now common paediatric practice to protect all infants by giving vitamin K supplements in the immediate perinatal period. Vitamin K prophylaxis has had a chequered history but in recent years has become a high-profile issue of public health in many countries throughout the world. The reasons for this are twofold. First there is now a convincing body of evidence showing that without vitamin K prophylaxis, infants have a small but real risk of dying from or being permanently brain damaged by vitamin K deficiency in the first 6 months of life (*19, 22, 23*). The other, much less certain evidence stems from a reported epidemiologic association between vitamin K given intramuscularly (but not orally) and the later development of childhood cancer (*32*). The debate, both scientific and public, which followed this and other publications has led to an increase in the use of multiple oral supplements instead of the traditional single intramuscular injection (usually of 1 mg of phylloquinone) given at birth. Although most of the subsequent epidemiologic studies have not confirmed any cancer link with vitamin K, the issue is still not resolved (*33, 34*).

Vitamin K in adults

In adults, primary vitamin K–deficient states that manifest as bleeding are almost unknown except when the absorption of the vitamin is impaired as a result of an underlying pathology (*1*).

Dietary sources

High-performance liquid chromatography can be used to accurately determine the major dietary form of vitamin K (phylloquinone) in foods, and food tables are being compiled for Western diets (*16, 35, 36*). Phylloquinone is distributed ubiquitously throughout the diet, and the range of concentrations in different food categories is very wide. In general, the relative values in vegetables confirm the known association of phylloquinone with photosynthetic tissues, with the highest values (normally in the range 400–700 μg/100 g) being found in green leafy vegetables. The next best sources are certain vegetable oils (e.g., soybean, rapeseed, and olive oils) which contain 50–200 μg/100 g. Some vegetable oils, such as peanut, corn, sunflower and safflower oils, have a much lower phylloquinone content (1–10 μg/100 g). The great differences between vegetable oils obviously presents problems for calculating the phylloquinone contents of oil-containing foods when the type of oil (or its storage condition) is not known.

Menaquinones seem to have a more restricted distribution in the diet than does phylloquinone. In the Western diet nutritionally significant amounts of long-chain menaquinones have been found in animal livers and fermented foods such as cheeses. Yeasts do not synthesise menaquinones and menaquinone-rich foods are those with a bacterial fermentation stage. The Japanese food *natto* (fermented soybeans) has a menaquinone content even higher than that of phylloquinone in green leafy vegetables.

The relative dietary importance of MK-4 is more difficult to evaluate because concentrations in foods may well depend on geographic differences in the use of menadione in animal husbandry, menadione from which MK-4 may be synthesised in animal tissues.

Another imponderable factor is the evidence that animal tissues and dairy produce may contain some MK-4 as a product of tissue synthesis from phylloquinone itself (*37*).

Knowledge of the vitamin K content of human milk has been the subject of methodologic controversies with a 10-fold variation in reported values of phylloquinone concentrations of mature human milk (*38*). Where milk sampling and analytical techniques have met certain criteria for their validity, the phylloquinone content of mature milk have generally ranged between 1 and 4 μg/l, with average concentrations near the lower end of this range (*28, 29, 38*). However, there is considerable intra- and inter-subject variation, and levels higher are in colostral milk than in mature milk (*28*). Menaquinone concentrations in human milk have not been accurately determined but appear to be much lower than those of phylloquinone. Phylloquinone concentrations in infant formula milk range from 3 to 16 μg/l in unsupplemented formulas and up to 100 μg/l in fortified formulas (*26*). Nowadays most formulas are fortified; typical phylloquinone concentrations are about 50 μg/l.

Bio-availability of vitamin K from foods

Very little is known about the bio-availability of the K vitamins from different foods. It has been estimated that the efficiency of absorption of phylloquinone from boiled spinach (eaten with butter) is no greater than 10 percent (*39*) compared with an estimated 80 percent when phylloquinone is given in its free form (*10, 11*). This poor absorption of phylloquinone from green leafy vegetables may be explained by its location in chloroplasts and tight association with the thylakoid membrane, where this naphthoquinone plays a role in photosynthesis. In comparison, the bio-availability of MK-4 from butter artificially enriched with this vitamer was more than twofold higher than that of phylloquinone from spinach (*39*). The poor extraction of phylloquinone from leafy vegetables, which as a category represents the single greatest food source of phylloquinone, may place a different perspective on the relative importance of other foods with lower concentrations of phylloquinone (e.g., containing soybean and rapeseed oils) but in which the vitamin is not tightly bound and its bio-availability is likely to be greater. Even before bio-availability was taken into account, fats and oils that are contained in mixed dishes were found to make an important contribution to the phylloquinone content of the US diet (*40*) and in a UK study contributed 30 percent of the total dietary intake (*41*).

No data exist on the efficiency of intestinal absorption of dietary long-chain menaquinones. Because the lipophilic properties of menaquinones are greater than those of phylloquinone, it is likely that the efficiency of their absorption, in the free form, is low, as suggested by animal studies (*18, 21*).

Importance of intestinal bacterial synthesis as a source of vitamin K

Intestinal microflora synthesise large amounts of menaquinones, which are potentially available as a source of vitamin K (*42*). Quantitative measurements at different sites of the human intestine have demonstrated that most of these menaquinones are present in the distal colon (*42*). Major forms produced are MK-10 and MK-11 by *Bacteroides*, MK-8 by *Enterobacter*, MK-7 by *Veillonella,* and MK-6 by *Eubacterium lentum*. It is noteworthy that menaquinones with very long chains (MKs 10–13) are known to be synthesised by members of the anaerobic genus *Bacteroides* and are major inhabitants of the intestinal tract but have not been detected in significant amounts in foods. The widespread presence of MKs 10–13 in human livers at high concentrations (*14, 15*) therefore suggests that these forms, at least, originate from intestinal synthesis (*16*).

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It is commonly held that animals and humans obtain a significant fraction of their vitamin K requirement from direct absorption of menaquinones produced by microfloral synthesis (*43*), but hard experimental evidence documenting the site and extent of any absorption is singularly lacking (*18, 19, 23*). The most promising site of absorption is the terminal ileum, where there are some menaquinone-producing bacteria as well as bile salts. The evidence overall suggests that the bio-availability of bacterial menaquinones is poor because they are mostly tightly bound to the bacterial cytoplasmic membrane and the largest pool is present in the colon, which lacks bile salts for their solubilisation (*19, 23*).

Evidence on which recommendations can be based

Assessment of vitamin K status

Conventional coagulation assays are useful for detecting overt vitamin K-deficient states which are associated with a risk of bleeding. However, they offer only a relatively insensitive insight into vitamin K nutritional status and the detection of sub-clinical vitamin K-deficient states. A more sensitive measure of vitamin K sufficiency can be obtained from tests that detect under-carboxylated species of vitamin K-dependent proteins. In states of vitamin K deficiency, under-carboxylated species of the vitamin K-dependent coagulation proteins are released from the liver into the blood; their levels increase with the degree of severity of vitamin K deficiency. These under-carboxylated forms (PIVKA) are unable to participate in the normal coagulation cascade because they are unable to bind calcium. The measurement of under-carboxylated prothrombin (PIVKA-II) is the most useful and sensitive homeostatic marker of sub-clinical vitamin K deficiency. Importantly, PIVKA-II is detectable in plasma before any changes occur in conventional coagulation tests. Several types of assay for PIVKA-II have been developed which vary in their sensitivity (*44*).

In the same way that vitamin K deficiency causes PIVKA-II to be released into the circulation from the liver, a deficit of vitamin K in bone will cause the osteoblasts to secrete under-carboxylated species of osteocalcin (ucOC) into the bloodstream. It has been proposed that the concentration of circulating ucOC reflects the sufficiency of vitamin K for the carboxylation of this Gla protein in bone tissue (*7, 45*). Most assays for ucOC have been indirect because they rely on the differential absorption of carboxylated and under-carboxylated forms to hydroxyapatite and are difficult to interpret (*46*).

Other criteria of vitamin K sufficiency that have been used are plasma measurements of phylloquinone and the measurement of urinary Gla. It is expected and found that the excretion of urinary Gla is decreased in vitamin K deficiency.

Dietary intakes in infants and their adequacy

The average intake of phylloquinone in infants fed human milk during the first 6 months of life has been reported to be less than 1 μg/day; this is approximately 100-fold lower than the intake in infants fed a typical supplemented formula (29). This big disparity between intakes is reflected in plasma levels (***Table 27***).

Using the detection of PIVKA-II as a marker of sub-clinical deficiency, a study from Germany concluded that a minimum daily intake of about 100 ml of colostral milk (that supplies about 0.2-0.3 μg of phylloquinone) is sufficient for normal haemostasis in a baby of about 3 kg during the first week of life (*30, 47*). Similar conclusions were reached in a Japanese study which showed a linear correlation between the prevalence of PIVKA-II and the volume of breast milk ingested over 3 days (*48*); 95 percent of infants with detectable PIVKA-II had average daily intakes of less than about 120 ml, but the marker was not detectable when intakes reached 170 ml/day.

Table 27

Dietary intakes and plasma levels of phylloquinone in human-milk-fed *versus* formula-fed infants aged 0–6 months

Age (weeks)	Phylloquinone intake (μg/day)		Plasma phylloquinone (μg/l)	
	Human milk fed[a]	Formula fed[b]	Human milk fed	Formula fed
6	0.55	45.4	0.13	6.0
12	0.74	55.5	0.20	5.6
26	0.56	52.2	0.24	4.4

[a]Breast-milk concentrations averaged 0.86, 1.14, and 0.87 μg/l of phylloquinone at 6, 12, and 26 weeks, respectively.

[b]All infants were fed a formula containing phylloquinone at 55 μg/l.

Source: Greer FR *et al*. (*29*).

Factors of relevance to classical vitamin K deficiency bleeding

The liver stores of vitamin K in the newborn infant differ both qualitatively and quantitatively from that in adults. First, phylloquinone levels at birth are about one-fifth those in adults and second, bacterial menaquinones are undetectable (*14*). The resistance to placental transport of vitamin K to the human foetus is well-established (*19, 22*). Thereafter, the limited available data suggest that hepatic stores of menaquinones build up gradually, becoming detectable at around the second week of life but only reaching adult concentrations after 1 month of age (*14, 49*). A gradual increase in liver stores of menaquinones may reflect the gradual colonisation of the gut by enteric microflora.

A practical problem in assessing the functional status of vitamin K in the neonatal period is that there are both gestational and postnatal increases in the four vitamin K-dependent procoagulant factors which are unrelated to vitamin K status (*50*). This means that unless the deficiency state is quite severe, it is very difficult to interpret clotting factor activities as a measure of vitamin K sufficiency. The best diagnostic tool of the adequacy of vitamin K stores for neonates is by the detection of PIVKA-II by immunoassays. The use of this marker has clearly shown that there is a temporary dip in the vitamin K status of infants exclusively fed human milk in the first few days after birth (*30, 47, 48, 51, 52*). The fact that the degree of this dip is associated with human-milk intakes (*30, 47, 48*) and is less evident or abolished in infants given artificial feeds (*30, 48, 52*) or prophylactic vitamin K at birth (*48, 51, 52*) shows that the detection of PIVKA-II reflected a dietary lack of vitamin K.

Factors of relevance to late vitamin K deficiency bleeding

The natural tendency for human-milk-fed infants to develop a sub-clinical vitamin K deficiency in the first 2–3 days of life is self-limiting. Comparisons between untreated human-milk-fed infants with others who had received vitamin K or supplementary feeds clearly suggest that this improvement in vitamin K–dependent clotting activity is due to an improved vitamin K status. After the first week, vitamin K–dependent clotting factor increases are more gradual, and it is not possible from clotting factor assays to differentiate between the natural post-natal increase in the synthesis of the core proteins from an improved vitamin K status leading to greater functional activity.

Use of the most sensitive assays for PIVKA-II shows that there is still evidence of suboptimal vitamin K status in infants solely fed human milk between the ages of 1 and 2

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months (*52, 53*). Deficiency signs are less common in infants who have received adequate vitamin K supplementation (*52, 53*) or who have been formula fed (*52*).

Dietary intakes in older infants, children, and adults and their adequacy

The only comprehensive national survey of phylloquinone intakes across all age groups (except infants aged 0–6 months) is that of the US Food and Drug Administration Total Diet Study, which was based on the 1987–88 Nationwide Food Consumption Survey (*40*). For infants and children from the age of 6 months to 16 years, average phylloquinone intakes were above the current US recommended dietary allowance (RDA) values for their respective age groups, more so for children up to 10 years than from 10 to 16 years (***Table 28***) (*40*). There have been no studies of intakes in children in relation to functional markers of vitamin K sufficiency in children.

Intakes for adults in The Total Diet Study (***Table 28***) were also close to or slightly higher than the current US RDA values of 80 μg for men and 65 μg for women, although intakes were slightly lower than the RDA in the 25–30 years age group (*54*). There is some evidence from an evaluation of all the US studies that older adults have higher dietary intakes of phylloquinone than do younger adults (*55*).

The US results are very comparable with a detailed, seasonality study in the United Kingdom in which mean intakes in men and women (aged 22–54 years) were 72 and 64 μg/day, respectively; no significant sex or seasonal variations were found (*56*). Another UK study suggested that intakes were lower in manual workers and in smokers, reflecting their lower intakes of green vegetables and high-quality vegetable oil (*57*).

Several dietary restriction and repletion studies have attempted to assess the adequacy of vitamin K intakes in adults (*55, 58*). It is clear from these studies that volunteers consuming less than 10 μg/day of phylloquinone do not show any changes in conventional coagulation tests even after several weeks unless other measures to reduce the efficiency of absorption are introduced. However, a diet containing only 2–5 μg/day of phylloquinone fed for 2 weeks did result in an increase of PIVKA-II and a 70 percent decrease in plasma phylloquinone (*59*). Similar evidence of a sub-clinical vitamin K deficiency together with an increased urinary excretion of Gla was found when dietary intakes of phylloquinone were reduced from about 80 to about 40 μg/day for 21 days (*60*). A repletion phase in this study was consistent with a human dietary vitamin K requirement (for its coagulation role) of about 1 μg/kg body weight/day.

The most detailed and controlled dietary restriction and repletion study in healthy human subjects is that by Ferland *et al*. (*61*). In this study 32 healthy subjects in two age groups (20–40 and 60–80 years) were fed a mixed diet containing about 80 μg/day of phylloquinone, which is the RDA for adult males in the United States (*54*). After 4 days on this baseline diet there was a 13-day depletion period during which the subjects were fed a diet containing about 10 μg/day. After this depletion phase the subjects entered a 16-day repletion period during which, over 4-day intervals, they were sequentially repleted with 5, 15, 25, and 45 μg phylloquinone. The depletion protocol had no effect on conventional coagulation and specific factor assays but did induce a significant increase in PIVKA-II in both age groups. The most dramatic change was in plasma levels of phylloquinone, which fell to about 15 percent of the values on day 1. The drop in plasma phylloquinone also suggested that the average dietary intake of these particular individuals before they entered the study had been greater than the baseline diet of 80 μg/day. The repletion protocol failed to bring the plasma phylloquinone levels of the young subjects back above the lower limit of the normal range (previously established in healthy, free-living adults) whereas the plasma levels in the elderly group only rose slightly above this lower limit in the last 4 days. Another indication of

a reduced vitamin K status in the young group was the fall in urinary output of Gla (90 percent of baseline) that was not seen in the elderly group; this suggested that younger subjects are more susceptible to the effects of an acute deficiency than are older subjects.

Table 28

Mean dietary intakes of phylloquinone from US Food and Drug Administration Total Diet Study based on 1987-88 Nationwide Food Consumption Survey *versus* US RDA

Group	No.[a]	Phylloquinone Intake (μg/day) 1990 TDS[b]	RDA[c]
Infants			
6 months	141	77	10
Children			
2 years	152	24	15
6 years	154	46	20
10 years	119	45	30
Females, 14–16 years	188	52	45-55
Males, 14–16 years	174	64	45-65
Younger adults			
Females, 25–30 years	492	59	65
Males, 25–30 years	386	66	80
Females, 40–45years	319	71	65
Males, 40–45years	293	86	80
Older adults			
Females, 60–65 years	313	76	65
Males, 60–65 years	238	80	80
Females, 70+ years	402	82	65
Males, 70+ years	263	80	80

[a]The number of subjects as stratified by age and/or sex.
[b]Total Diet Study (*40*).
[c]Recommended Dietary Allowance, 1989 (*54*).

One important dietary intervention study measured the carboxylation status of the bone vitamin K-dependent protein, osteocalcin, in response to altered dietary intakes of phylloquinone (*62*). This was a crossover study, which evaluated the effect in young adults of increasing the dietary intake of phylloquinone to 420 μg/day for 5 days from a baseline intake of 100 μg/day. Although total concentrations of osteocalcin were not affected by either of the dietary treatments, ucOC fell dramatically in response to the 420 μg diet and by the end of the 5-day supplementation period was 41 percent lower than the baseline value. After the return to the mixed diet, the ucOC percent rose significantly but after 5 days had not returned to pre-supplementation values. This study suggested that the carboxylation of osteocalcin in bone may require higher dietary intakes of vitamin K than those needed to sustain its haemostatic function.

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Recommendations for vitamin K intakes

Table 29

Recommended nutrients intakes for vitamin K

Age group	Recommended Nutrient Intake† μg /day
Infants and children	
0–6 months	5*
7–12 months	10
1–3 years	15
4–6 years	20
7–9 years	25
Adolescents, 10–18 years	
Females	35-55
Males	35-55
Adults	
Females, 19–65 years	55
65+ years	55
Males, 19–65 years	65
65+ years	65
Pregnancy	55
Lactation	55

†The RNI for each age group is based on a daily intake of approximately 1 microgram/body weight of phylloquinone.
*This intake cannot be met by infants who are exclusively breast-fed.

To prevent bleeding due to vitamin K deficiency, the panel recommends that all breast babies should receive vitamin K supplementation at birth according to nationally approved guidelines. Vitamin K formulations and prophylactic regimes differ from country to country. Guidelines range from a single intramuscular injection (usually 1 mg of phylloquinone) given at birth to multiple oral doses given over the first few weeks (***Table 29***).

Infants 0–6 months

Consideration of the requirements of vitamin K for infants up to age 6 months is complicated by the need to prevent a rare but potentially devastating bleeding disorder, which is caused by vitamin K deficiency. To protect the few affected infants, most developed and some developing countries have instituted a blanket prophylactic policy to protect infants at risk. The numbers of infants at risk without such a programme has a geographic component, being more prevalent in the Far East, and a dietary component with solely human-milk-fed babies having the highest risk (*22, 23, 27*). Of the etiologic factors, some of which may still be unrecognised, one factor in some infants is mild cholestasis. The problem of overcoming a variable and, in some infants, inefficient absorption is the likely reason that oral prophylactic regimens, even with two or three pharmacologic doses (1 mg phylloquinone), have occasionally failed to prevent VKDB (*63*). This makes it difficult to design an effective oral prophylaxis regimen that is comparable in efficacy with the previous "gold standard" of 1 mg phylloquinone given by intramuscular injection at birth. In several countries intramuscular prophylaxis fell out of favour after the epidemiologic report and subsequent controversy that this route may be linked to childhood cancer (*32-34*).

Infants who have been entirely fed with supplemented formulas are well protected against VKDB and on intakes of around 50 μg/day have plasma levels that are about 10-fold higher than the adult average of about 1.0 nmol/l (0.5 μg/l) (*29*) (***Table 29***). Clearly then, an optimal intake would lie below an intake of 50 μg/day. Cornelissen *et al*. (*64*) evaluated the effectiveness of giving infants a daily supplement of 25 μg phylloquinone after they had received a single oral dose of 1 mg at birth. This regimen resulted in median plasma levels at ages 4, 8, and 12 weeks of around 2.2 nmol/l (1.0 μg/l) when sampled 20–28 hours after the most recent vitamin K dose; this level corresponds to the upper end of the adult fasting range. In 12-week-old infants supplemented with this regime, the median plasma level was about fourfold higher than that in a control group of unsupplemented infants (1.9 *versus* 0.5 nmol/l). Also none of the 50 supplemented infants had detectable PIVKA-II at 12 weeks compared with 15 of 131 infants (11.5 percent) in the control group. This regime has now been implemented in The Netherlands and surveillance data on late VKDB suggest that it may be as effective as parenteral vitamin K prophylaxis (*63*).

The fact that VKDB is epidemiologically associated with breast feeding means that it is not prudent to base requirements solely on normal intakes of human milk and justifies the setting of a higher value that can only be met by some form of supplementation. The current US RDA for infants is 5 μg/day for the first 6 months (the greatest period of risk for VKDB) and 10 μg/day during the second 6 months (*54*). This is based on the adult RDA of 1 μg/kg body weight/day. However, if the vitamin K content of human milk is assumed to be about 2 μg/l, exclusively breast-fed infants aged 0–6 months may ingest only 20 percent of their presumed daily requirement of 5 μg (*54*). Whether a figure of 5 μg/day is itself safe is uncertain. In the United Kingdom the dietary reference value for infants was set at 10 μg/day, which in relation to body weight (2 μg/kg) is about double the estimate for adults (*65*). It was set with reference to the upper end of possible human milk concentrations plus a further qualitative addition to allow for the absence of hepatic menaquinones in early life and the presumed reliance on dietary vitamin K alone.

The association of VKDB with breast-feeding does not mean that most infants are at risk of developing VKDB, because this is a rare vitamin K deficiency syndrome. In contrast to measurements of PIVKA-II levels, comparisons of vitamin K–dependent clotting activities have shown no detectable differences between infants fed human milk and those fed artifical formula. The detection of PIVKA-II with normal functional levels of vitamin K–dependent coagulation factors does not imply immediate or even future haemorrhagic risk for a particular individual. The major value of PIVKA-II measurements in infants is to assess the prevalence of suboptimal vitamin K status in population studies. However, because of the potential consequences of VKDB, the paediatric profession of most countries agrees that some form of vitamin K supplementation is necessary even though there are widespread differences in actual practice.

Infants (7–12 months), children, and adults

In the past, the requirements for vitamin K only considered its classical function in coagulation; an RDA was given for vitamin K in the United States (*54, 58*) and a safe and adequate intake level was given in the United Kingdom (*65*). In both countries the adult RDA or adequate intake was set at a value of 1 μg/kg body weight/day. Thus in the United States the RDA for a 79-kg man is listed as 80 μg/day and for a 63-kg women as 65 μg/day (*54*).

At the time previous recommendations were set there were few data on dietary intakes of vitamin K (mainly phylloquinone) in different populations. The development of more accurate and wide-ranging food databases is now helping to address this question. The results of several dietary intake studies in the United States and the United Kingdom suggest that the

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average intakes for adults are very close to the respective recommendations of each country. In the United States, preliminary intake data also suggest that average intakes of phylloquinone in children and adolescents also exceed the RDA; in 6-month-old infants the intakes exceeded the RDA of 10 μg by nearly eightfold (*40*), reflecting the use of supplemented formula foods. Because there is no evidence of even sub-clinical deficiencies of haemostatic function, a daily intake of 1 μg/kg may still be used as the basis for the recommended nutrient intake (RNI). There is no basis as yet for making different recommendations for pregnant and lactating women.

A relevant question is whether the RNI should be raised to take into account recent evidence that the requirements for the optimal carboxylation of vitamin K–dependent proteins in other tissues are greater than those for coagulation. There is certainly evidence that the γ-carboxylation of osteocalcin can be improved by intakes somewhere between 100 and 420 μg/day (*62*). If an RNI for vitamin K sufficiency is to be defined as that amount necessary for the optimal carboxylation of all vitamin K-dependent proteins, including osteocalcin, then it seems clear that this RNI would lie somewhere above the current intakes of many, if not most, of the population in the United States and the United Kingdom. Because a clearly defined metabolic role and biochemical proof of the necessity for fully γ-carboxylated osteocalcin for bone health is currently lacking, it would be unwise to make such a recommendation.

Toxicity

When taken orally, natural K vitamins seem free of toxic side effects. This safety is illustrated by the common clinical administration of phylloquinone at doses of 10–20 mg or greater. Some patients with chronic fat malabsorption regularly ingest doses of this size without evidence of any harm. However, synthetic preparations of menadione or its salts are best avoided for nutritional purpose, especially for vitamin prophylaxis in the newborn. Besides lacking intrinsic biologic activity, the high reactivity of its unsubstituted 3-position has been associated with neonatal haemolysis and liver damage.

Future research

The following are recommended areas for future research:

- prevalence, causes, and prevention of VKDB in infants in different population groups;
- bio-availability of dietary phylloquinone (and menaquinones) from foods and menaquinones from gut flora;
- significance of menaquinones to human requirements for vitamin K;
- the physiologic roles of vitamin K-dependent proteins in functions other than coagulation; and
- the significance of under-carboxylated vitamin K-dependent proteins and sub-optimal vitamin K status to bone and cardiovascular health.

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Chapter 11
Calcium

It is nearly 30 years since the last FAO/WHO recommendations on calcium intake were published in 1974 (*1*) and nearly 40 years since the experts' meeting in Rome (*2*) on which these recommendations were based. During this generation gap, a paradigm shift has occurred with respect to the involvement of calcium in the aetiology of osteoporosis. The previous reports were written against the background of the Albright paradigm (*3*), according to which osteomalacia and rickets were due to calcium deficiency, vitamin D deficiency, or both, whereas osteoporosis was attributed to failure of new bone formation secondary to negative nitrogen balance, osteoblast insufficiency, or both. The rediscovery of earlier information that calcium deficiency led to the development of osteoporosis (not rickets and osteomalacia) in experimental animals (*4*) resulted in a reexamination of osteoporosis in humans, notably in postmenopausal women. This reexamination yielded evidence in the late 1960s that menopausal bone loss was not due to a decrease in bone formation but rather to an increase in bone resorption (*5-8*), and this has had a profound effect on our understanding of other forms of osteoporosis. Although reduced bone formation may aggravate the bone loss process in elderly people (*9*) and probably plays a major role in corticosteroid osteoporosis (*10*) – and possibly in osteoporosis in men (*11*) – bone resorption is increasingly held responsible for osteoporosis in women and for the bone deficit associated with hip fractures in elderly people of both sexes (*12*). Because bone resorption is also the mechanism whereby calcium deficiency destroys bone, it is hardly surprising that the role of calcium in the pathogenesis of osteoporosis has received increasing attention and that recommended calcium intakes have risen steadily in the past 35 years from the nadir which followed the publication of the report from Rome in 1962 (*13*). The process has been accelerated by the growing realisation that insensible losses of calcium (via skin, hair, nails, etc.) need to be taken into account in the calculation of calcium requirement.

As the calcium allowances recommended for developed nations have been rising – and may still not have reached their peak – the gap between them and the actual calcium intakes in developing countries has widened. The concept that calcium requirement may itself vary from culture to culture for dietary, genetic, lifestyle, and geographical reasons is emerging. This report therefore seeks to make it clear that our main recommendations – like the latest recommendations from USA and Canada (*14*), Great Britain (*15*), the European Union (*16*), and Australia (*17*) – are largely based on data derived from the developed world and are not necessarily applicable to nations with different dietary cultures, different lifestyles, and different environments for which different calculations may be indicated.

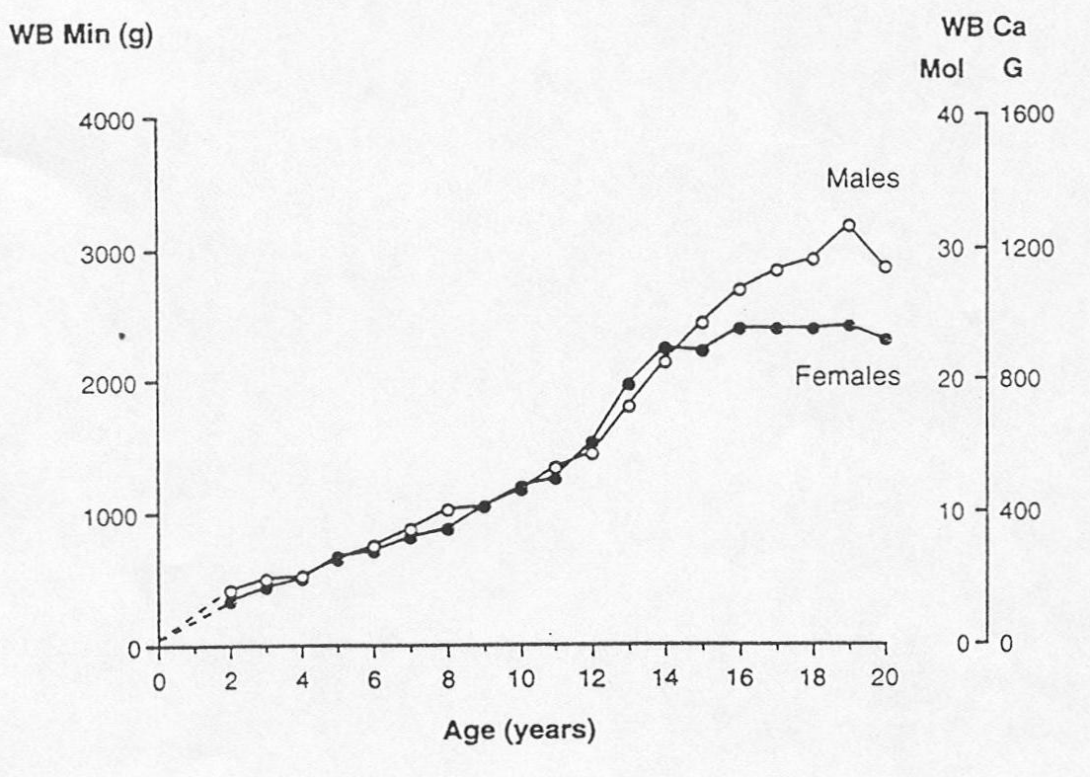
Chemistry and distribution of calcium

Calcium is a divalent cation with an atomic weight of 40. In the elementary composition of the human body, it ranks fifth after oxygen, carbon, hydrogen, and nitrogen, and it makes up 1.9 percent of the body by weight (18). Carcass analyses show that it constitutes 0.1–0.2 percent of early foetal fat-free weight, rising to about 2 percent of adult fat-free weight. In absolute terms, this represents a rise from about 24 g (600 mmol) at birth to 1300 g (32.5 mol) at maturity, requiring an average daily positive calcium balance of 180 mg (4.5 mmol) during the 20 years of growth (***Figure 12***).

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Figure 12

Whole-body bone mineral (WB Min) (left axis) and calcium (right axis) as a function of age as determined by total-body dual-energy X-ray absorptiometry



Note: Data supplied by Dr Zanchetta, IDIM, Buenos Aires, Argentina).

Ninety-nine percent of the body calcium is located in the skeleton. The remaining 1 percent is equally distributed between the teeth and soft tissues, with only 0.1 percent in the extracellular fluid (ECF). In the skeleton it constitutes 25 percent of the dry weight and 40 percent of the ash weight. The ECF contains ionised calcium at about 4.8 mg/100 ml (1.20 mmol/l) maintained by the parathyroid – vitamin D system as well as complexed calcium at about 1.6 mg/100 ml (0.4 mmol/l). In the plasma there is an additional protein-bound calcium fraction of 3.2 mg/100 ml (0.8 mmol/l). In the cellular compartment the total calcium concentration is comparable with that in the ECF, but the free calcium concentration is lower by several orders of magnitude (*19*).

Biological role of calcium

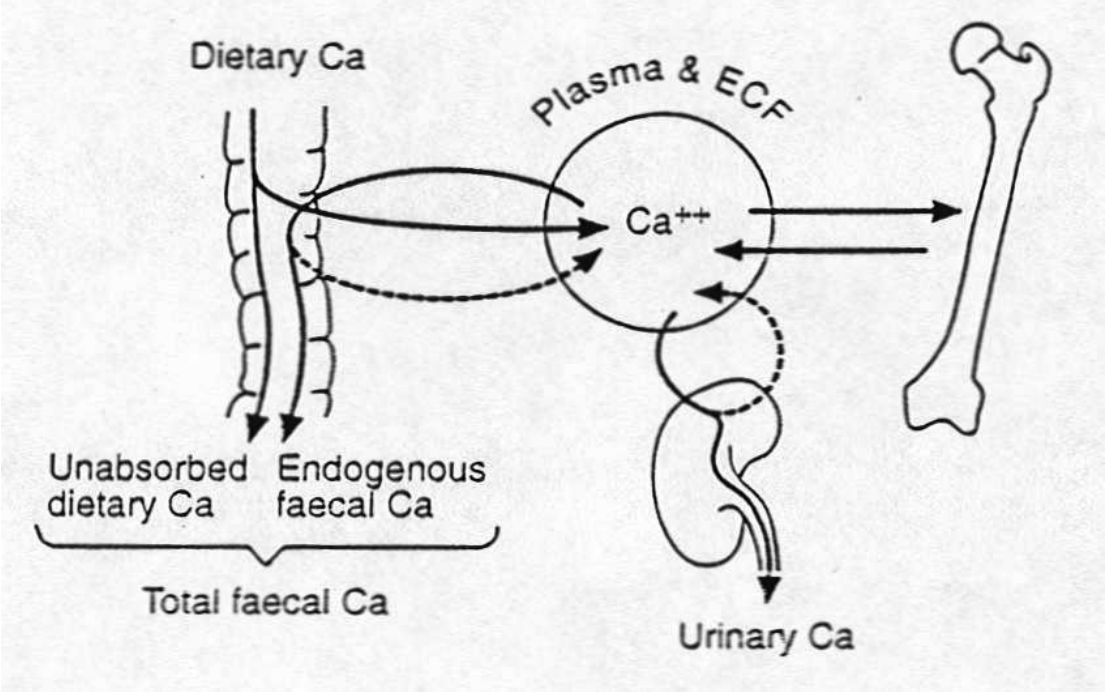
Calcium salts provide rigidity to the skeleton and calcium ions play a role in many if not most metabolic processes. In the primitive exoskeleton and in shells, rigidity is generally provided by calcium carbonate, but in the vertebrate skeleton it is provided by a form of calcium phosphate which approximates hydroxyapatite [$Ca_{10}(OH)_2(PO_4)_6$] and is embedded in collagen fibrils.

Bone mineral serves as the ultimate reservoir for the calcium circulating in the ECF. Calcium enters the ECF from the gut by absorption and from bone by resorption. Calcium leaves the ECF via the gastrointestinal tract, kidneys, and skin and enters into bone via bone formation (***Figure 13***). In addition, calcium fluxes occur across all cell membranes. Many neuromuscular and other cellular functions depend on the maintenance of the ionised calcium concentration in the ECF. Calcium fluxes are also important mediators of hormonal effects on target organs through several intracellular signalling pathways, such as the phosphoinositide and cyclic adenosine monophosphate systems. The cytoplasmic calcium concentration is kept

down by a series of calcium pumps, which concentrate calcium within the intracellular storage sites or extrude from the cells the calcium which flows in by diffusion. The physiology of calcium metabolism is primarily directed towards the maintenance of the concentration of ionised calcium in the ECF. This is protected and maintained by a feedback loop through calcium receptors in the parathyroid glands (*20*), which control the secretion of parathyroid hormone (see ***Figure 10*** of Chapter 8). This hormone increases the renal tubular reabsorption of calcium, promotes intestinal calcium absorption by stimulating the renal production of 1,25-dihyroxycolecaliferol [$1,25(OH)_2D$], and, if necessary, resorbs bone. However, the integrity of the system depends critically on vitamin D status; if there is a deficiency of vitamin D, the loss of its calcaemic action (*21*) leads to a decrease in the ionised calcium and secondary hyperparathyroidism and hypophosphataemia. This is why experimental vitamin D deficiency results in rickets and osteomalacia whereas calcium deficiency gives rise to osteoporosis (*4,22*).

Figure 13

Major calcium movements in the body



Determinants of calcium balance

Calcium intake

In a strictly operational sense, calcium balance is determined by the relationship between calcium intake and calcium absorption and excretion. A striking feature of the system is that relatively small changes in calcium absorption and excretion can neutralise a high intake or compensate for a low one. There is a wide variation in calcium intake among nations, generally following the animal protein intake and depending largely on dairy product consumption. The lowest calcium intakes are in developing countries, particularly in Asia, and the highest are in developed countries, particularly in USA, Canada and Europe (***Table 30***).

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Table 30

Protein and calcium intakes in different regions of the world 1987–1989

Region	Protein (g)			Calcium (mg)		
	Total	Animal	Vegetable	Total	Animal	Vegetable
USA and Canada	108.7	72.2	36.5	1031	717	314
Europe	102.0	59.6	42.4	896	684	212
Oceania	98.3	66.5	31.8	836	603	233
Other developed	91.1	47.3	43.8	565	314	251
USSR	106.2	56.1	50.1	751	567	184
All developed	103.0	60.1	42.9	850	617	233
Africa	54.1	10.6	43.5	368	108	260
Latin America	66.8	28.6	38.2	476	305	171
Near East	78.7	18.0	60.7	484	223	261
Far East	58.2	11.0	47.2	305	109	196
Other developing	55.8	22.7	33.1	432	140	292
All developing	59.9	13.3	46.6	344	138	206

Source: Adapted from FAO Yearbook, 1990 (*23*).

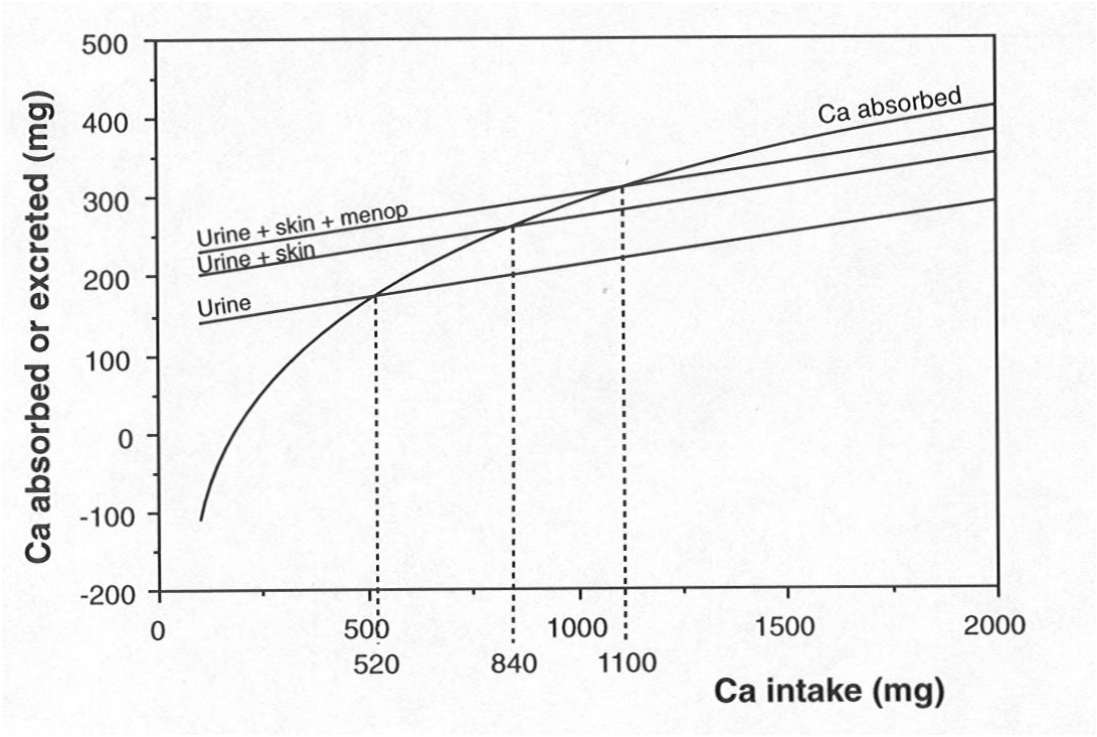
Calcium absorption

Ingested calcium mixes with digestive juice calcium in the proximal small intestine from where it is absorbed by a process, which has an active saturable component and a diffusion component (*24-27*). At low calcium intakes calcium is mainly absorbed by active (transcellular) transport, but at higher intakes an increasing proportion of calcium is absorbed by simple (paracellular) diffusion. The unabsorbed component appears in the faeces together with the unabsorbed component of digestive juice calcium known as endogenous faecal calcium. Thus, the faeces contain unabsorbed dietary calcium and unreabsorbed digestive juice calcium (***Figure 14***).

True absorbed calcium is the total calcium absorbed from the calcium pool in the intestines and therefore contains both dietary and digestive juice components. Net absorbed calcium is the difference between dietary calcium and faecal calcium and is numerically the same as true absorbed calcium minus endogenous faecal calcium. At zero calcium intake, all the faecal calcium is endogenous and represents the digestive juice calcium which has not been reabsorbed; net absorbed calcium at this intake is therefore negative to the extent of about 200 mg (5 mmol) (*28,29*). When the intake reaches about 200 mg (5 mmol), dietary and faecal calcium become equal and net absorbed calcium is zero. As calcium intake increases, net absorbed calcium also increases, steeply at first but then, as the active transport becomes saturated, more slowly until the slope of absorbed on ingested calcium approaches linearity with an ultimate gradient of about 5–10 percent (*24,25,30,31*). The relationship between intestinal calcium absorption and calcium intake, derived from 210 balance studies performed in 81 individuals collected from the literature (*32-39*), is shown in ***Figure 14***.

Figure 14

Relationship between calcium absorption and calcium intake



The relationships between calcium intake and calcium absorbed and excreted calcium calculated from 210 balance experiments in 81 subjects (32-39). Equilibrium is reached at an intake of 520 mg, which rises to 840 mg when skin losses of 60 mg are added and to 1100 mg when menopausal loss is included. The curvilinear relationship between intestinal calcium absorption and calcium intake can be made linear by using the logarithm of calcium intake to yield the equation: Caa = 174 loge Cai -909 ± 71 (SD) mg/day, where Cai represents ingested calcium and Caa net absorbed calcium. The relationship between urinary calcium excretion and calcium intake is given by the equation: Cau = 0.078 Cai + 137 ± 11.2 (SD) mg/day, where Cau is urinary calcium and Cai calcium intake.

True absorption is an inverse function of calcium intake, falling from some 70 percent at very low intakes to about 35 percent at high intakes (***Figure 15***). Percent net absorption is negative at low intakes, becomes positive as intake increases, reaches a peak of about 30 percent at an intake of about 400 mg, and then falls off as the intake increases. The two lines converge as intake rises because the endogenous faecal component (which separates them) becomes proportionately smaller.

Many factors influence the availability of calcium for absorption and the absorptive mechanism itself. The former includes substances, which form insoluble complexes with calcium, such as the phosphate ion. The relatively high calcium-phosphate ratio of 2.2 in human milk compared with 0.77 in cow milk (*18*) may be a factor in the higher absorption of calcium from human milk than cow milk (see below).

Intestinal calcium absorption is mainly controlled by the serum concentration of $1,25(OH)_2D$ (see ***Chapter 8***). The activity of the 1-α-hydroxylase, which catalyses $1,25(OH)_2D$ production from 25-hydroxycolecalciferol (25OHD) in the kidneys, is negatively related to the plasma calcium and phosphate concentrations and positively to plasma parathyroid hormone (*21*). Thus the inverse relationship between calcium intake and

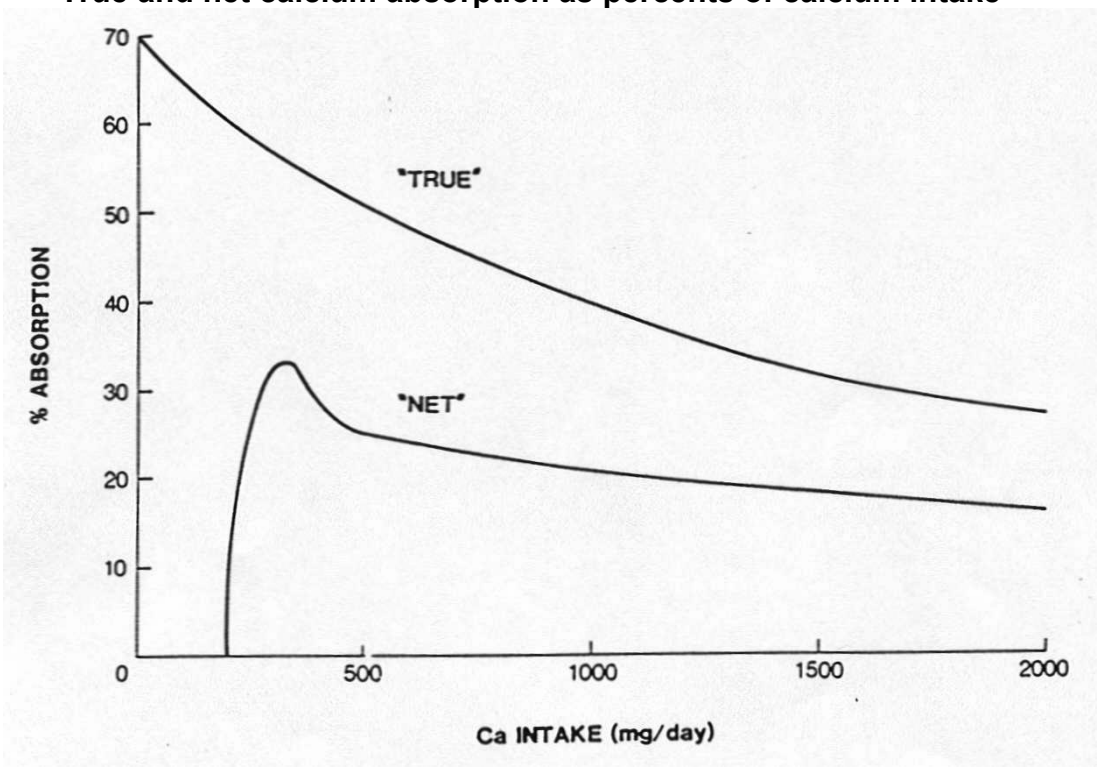
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fractional absorption described above is enhanced by the inverse relationship between dietary calcium and serum $1,25(OH)_2D$ (*21,40,41*).

Phytates, present in the husks of many cereals as well as in nuts, seeds, and legumes, can form insoluble calcium phytate salts in the gastrointestinal tract. Excess oxalates can precipitate calcium in the bowel but are not an important factor in most diets.

Figure 15

True and net calcium absorption as percents of calcium intake



Note: The great differences between these functions at low calcium intakes and their progressive convergence as calcium intake increases.

Urinary calcium

Urinary calcium is the fraction of the filtered plasma water calcium, which is not reabsorbed in the renal tubules. At a normal glomerular filtration rate of 120 ml/min and ultrafiltrable calcium of 6.4 mg/100 ml (1.60 mmol/l), the filtered load of calcium is about 8 mg/min (0.20 mmol/min) or 11.6 g/day (290 mmol/day). Because the usual 24-hour calcium excretion in developed countries is about 160–200 mg (4–5 mmol), it follows that 98–99 percent of the filtered calcium is usually reabsorbed in the renal tubules. However, calcium excretion is extremely sensitive to changes in filtered load. A decrease in plasma water calcium of only 0.17 mg/100 ml (0.043 mmol/l), which is barely detectable, was sufficient to account for a decrease in urinary calcium of 63 mg (1.51 mmol) when 27 subjects changed from a normal- to a low-calcium diet (*42*). This very sensitive renal response to calcium deprivation combines with the inverse relationship between calcium intake and absorption to stabilise the plasma ionised calcium concentration and to preserve the equilibrium between calcium entering and leaving the ECF over a wide range of calcium intakes. However, there is always a significant obligatory loss of calcium in the urine (as there is in the faeces), even on a low calcium intake, simply because maintenance of the plasma ionised calcium and, therefore, of the filtered load, prevents total elimination of the calcium from the urine. The lower limit for urinary calcium in developed countries is about 140 mg (3.5 mmol) but

depends on protein and salt intakes. From this obligatory minimum, urinary calcium increases on intake with a slope of about 5–10 percent (*30,31,43*). In the graph derived from 210 balance studies referred to above (***Figure 14***), the relationship between urinary calcium excretion and calcium intake is represented by the line which intersects the absorbed calcium line at an intake of 520 mg.

Insensible losses

Urinary and endogenous faecal calcium are not the only forms of excreted calcium; losses through skin, hair, and nails need to be taken into account. These are not easily measured, but a combined balance and isotope procedure has yielded estimates of daily insensible calcium losses in the range of 40–80 mg (1–2 mmol), which are unrelated to calcium intake (*44,45*). The addition of a loss of 60 mg (1.5 mmol) as a constant to urinary calcium loss raises the dietary calcium at which absorbed and excreted calcium reach equilibrium from 520 to 840 mg (13 to 21 mmol) (***Figure 14***).

Calcium requirements and recommended intakes

Methodology

Although it is well established that calcium deficiency causes osteoporosis in experimental animals, the contribution that calcium deficiency makes to osteoporosis in humans is much more controversial, not least because of the great variation in calcium intakes across the world (***Table 30***), which does not appear to be associated with any corresponding variation in the prevalence of osteoporosis. This issue is dealt with at greater length below in the section on nutritional factors; in this section we will simply define what is meant by calcium requirement and how it may be calculated.

The calcium requirement of an adult is generally recognised to be the intake required to maintain calcium balance and therefore skeletal integrity. The mean calcium requirement of adults is therefore the mean intake at which intake and output are equal, which at present can only be determined by balance studies conducted with sufficient care and over a sufficiently long period to ensure reasonable accuracy and then corrected for insensible losses. The reputation of the balance technique has been harmed by a few studies with inadequate equilibration times and short collection periods, but this should not be allowed to detract from the value of the meticulous work of those who have collected faecal and urinary samples for weeks or months from subjects on well-defined diets. This meticulous work has produced valuable balance data, which are clearly valid; the mean duration of the balances in the 210 studies from eight publications used in this report was 90 days with a range of 6–480 days. (The four 6-day balances in the series used a non-absorbable marker and are therefore acceptable.)

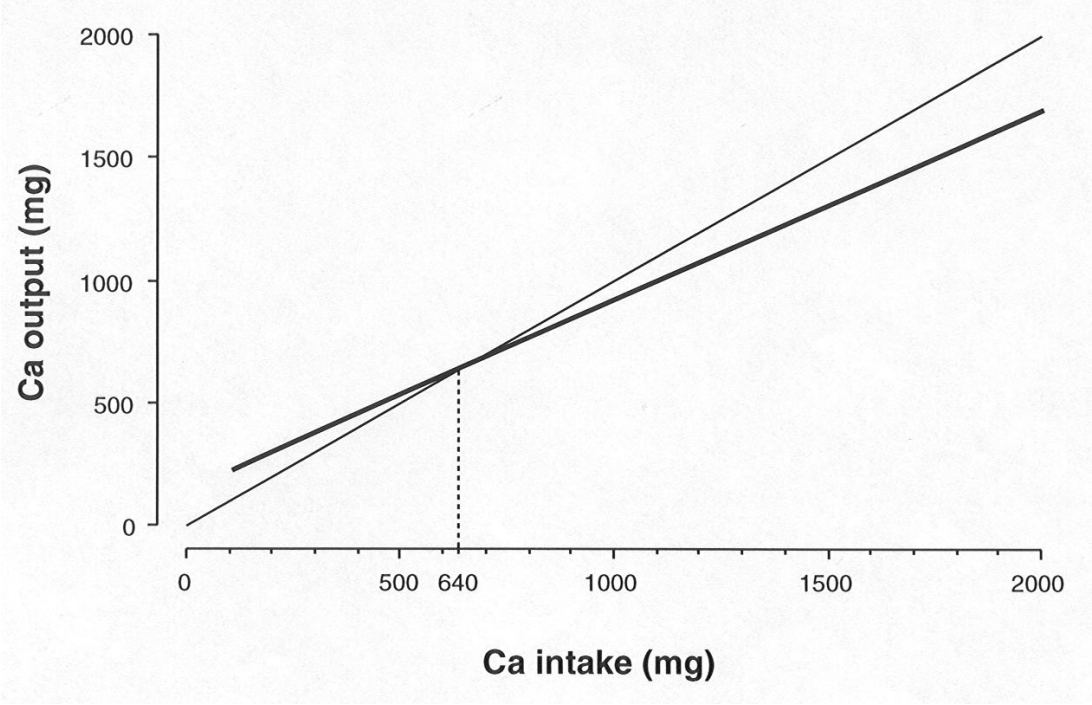
The usual way of determining mean calcium requirement from balance studies has been by linear regression of calcium output (or calcium balance) on intake and calculation of the mean intake at which intake and output are equal (or balance is zero). This was probably first done in 1939 by Mitchell and Curzon (*46*), who arrived at a mean requirement of 9.8 mg/kg/day or about 640 mg (16 mmol) at a mean body weight of 65 kg. The same type of calculation was subsequently used by many other workers who arrived at requirements ranging from 200 mg/day (5 mmol/day) in male Peruvian prisoners (*47*) to 990 mg (24.75 mmol) in premenopausal women (*48*), but most values were about 600 mg (15 mmol) (*31*) without allowing for insensible losses. However, this type of simple linear regression yields a higher mean calcium requirement (640 mg in the same 210 balances) (***Figure 16a***) than the intercept of absorbed and excreted calcium (520 mg) (***Figure 14***) because it tends to underestimate the negative calcium balance at low intake and overestimate the positive balance at high intake. A better reflection of biological reality is obtained by deriving calcium

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output from the functions given in the previous section and then regressing that output on calcium intake. This yields the result shown in ***Figure 16b*** where the negative balance is more severe at low intakes and less positive at high intakes than in the linear model and in which zero balance occurs at 520 mg as in ***Figure 14***.

Figure 16a

Calcium output as a linear (A) function of calcium intake calculated from the same balances as Figure 14



Note: The regression line crosses the line of equality at an intake of 640 mg.
The equation is: $Ca_o = 0.779\ Ca_i + 142$ mg where Ca_o is calcium output.

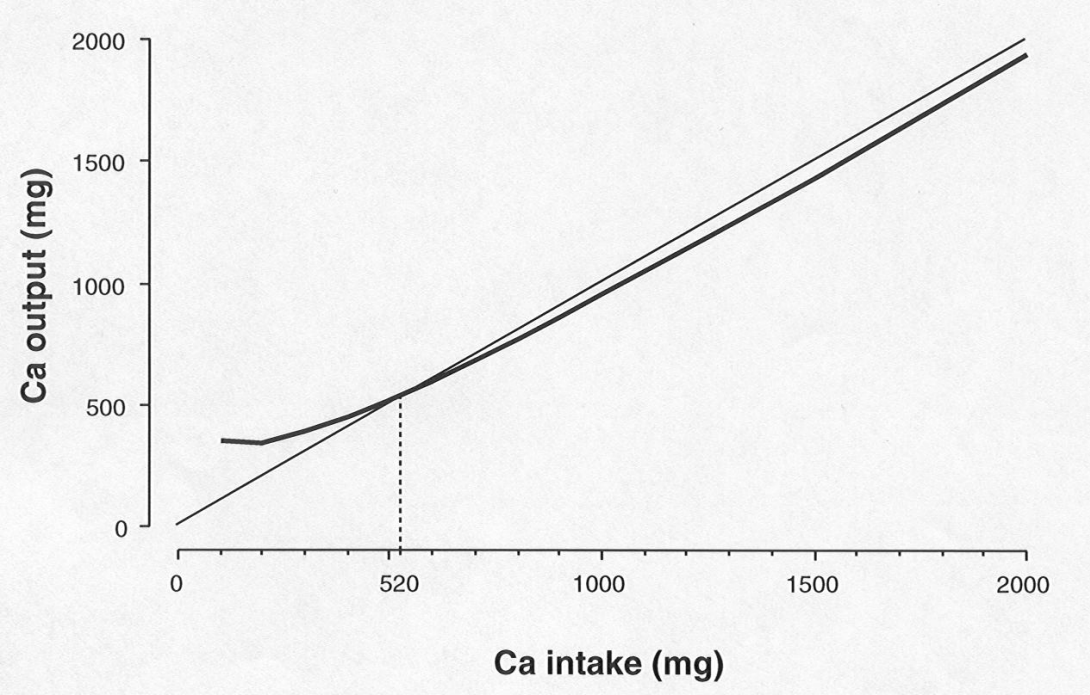
An alternative way of calculating calcium requirement is to determine the intake at which the mean maximum positive balance occurs. This has been done with a two-component, split, linear regression model in which calcium balance is regressed on intake to determine the threshold intake above which no further increase in calcium retention occurs (*49*). This may well be an appropriate way of calculating the calcium requirement of children and adolescents (and perhaps pregnant and lactating women) who need to be in positive calcium balance and in whom the difference between calcium intake and output is therefore relatively large and measurable by the balance technique. However, in normal adults the difference between calcium intake and output at high calcium intakes represents a very small difference between two large numbers, and this calculation therefore carries too great an error to calculate their requirement.

We are inclined to think that the most satisfactory way of calculating calcium requirement from current data is as the intake at which excreted calcium equals net absorbed calcium, which has the advantage of permitting separate analysis of the effects of changes in calcium absorption and excretion. This intercept has been shown in ***Figure 14*** to occur at an intake of about 520 mg, but when insensible losses of calcium of 60 mg (1.5 mmol) (*44,45*) are taken into account, the intercept rises to 840 mg, which we believe is as close as it is possible to get at present to the calcium requirement of adults on Western-style diets. The addition to this excretion line of an additional obligatory urinary calcium of 30 mg (0.75 mmol) at menopause (*50*) raises the amount to about 1100 mg, which we suggest is the mean calcium requirement of postmenopausal women (see below). However, this type of

calculation cannot easily be applied to other high-risk populations (such as children) because there are not sufficient published data from these groups to permit a similar analysis of the relationship among calcium intake, absorption, and excretion. An alternative is to estimate how much calcium each population group needs to absorb to meet obligatory calcium losses and desirable calcium retention and then to calculate the intake required to provide this rate of calcium absorption. This is what has been done in the following section.

Figure 16b

Calcium output as a non-linear function of calcium intake calculated from the same balances as Figure 14



Note: The regression line crosses the line of equality at an intake of 520 mg.
The equation is: $Ca_o = Ca_i - 174\ Log_e\ Ca_I - 909 + 0.078\ Ca_u + 137$ mg.

Populations at risk

It is clear from ***Figure 12*** that a positive calcium balance (i.e., net calcium retention) is required throughout growth, particularly during the first 2 years of life and during puberty and adolescence. These age groups therefore constitute populations at risk for calcium deficiency, as are pregnant women (especially in the last trimester), lactating women, postmenopausal women, and, possibly, elderly men. Our calculations for these groups, ultimately derived from Western European and North American data, are given below.

Recommendations by group

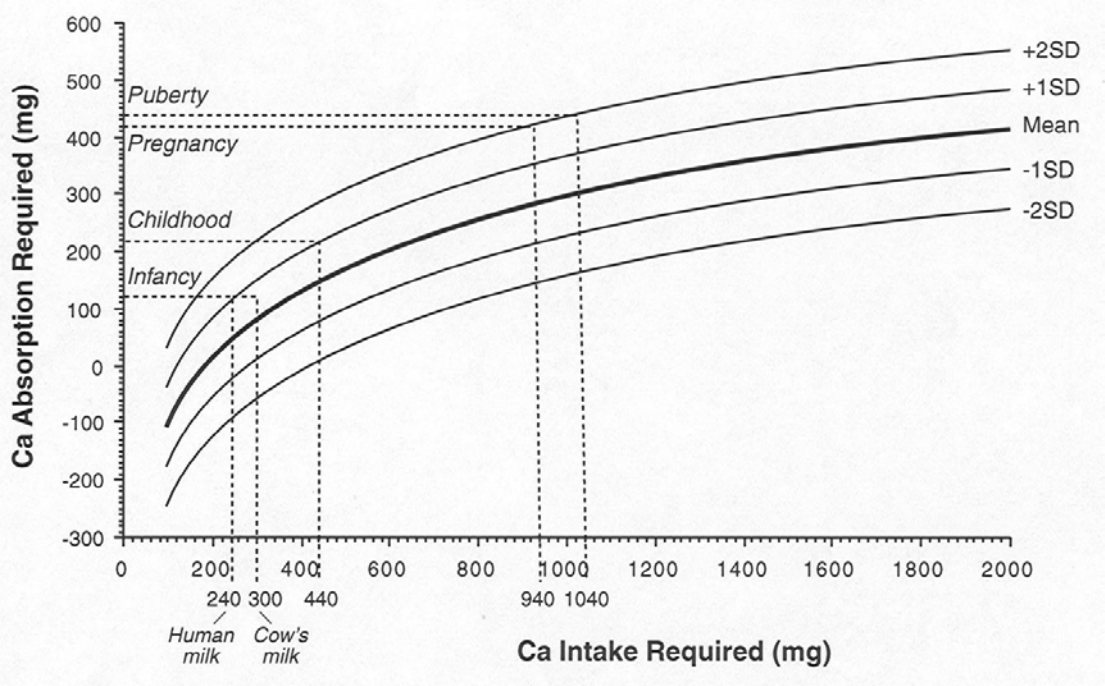
Infancy

In the first 2 years of life, the daily calcium increment in the skeleton is about 100 mg (2.5 mmol) (*51*). The urinary calcium of infants is about 10 mg/day (0.25 mmol/day) and is virtually independent of intake (*52-56*) and insensible losses are perhaps the same. Therefore, infants need to absorb some 120 mg (3 mmol) of calcium daily to allow for normal growth. What this represents in dietary terms can be calculated from calcium absorption studies in newborn infants (*52-56*), which suggest that the absorption of calcium from cow milk by

infants is about 0.5 SD above the normal adult slope and from human milk is more than 1 SD above the normal adult slope. If this information is correct, different recommendations need to be made for infants depending on milk source. With human milk, an absorption of 120 mg (3 mmol) of calcium requires a mean intake of 240 mg (6 mmol) (***Figure 17***) and a recommended intake of say 300 mg (7.5 mmol), which is close to the amount provided in the average daily milk production of 750 ml. With cow milk, calcium intake needs to be about 300 mg (7.5 mmol) to meet the requirement (***Figure 17***) and the allowance should be 400 mg (10 mmol) (***Table 31***).

Figure 17

Calcium intakes required to provide the absorbed calcium necessary to meet calcium requirements at different stages in the lifecycle



Note: The solid lines represent the mean and range of calcium absorption as a function of calcium intake derived from the equation in ***Figure 14***. The interrupted lines represent the estimated calcium requirements based on Western European and North American data.

Childhood

The accumulation of whole-body calcium with skeletal growth is illustrated in ***Figure 12***. It rises from about 120 g (3 mol) at age 2 years to 400 g (10 mol) at age 9 years. These values can be converted into a daily rate of calcium accumulation from ages 2 to 9 of about 120 mg (3 mmol), which is very similar to the amount calculated by Leitch and Aitken (*57*) from growth analyses. Although urinary calcium must rise with the growth-related rise in glomerular filtration rate, a reasonable estimate of the mean value from ages 2 to 9 might be 60 mg (1.5 mmol) (*58*). When this is added to a daily skeletal increment of 120 mg (3 mmol) and a dermal loss of perhaps 40 mg (1.0 mmol), the average daily net absorbed calcium needs to be 220 mg (5.5 mmol) during this period. If the net absorption of calcium by children is 1 SD above that of adults, the average daily requirement during this period is about 440 mg (11 mmol) (***Figure 17***) and the average recommended intake is 600 mg (15 mmol) – somewhat lower in the earlier years and somewhat higher in the later years (***Table 31***).

Table 31

Recommended calcium allowances based on Western European, American and Canadian data

Group	Recommended intake mg/day
Infants and children	
0–6 months	
Human milk	300
Cow milk	400
7–12 months	400
1–3 years	500
4–6 years	600
7–9 years	700
Adolescents, 10–18 years	1300[a]
Adults	
Females	
19 years to menopause	1000
Postmenopause	1300
Males	
19–65 years	1000
65 +	1300
Pregnancy (last trimester)	1200
Lactation	1000

[a] Particularly during the growth spurt.

Puberty and adolescence

As can be seen in ***Figure 12***, a striking increase in the rate of skeletal calcium accretion occurs at puberty – from about ages 10 to 17 years. The peak rate of calcium retention in this period is 300–400 mg (7.5–10 mmol) daily (*57*); it occurs earlier in girls but continues longer in boys. For a target value of 300 mg (7.5 mmol) for the skeleton, 100 mg (2.5 mmol) for urinary calcium (*58*), and insensible losses of 40 mg (1.0 mmol), the net absorbed calcium during at least part of this period needs to be 440 mg (11 mmol) daily. Even with assuming high calcium absorption (+2 SD), this requires an intake of 1040 mg (26.0 mmol) daily (***Figure 17***) and an allowance of 1300 mg (32.5 mmol) during the peak growth phase (***Table 31***). It is difficult to justify any difference between the allowances for boys and girls because, as mentioned above, although the growth spurt starts earlier in girls, it continues longer in boys. This recommended intake (which is close to that derived differently by Matkovic and Heaney [*49,58*]) is not achieved by many adolescents even in developed countries (*59-61*), but the effects of this shortfall on their growth and bone status are unknown.

Adults

As indicated earlier and for the reasons given, we accept that the mean apparent calcium requirement of adults in developed countries is about 520 mg (13 mmol) but that this is increased by insensible losses to some 840 mg (21 mmol) (***Figure 14***). This reasoning forms the basis of our recommended intake for adults of 1000 mg (***Table 31***).

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Menopause

The most important single cause of osteoporosis – at least in developed countries – is probably menopause, which is accompanied by an unequivocal and sustained rise in obligatory urinary calcium of about 30 mg (0.75 mmol) daily (*50,62,63*). Because calcium absorption certainly does not increase at this time – and probably decreases (*64,65*) – this extra urinary calcium represents a negative calcium balance which is compatible with the average bone loss of about 0.5–1.0 percent per year after menopause. There is a consensus that these events are associated with an increase in bone resorption but controversy continues about whether this is the primary event, the response to an increased calcium demand, or both. The results of calcium trials are clearly relevant. Before 1997, there had been 20 prospective trials of calcium supplementation in 857 postmenopausal women and 625 control subjects; these trials showed highly significant suppression of bone loss by calcium (*65*). Another meta-analysis covering similar numbers showed that calcium supplementation significantly enhanced the effect of oestrogen on bone (*66*). It is therefore logical to recommend sufficient additional calcium after the menopause to cover at least the extra obligatory loss of calcium in the urine. The additional dietary calcium needed to meet an increased urinary loss of 30 mg (0.75 mmol) is 260 mg/day (6.5 mmol/day) (***Figure 14***), which raises the daily requirement from 840 mg (21 mmol) to 1100 mg (27.5 mmol) and the recommended intake from 1000 to 1300 mg/day (25 to 32.5 mmol/day), which is a little higher than that recommended by the United States and Canada (*14*) (***Table 31*** and ***32***).

Table 32

Current calcium intake recommendations (mg/day)

	Australia 1991 Recommended Dietary Intake	**United Kingdom 1991 Reference Nutrient Intake**	**European Union 1993 Population Reference Intake**	**United States and Canada 1997 Adequate Intake**
Pregnancy (last trimester)	1100	700	700	1000–1300
Lactation	1200	1250	1200	1000–1300
Infancy	300 (human milk) 500 (cow milk)	525	400	210–270
Childhood	530-800	350–550	400-550	500–800
Puberty and adolescence				
Boys	1000–1200	1000	1000	1300
Girls	800–1000	800	800	1300
Maturity				
Males	800	700	700	1000
Females	800	700	700	1000
Later life				
Males >65 years	800	700	700	1200
Postmenopausal women	1000	700	700	1200

Ageing

Not enough is known about bone and calcium metabolism during ageing to enable calculation of the calcium requirements of older men and women with any confidence. Calcium absorption tends to decrease with age in both sexes (*67-69*) but whereas the evidence that calcium requirement goes up at the menopause is strong, corresponding evidence about ageing men is less convincing (*32,36*). Nonetheless, as a precaution we propose an extra allowance of 300 mg/day (7.5 mmol/day) for men over 65 to bring them into line with postmenopausal women (***Table 31***).

Pregnancy

The calcium content of the newborn infant is about 24 g (600 mmol). Most of this calcium is laid down in the last trimester of pregnancy, during which the foetus retains about 240 mg (6 mmol) of calcium daily (*51*). There is some evidence that pregnancy is associated with an increase in calcium absorption [associated with a rise in the plasma $1,25(OH)_2$ D level] (*70-72*). For a maternal urinary calcium of 120 mg (3 mmol) and a maternal skin loss of 60 mg (1.5 mmol), the absorbed calcium should be 420 mg (10.5 mmol) daily. Even at optimal calcium absorption, the corresponding calcium intake would need to be 940 mg (23.5 mmol) (***Figure 17***) and the recommended allowance would need to be 1200 mg (30 mmol) (***Table 31***), which is similar to that proposed by the United States and Canada (*14*) (***Table 32***).

Lactation

The calcium content of human milk is about 36 mg per 100 ml (9 mmol/l) (*18*). A lactating woman produces about 750 ml of milk daily, which represents about 280 mg (7.0 mmol) of calcium. For a maternal urinary calcium of 100 mg/day (2.5 mmol/day) and maternal skin loss of 60 mg/day (1.5 mmol/day), the required absorption is 440 mg/day (11 mmol/day) – the same as at puberty. If calcium absorption efficiency is maximal (i.e., 2 SD above the normal mean) – possibly because of the effect of prolactin on the production of $1,25(OH)_2D$ (*72*) – the requirement would be about 1040 mg (26.0 mmol) and the recommended intake would be about 1300 mg (32.5 mmol). However, although it is known that bone is lost during lactation and restored after weaning (*73,74*), early reports that this bone loss could be prevented by calcium supplementation (*75*) have not been confirmed in controlled studies (*76-78*). The prevailing view now is that calcium absorption does not increase and may decrease during lactation. It is increasingly thought that lactational bone loss is not a nutritional problem but may be due to parathyroid hormone-related peptide secreted by the breast (*79*) and therefore beyond the control of dietary calcium. In view of this uncertainty, we do not at present recommend any extra calcium allowance during lactation; any risk to adolescent mothers is covered by our general recommendation of 1300 mg for adolescents.

Upper limits

Because of the inverse relationship between fractional calcium absorption and calcium intake (***Figure 15***), a calcium supplement of 1000 mg (2.5 mmol) added to a Western-style diet only increases urinary calcium by about 60 mg (1.5 mmol). Urinary calcium also rises very slowly with intake (slope of 5–10 percent) and the risk of kidney stones from dietary hypercalciuria must therefore be negligible. In fact, it has been suggested that dietary calcium may protect against renal calculi because it binds dietary oxalate and reduces oxalate excretion (*80,81*). Toxic effects of a high calcium intake have only been described when the calcium is given as the carbonate in very high doses; this toxicity is caused as much by the alkali as by the calcium and is due to precipitation of calcium salts in renal tissue (milk-alkali syndrome) (*82*). However, in practice we recommend an upper limit on calcium intake of 3 g (75 mmol).

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Comparisons with other recommendations

Our recommendations in ***Table 31*** can be compared with the current recommendations of Australia, the United Kingdom, the European Union, and the United States and Canada in ***Table 32***. Our recommendations for adults are very close to those of the United States and Canada but higher than those of the United Kingdom and Australia, which do not take into account insensible losses, and higher than those of the European Union, which assumed 30 percent absorption of dietary calcium. The British and European values make no allowance for ageing or menopause. Recommendations for other high-risk groups are very similar in all five sets of recommendations except for the rather low allowance for infants by the United States and Canada. Nonetheless, and despite this broad measure of agreement, we have some misgivings about the application of these recommendations, all of which rely ultimately on data from developed nations, to developing countries where other dietary constituents – such as animal protein and sodium – and environmental factors may be very different. We shall therefore in the next sections briefly review current knowledge about the prevalence of osteoporosis across racial national boundaries and its relevance to calcium requirement.

Ethnic and environmental variations in the prevalence of osteoporosis

Intakes of calcium have been known for many years to vary greatly from one country to another, as is clearly shown in FAO food balance sheets (***Table 30***). Until fairly recently, it was widely assumed that low calcium intakes had no injurious consequences. This view of the global situation underlay the very conservative adequate calcium intakes recommended by WHO/FAO in 1962 (*2*). At that time, osteoporosis was still regarded as a bone matrix disorder and the possibility that it could be caused by calcium deficiency was barely considered. The paradigm has changed since then. Calcium deficiency is taken more seriously than it was and the apparent discrepancy between calcium intake and bone status across the world has attracted more attention. In general, recent investigations have sought for evidence of low bone density and high fracture incidence in countries where calcium intake is low; rickets has not been looked for, but the low calcium rickets recently reported from Nigeria (*83*) will no doubt attract attention.

This issue can be considered at several levels. The first level is genetic: Is there a genetic (ethnic) difference in the prevalence of osteoporosis between racial groups within a given society? The second level might be termed environmental-cultural (e.g., dietary): Is there a difference in the prevalence of osteoporosis between national groups of similar ethnic composition? The third level is environmental-geographical (e.g., latitude, affluence, and lifestyle): Is there a difference in the prevalence of osteoporosis between countries regardless of ethnic composition? At each of these levels, the prevalence of osteoporosis can in theory be determined in at least two ways – from the distribution of bone density within the population and from the prevalence of fractures, notably hip fractures. In practice, hip fracture data (or mortality from falls for elderly people which has been used as a surrogate [*84*]) are more readily available than bone densitometry.

Ethnicity

Comparisons between racial groups within countries suggest substantial racial differences in the prevalence of osteoporosis. This was probably first noted by Trotter (*85*) when she showed that bone density (weight/volume) was significantly higher in skeletons from black than from Caucasian subjects in the United States. It was later shown that hip fracture rates were lower in blacks than Caucasians in South Africa (*86*) and the United States (*87*). These observations have been repeatedly confirmed (*88,89*) without being fully explained but appear to be genetic in origin because the difference in bone status between blacks and Caucasians in the United States is already apparent in childhood (*90*) and cannot be explained by differences in body size (*91*). The difference in fracture rates between blacks and Caucasians cannot be

explained by differences in hip axis length (*91*); it seems to be largely or wholly due to real differences in bone density. Comparisons between Caucasians and Samoans in New Zealand (*92*) have also shown the latter to have the higher bone densities whereas the lower bone densities of Asians than Caucasians in New Zealand are largely accounted for by differences in body size (*92*). In the United States, fracture rates are lower among Japanese than among Caucasians but may be accounted for by their shorter hip axis length (*93*) and their lower incidence of falls (*94*). Bone density is generally lower in Asians than Caucasians within the United States (*95*) but this again is largely accounted for by differences in body size (*96*). There are also lower hip fracture rates for Hispanics, Chinese, Japanese, and Koreans than Caucasians in the United States (*97,98*). The conclusion must be that there are probably genetic factors influencing the prevalence of osteoporosis and fractures, but it is impossible to exclude the role of differences in diet and lifestyle between ethnic communities within a country.

Geography

There are wide geographical variations in hip fracture incidence, which cannot be accounted for by ethnicity. In the United States, the age-adjusted incidence of hip fracture in Caucasian women aged 65 and over varied with geography but was high everywhere – ranging from 700 to 1000 per 100 000 per year (*99*). Within Europe, the age-adjusted hip fracture rates ranged from 280 to 730 per 100 000 women in one study (*100*) and from 419 to 545 per 100 000 in another study (*97*) in which the comparable rates were 52.9 in Chile, 94.0 in Venezuela, and 247 in Hong Kong. In another study (*101*) age-adjusted hip fracture rates in women in 12 European countries ranged from 46 per 100 000 per year in Poland to 504 per 100 000 in Sweden, with a marked gradient from south to north and from poor to rich. In Chinese populations, the hip fracture rate is much lower in Beijing (87–97 per 100 000) than in Hong Kong (181–353 per 100 000) (*102*), where the standard of living is higher. Thus there are marked geographic variations in hip fracture rates within the same ethnic groups.

Ethnicity, environment, and lifestyle

The conclusion from the above is that there are probably ethnic differences in hip fracture rates within countries but also environmental differences within the same ethnic group which may complicate the story. For international comparisons on a larger scale, it is impossible to separate genetic from environmental factors, but certain patterns emerge which are likely to have biological meaning. The most striking of these is the positive correlation between hip fracture rates and standard of living noted by Hegsted when he observed that osteoporosis was largely a disease of affluent Western cultures (*103*). He based this conclusion on a previously published review of hip fracture rates in 10 countries (*104*), which strongly suggested a correlation between hip fracture rate and affluence. Another review of 19 regions and racial groups (*105*) confirmed this by showing a gradient of age- and sex-adjusted hip fracture rates from 31 per 100 000 in South African Bantu to 968 per 100 000 in Norway. In the analysis of hip fracture rates in Beijing and Hong Kong referred to above (*102*), it was noted that the rates in both cities were much lower than in the United States. Many other publications point to the same conclusion – that hip fracture prevalence (and by implication osteoporosis) is related to affluence and, consequently, to animal protein intake, as Hegsted pointed out, but also and paradoxically to calcium intake.

The calcium paradox

The paradox that hip fracture rates are higher in developed nations where calcium intake is high than in developing nations where calcium intake is low clearly calls for an explanation. Hegsted (*103*) was probably the first to note the close relation between calcium and protein intakes across the world (which is also true within nations [*63*]) and to hint at but dismiss the

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possibility that the adverse effect of protein might outweigh the positive effect of calcium on calcium balance. Only recently has fracture risk been shown to be a function of protein intake in American women (*106*). There is also suggestive evidence that hip fracture rates (as judged by mortality from falls in elderly people across the world) are a function of protein intake, national income, and latitude (*107*). The latter is particularly interesting in view of the strong evidence of vitamin D deficiency in hip fracture patients in the developed world (*108-114*) and the successful prevention of such fractures with small doses of vitamin D and calcium (*115,116*) (see ***Chapter 8***). It is therefore possible that hip fracture rates may be related to protein intake, vitamin D status, or both and that either of these factors could explain the calcium paradox. We shall therefore consider how these and other nutrients (notably sodium) affect calcium requirement.

Nutritional factors affecting calcium requirements

The calculations of calcium requirements proposed above were based on data from developed countries (notably the United States and Norway) and can only be applied with any confidence to nations and populations with similar dietary cultures. Other dietary cultures may entail different calcium requirements and call for different recommendations. In particular, the removal or addition of any nutrient that affects calcium absorption or excretion must have an effect on calcium requirement. Two such nutrients are sodium and animal protein, both of which increase urinary calcium and must be presumed therefore to increase calcium requirement. A third candidate is vitamin D because of its role in calcium homeostasis and calcium absorption.

Sodium

It has been known at least since 1961 that urinary calcium is related to urinary sodium (*117*) and that sodium administration raises calcium excretion, presumably because sodium competes with calcium for reabsorption in the renal tubules. Regarding the quantitative relationships between the renal handling of sodium and calcium, the filtered load of sodium is about 100 times that of calcium (in molar terms) but the clearance of these two elements is similar at about 1 ml/min, which yields about 99 percent reabsorption and 1 percent excretion for both (*118*). However, these are approximations, which conceal the close dependence of urinary sodium on sodium intake and the weaker dependence of urinary calcium on calcium intake. It is an empirical fact that urinary sodium and calcium are significantly related in normal and hypercalciuric subjects on freely chosen diets (*119-122*). The slope of urinary calcium on sodium varies in published work from about 0.6 percent to 1.2 percent (in molar terms); a representative figure is about 1 percent – that is, 100 mmol of sodium (2.3 g) takes out about 1 mmol (40 mg) of calcium (*63,120*). The biological significance of this relationship is supported by the accelerated osteoporosis induced by feeding salt to rats on low-calcium diets (*123*) and the effects of salt administration and salt restriction on markers of bone resorption in postmenopausal women (*124,125*). Because salt restriction lowers urinary calcium, it is likely also to lower calcium requirement and, conversely, salt feeding is likely to increase calcium requirement. This is illustrated in ***Figure 18***, which shows that lowering sodium intake by 100 mmol (2.3 g) from, for example, 150 to 50 mmol (3.45 to 1.15 g), reduces the theoretical calcium requirement from 840 mg (21 mmol) to 600 mg (15 mmol). However, the implications of this on calcium requirement across the world cannot be computed because information about sodium intakes is available from very few countries (*126*).

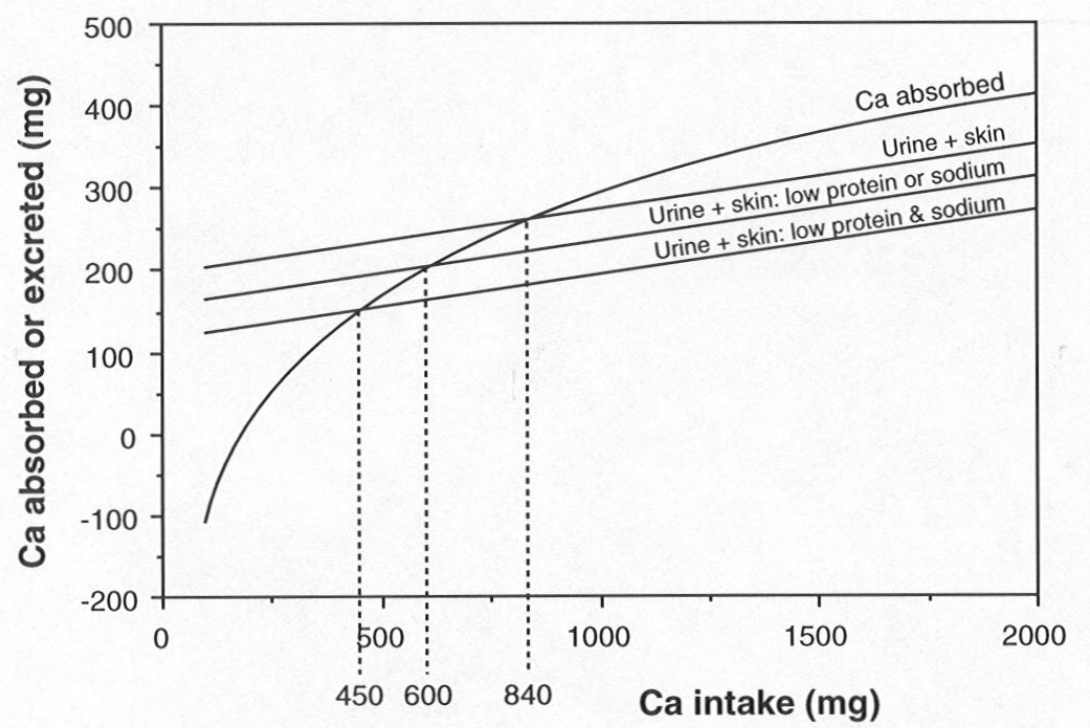
Protein

The positive effect of dietary protein – particularly animal protein – on urinary calcium has also been known at least since the 1960s (*127-129*). One study found that 0.85 mg of calcium

was lost for each gram of protein in the diet (*130*). A meta-analysis of 16 studies in 154 adult humans on protein intakes up to 200 g found that 1.2 mg of calcium was lost in the urine for every 1g rise in dietary protein (*131*). A small but more focussed study showed a rise of 40 mg in urinary calcium when dietary animal protein was raised from 40 to 80 g (i.e., within the physiological range) (*132*). This ratio of urinary calcium to dietary protein ratio (1mg to 1g) is a representative value, which we have adopted. This means that a 40g reduction in animal protein intake from 60 to 20 g (or from the developed to the developing world [***Table 30***]) would reduce calcium requirement by the same amount as a 2.3g reduction in dietary sodium, i.e. from 840 to 600 mg. (***Figure 18***).

Figure 18

The effect of varying protein or sodium intake on theoretical calcium requirement.



Note: In a western-style diet, absorbed calcium matches urinary and skin calcium at an intake of 840 mg as in ***Figure 14***. Reducing animal protein intakes by 40 g reduces the intercept value and requirement to 600 mg. Reducing both sodium and protein reduces the intercept value to 450 mg.

How animal protein exerts its effect on calcium excretion is not fully understood. A rise in glomerular filtration rate in response to protein has been suggested as one factor (*128*) but this is unlikely to be important in the steady state. The major mechanisms are thought to be the effect of the acid load contained in animal proteins and the complexing of calcium in the renal tubules by sulphate and phosphate ions released by protein metabolism (*133,134*). Urinary calcium is significantly related to urinary phosphate (as well as to urinary sodium), particularly in subjects on restricted calcium intakes or in the fasting state, and most of the phosphorus in the urine of people on Western-style diets comes from animal protein in the diet (*63*). Similar considerations apply to urinary sulphate but it is probably less important than the phosphate ion because the association constant for calcium sulphate is lower than that for calcium phosphate (*135*). The empirical observation that each 1 g of protein results in 1 mg of calcium in the urine agrees very well with the phosphorus content of animal protein (about 1 percent by weight) and the observed relationship between calcium and phosphate in the urine (*63*).

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Vitamin D

One of the first observations made on vitamin D after it had been identified in 1918 (*136*) was that it promoted calcium absorption (*137*). It is now well established that vitamin D (synthesised in the skin under the influence of sunlight) is converted to 25OHD in the liver and then to $1,25(OH)_2D$ in the kidneys and that the latter metabolite controls calcium absorption (*21*) (see ***Chapter 8***). However, plasma 25OHD closely reflects vitamin D nutritional status and because it is the substrate for the renal enzyme which produces $1,25(OH)_2D$, it could have an indirect effect on calcium absorption. The plasma level of $1,25\text{-}(OH)_2D$ is principally regulated is through increased gene expression of the 1-α-hydroxylase (CYP1α) and not by increased 25OHD levels. This has been seen consistently in animal studies, and the high calcium absorption (138) and high plasma $1,25\text{-}(OH)_2D$ (139) observed in Gambian mothers is consistent with this type of adaptation. However, increasing latitude may compromise vitamin D synthesis to the degree that 25OHD levels are no longer sufficient to sustain adequate $1,25\text{-}(OH)_2D$ levels and efficient intestinal calcium absorption, although this theory remains unproved. Regardless of the mechanism of compromised vitamin D homeostasis, the differences in calcium absorption efficiency have a major effect on theoretical calcium requirement, as illustrated in ***Figure 18***, which shows that an increase in calcium absorption of as little as 10 percent reduces the intercept of excreted and absorbed calcium (and therefore calcium requirement) from 840 to 680 mg. (The figure also shows the great increase in calcium requirement that must result from any impairment of calcium absorption.)

Implications

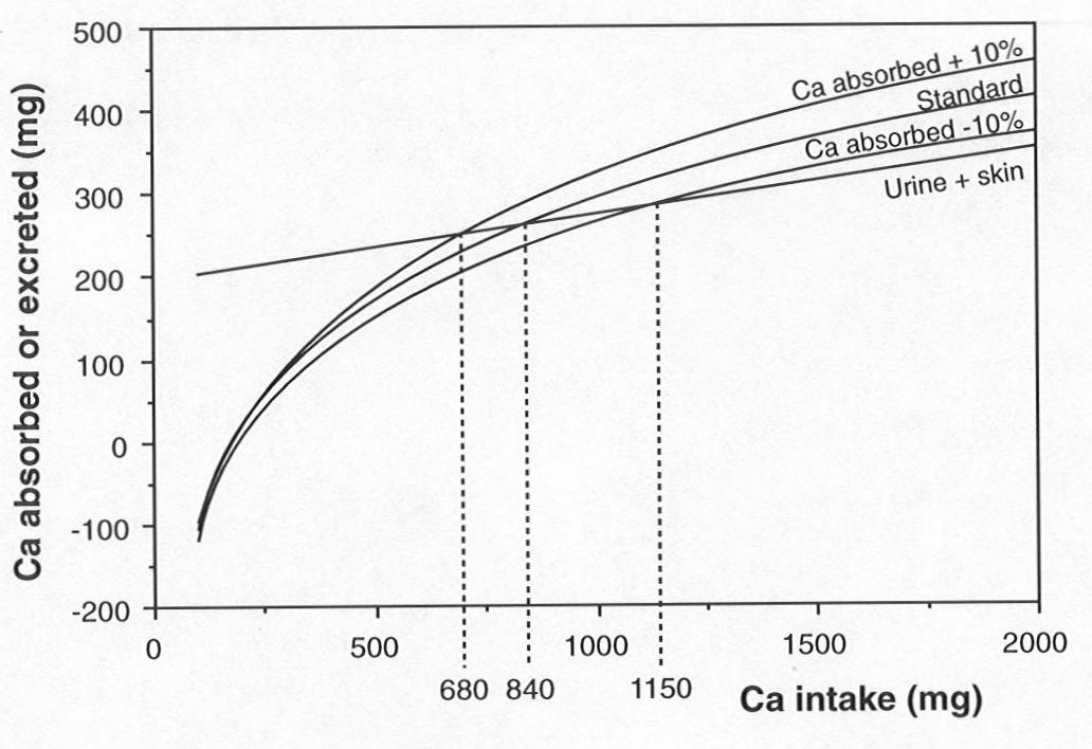
The major reduction in theoretical calcium requirement which follows animal protein restriction has led us to attempt to show in ***Table 33*** how the calcium allowances recommended in ***Table 31*** could be modified to apply to nations where the animal protein intake per capita is around 20–40 g rather than around the 60–80 g in developed countries. These hypothetical allowances take into account the need to protect children, in whom skeletal needs are much more important determinants of calcium requirement than are urinary losses and in whom calcium supplementation had a beneficial effect in the Gambia (*140*). However, adjustment for animal protein intake has a major effect on the recommended calcium allowances for adults as the table shows. It also brings the allowances nearer to what the actual calcium intakes are in many parts of the world.

If sodium intakes were also lower in developing than developed nations or urinary sodium were reduced for other reasons such as increased sweat losses, the calcium requirement might be even lower, for example, 450 mg (***Figure 18***). This would be reduced still further by any increase in calcium absorption, whether resulting from better vitamin D status because of increased sunlight exposure or for other reasons, as illustrated in ***Figure 19***. Because the increase in calcium absorption in the Gambia is much more than 10 percent (*138*), this is likely to have a major – although not at present calculable – effect on calcium requirement there. However, the adjusted bone mineral density in Gambian women is reported to be some 20 percent lower in the spine (but not in the forearm) than in British women (*141*), which emphasises the need for more data from developing countries.

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Figure 19

The effect of varying calcium absorptive efficiency on theoretical calcium requirement



Note: At normal calcium absorption, the intercept of urinary plus skin calcium meets absorbed calcium at an intake of 840 mg as in ***Figure 14***. A 10 percent reduction in calcium absorption raises the intercept and requirement to 1150 mg and a 10 percent increase in calcium absorption reduces it to 680 mg.

Table 33

Theoretical calcium allowances based on an animal protein intake of 20–40 g

Group	Recommended intake mg/day
Infants and children	
0–6 months	
Human milk	300
Cow milk	400
7–12 months	450
1–3 years	500
4–6 years	550
7–9 years	700
Adolescents, 10–18 years	1000[a]
Adults	
Females	
19 years to menopause	750
Postmenopause	800
Males	
19–65 years	750
65 +	800
Pregnancy (last trimester)	800
Lactation	750

[a] Particularly during the growth spurt.

Conclusions

Calcium is an essential nutrient that plays a vital role in neuromuscular function, many enzyme-mediated processes, blood clotting, and providing rigidity to the skeleton by virtue of its phosphate salts. Its non-structural roles require the strict maintenance of ionised calcium concentration in tissue fluids at the expense of the skeleton if necessary and it is therefore the skeleton which is at risk if the supply of calcium falls short of requirement.

Calcium requirements are essentially determined by the relationship between absorptive efficiency and excretory rate – excretion being through the bowel, kidneys, skin, hair, and nails. In adults, the rate of calcium absorption from the gastrointestinal tract needs to match the rate of all losses from the body if the skeleton is to be preserved; in children and adolescents, an extra input is needed to cover the requirements of skeletal growth.

Compared with that of other minerals, calcium economy is relatively inefficient. On most intakes, only about 25–30 percent of dietary calcium is effectively absorbed and obligatory calcium losses are relatively large. Absorbed calcium has to match these obligatory losses and the dietary intake has to be large enough to ensure this rate of absorption if skeletal damage is to be avoided. The system is subject to considerable inter-individual variation in both calcium absorption and excretion for reasons that are not fully understood but which include vitamin D status, sodium and protein intake, age, and menopausal status in women. Although it needs to be emphasised that calcium deficiency and negative calcium balance must sooner or later lead to osteoporosis, this does not mean that all osteoporosis can be attributed to calcium deficiency. On the contrary, there may be more osteoporosis in the world from other causes. Nonetheless, it would probably be generally agreed that any form of osteoporosis must inevitably be aggravated by negative external calcium balance. Such negative balance – even for short periods – is prejudicial because it takes so much longer to rebuild bone than to destroy it. Bone that is lost, even during short periods of calcium deficiency, is only slowly replaced when adequate amounts of calcium become available.

In seeking to define advisable calcium intakes on the basis of physiologic studies and clinical observations, nutrition authorities have to rely largely on data from developed nations living at relatively high latitudes. Although it is now possible to formulate recommendations that are appropriate to different stages in the life cycle of the populations of these nations, extrapolation from these figures to other cultures and nutritional environments can only be tentative and must rely on what is known of nutritional and environmental effects on calcium absorption and excretion. Nonetheless, we have made an attempt in this direction, knowing that our speculative calculations may be incorrect because of other variables not yet identified.

No reference has been made in this account to the possible beneficial effects of calcium in the prevention or treatment of pre-eclampsia (*142*), colon cancer (*143*), or hypertension (*144*) and no attempt has been made to use these conditions as endpoints on which to base calcium intakes. In each of the above conditions, epidemiologic data suggested an association with calcium intake, and experimentation with increased calcium intakes has now been tried. In each case the results have been disappointing, inconclusive, or negative (*145-147*) and have stirred controversy (*148-150*). Because there is no clear consensus about optimal calcium intake for prevention or treatment of these conditions and also no clear mechanistic ideas on how dietary calcium intakes affect them, it is not possible to allow for the effect of health outcomes in these areas on our calcium recommendations. However, although the anecdotal information and positive effects of calcium observed in these three conditions cannot influence our recommendations, they do suggest that generous calcium allowances may confer other benefits besides protecting the skeleton. Similarly, no reference has been made to the effects of physical activity, alcohol, smoking, or other known risk

factors on bone status because the effects of these variables on calcium requirement are beyond the realm of simple calculation.

Future research

Future research should:

- recognise that there is an overwhelming need for more studies of calcium metabolism in developing countries;
- investigate further the cultural, geographical, and genetic bases for differences in calcium intakes in different groups in developing nations;
- establish the validity of different recommended calcium intakes based on animal protein and sodium intakes;
- clarify the role of dietary calcium in pre-eclampsia, colon cancer, and hypertension; and
- investigate the relationship of latitude, sun exposure, and synthesis of vitamin D with intestinal calcium absorption in different geographical locations.

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Chapter 12
Iodine

Summary of the human metabolic processes requiring iodine

At present, the only physiologic role known for iodine in the human body is in the synthesis of thyroid hormones by the thyroid gland. Therefore, the dietary requirement of iodine is determined by normal thyroxine (T_4) production by the thyroid gland without stressing the thyroid iodide trapping mechanism or raising thyroid stimulating hormone (TSH) levels.

Iodine from the diet is absorbed throughout the gastrointestinal tract. Dietary iodine is converted into the iodide ion before it is absorbed. The iodide ion is bio-available and absorbed totally from food and water. This is not true for iodine within thyroid hormones ingested for therapeutic purposes. Iodine enters the circulation as plasma inorganic iodide, which is cleared from circulation by the thyroid and kidney. The iodide is used by the thyroid gland for synthesis of thyroid hormones, and the kidney excretes iodine with urine. The excretion of iodine in the urine is a good measure of iodine intake. In a normal population with no evidence of clinical iodine deficiency either in the form of endemic goitre or endemic cretinism, urinary iodine excretion reflects the average daily iodine requirement. Therefore, for determining the iodine requirements, the important indexes are serum T_4 and TSH levels (indicating normal thyroid status) and urinary iodine excretion. The simplified diagram of metabolic circuit of iodine is given in ***Figure 20*** (*1*).

Overview of significant scientific information

All biologic actions of iodide are attributed to the thyroid hormones. The major thyroid hormone secreted by the thyroid gland is T_4 (tetra-iodo-thyronine). T_4 in circulation is taken up by the cells and is de-iodinated by the enzyme 5' prime-mono-de-iodinase in the cytoplasm to convert it into tri-iodo-thyronine (T_3), the active form of thyroid hormone. T_3 traverses to the nucleus and binds to the nuclear receptor. All the biologic actions of T_3 are mediated through the binding to the nuclear receptor, which controls the transcription of a particular gene to bring about the synthesis of a specific protein.

The physiologic actions of thyroid hormones can be categorised as 1) growth and development and 2) control of metabolic processes in the body. Thyroid hormones play a major role in the growth and development of brain and central nervous systems in humans from the 15th week of gestation to age 3 years. If iodine deficiency exists during this period and results in thyroid hormone deficiency, the consequence is derangement in the development of brain and central nervous system. These derangements are irreversible, the most serious form being that of cretinism. The effect of iodine deficiency at different stages of life is given in ***Table 34*** (*2*).

The other physiologic role of thyroid hormone is to control several metabolic processes in the body. These include carbohydrate, fat, protein, vitamin, and mineral metabolism. For example, thyroid hormone increases energy production, increases lipolysis, and regulates neoglucogenesis, and glycolysis.

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Figure 20

Simplified diagram of the metabolic circuit of iodine

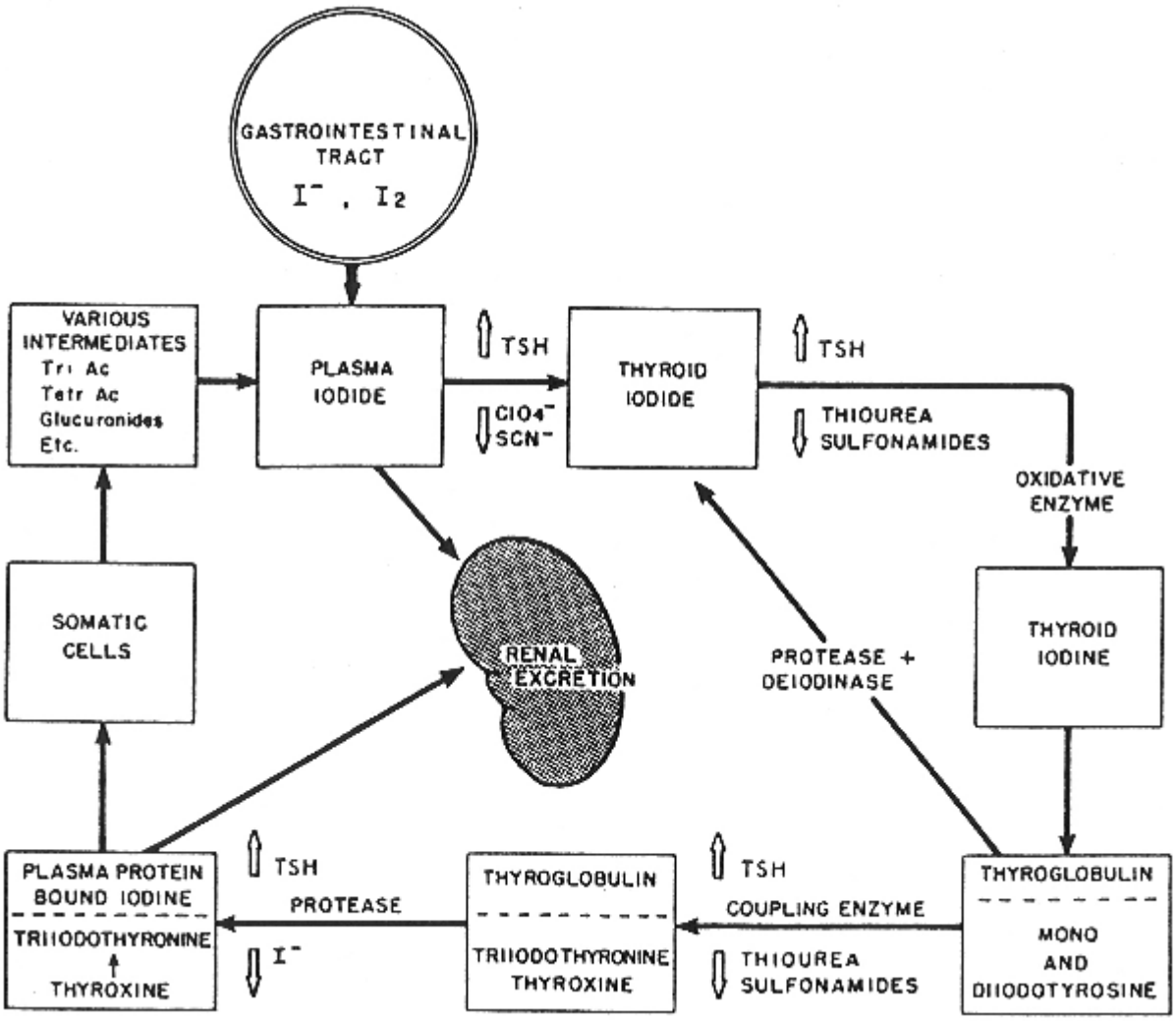


Table 34

The spectrum of iodine deficiency disorders

Life stage	Effects
Foetus	Abortions Stillbirths Congenital anomalies Increased perinatal mortality Increased infant mortality Neurological cretinism: mental deficiency, deaf mutism, spastic diplegia, and squint Myxedematous cretinism: mental deficiency and dwarfism Psychomotor defects
Neonate	Neonatal goitre Neonatal hypothyroidism
Child and Adolescent	Goitre Juvenile hypothyroidism Impaired mental function Retarded physical development
Adult	Goitre with its complications Hypothyroidism Impaired mental function

Population at risk

Iodine deficiency affects all stages of human life, from the intra-uterine stage to old age, as shown in ***Table 34***. However, pregnant women, lactating women, women of reproductive age, and children younger than 3 years are considered to be at high risk (*3*). During foetal and neonatal growth and development, iodine deficiency leads to irreversible damage to the brain and central nervous system.

Dietary sources

The iodine content of food depends on the iodine content of the soil in which it is grown. The iodine present in the upper crust of earth is leached by glaciation and repeated flooding and is carried to the sea. Sea water is, therefore, a rich source of iodine (*4*). The seaweed located near coral reefs has an inherent biologic capacity to concentrate iodine from the sea. The reef fish which thrive on seaweed are rich in iodine. Thus, a population consuming seaweed and reef fish has a high intake of iodine, as the case in Japan. The amount of iodine intake by the Japanese is in the range of 2–3 mg/day (*4*). In several areas of Asia, Africa, Latin America, and parts of Europe, iodine intake varies from 20 to 80 μg/day. In the United States and Canada and some parts of Europe, the intake is around 500 μg/day. The average iodine content of foods (fresh and dry basis) as reported by Koutras *et al.* (*4*) is given in ***Table 35.***

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Table 35

Average iodine content of foods (in µg/g)

Food	Fresh basis		Dry basis	
	Mean	**Range**	**Mean**	**Range**
Fish (fresh water)	30	17–40	116	68–194
Fish (marine)	832	163–3180	3715	471–4591
Shellfish	798	308–1300	3866	1292–4987
Meat	50	27–97	—	—
Milk	47	35–56	—	—
Eggs	93	—	—	—
Cereal grains	47	22–72	65	34–92
Fruits	18	10–29	154	62–277
Legumes	30	23–36	234	223–245
Vegetables	29	12–201	385	204–1636

The iodine content of food varies with geographic location because there is a large variation in the iodine content of the inorganic world (***Table 36***) (*4*). Thus, the average iodine content of foods shown in ***Table 35*** can not be used universally for estimating iodine intake.

Recommended intake

The daily intake of iodine recommended by the National Research Council of the US National Academy of Sciences in 1989 was 40 µg/day for young infants (0–6 months), 50 µg/day for older infants (6–12 months), 60–100 µg/day for children (1–10 years), and 150 µg/day for adolescents and adults (*5*). These values approximate 7.5 µg/kg/day for age 0–12 months, 5.4 µg/kg/day for age 1–10 years, and 2 µg/kg/day for adolescents and adults. These amounts are proposed to allow normal T_4 production without stressing the thyroid iodide trapping mechanism or raising TSH levels.

Iodine requirements in infancy

The US recommendation of 40 µg/day for infants aged 0–6 months (or 8 µg/kg/day, 7 µg/100 kcal, or 50 µg/l milk) is probably derived from the observation that until the late 1960s the iodine content of human milk was approximately 50 µg/l and from the concept that nutrition of the human-milk-fed infant growing at a satisfactory rate has been the standard against which nutrition requirements have been set (6, 7). However, more recent data indicate that the iodine content of human milk varies markedly as a function of the iodine intake of the population. For example, it ranges from 20 to 330 µg/l in Europe and from 30 to 490 µg/l in the United States (6, 8). It is as low as 12 µg/l under conditions of severe iodine deficiency (6, 8). An average human-milk intake of 750 ml/day would give an intake of iodine of about 60

µg/day in Europe and 120 µg/day in the United States. The upper US value (490 µg/l) would provide 368 µg/day or 68 µg/kg/day for a 5-kg infant. Positive iodine balance in the young infant, which is required for the increasing iodine stores of the thyroid, is achieved only when the iodine intake is at least 15 µg/kg/day in full-term infants and 30 µg/kg/day in pre-term infants (9). The iodine requirement of pre-term infants is twice that of term infants because of a 50 percent lower retention of iodine by pre-term infants. This corresponds approximately to an iodine intake of 90 µg/day. (This is probably based on the assumption of average body weight of 6 kg for a child of 6 months, the mid-age of an infant.) This value is twofold higher than the US recommendations.

On the basis of these considerations, a revision is proposed for the earlier World Health Organization (WHO), United Nations Children's Fund (UNICEF), and International Council for the Control of Iodine Deficiency Disorders (ICCIDD) recommendations (10): an iodine intake of 90 µg/day from birth onwards is suggested. To reach this objective, and based on an intake of milk of about 150 ml/kg/day, the iodine content of formula milk should be increased from 50 to 100 µg/l for full-term infants and to 200 µg/l for pre-term infants.

For a urine volume of about 4–6 dl/day from 0 to 3 years, the urinary concentration of iodine indicating iodine repletion should be in the range of 150–220 µg/l (1.18-1.73µmol/l) in infants aged 0–36 months. Such values have been observed in iodine-replete infants in Europe (11), Canada (12), and the United States (12). Under conditions of moderate iodine deficiency, as seen in Belgium, the average urinary iodine concentration is only 50–100 µg/l (0.39-0.79µmol/l) in this age group. It reaches a stable normal value of 180–220 µg/l (1.41-1.73µmol/l) only after several months of daily iodine supplementation with a physiologic dose of 90 µg/day (***Figure 21***).

Table 36

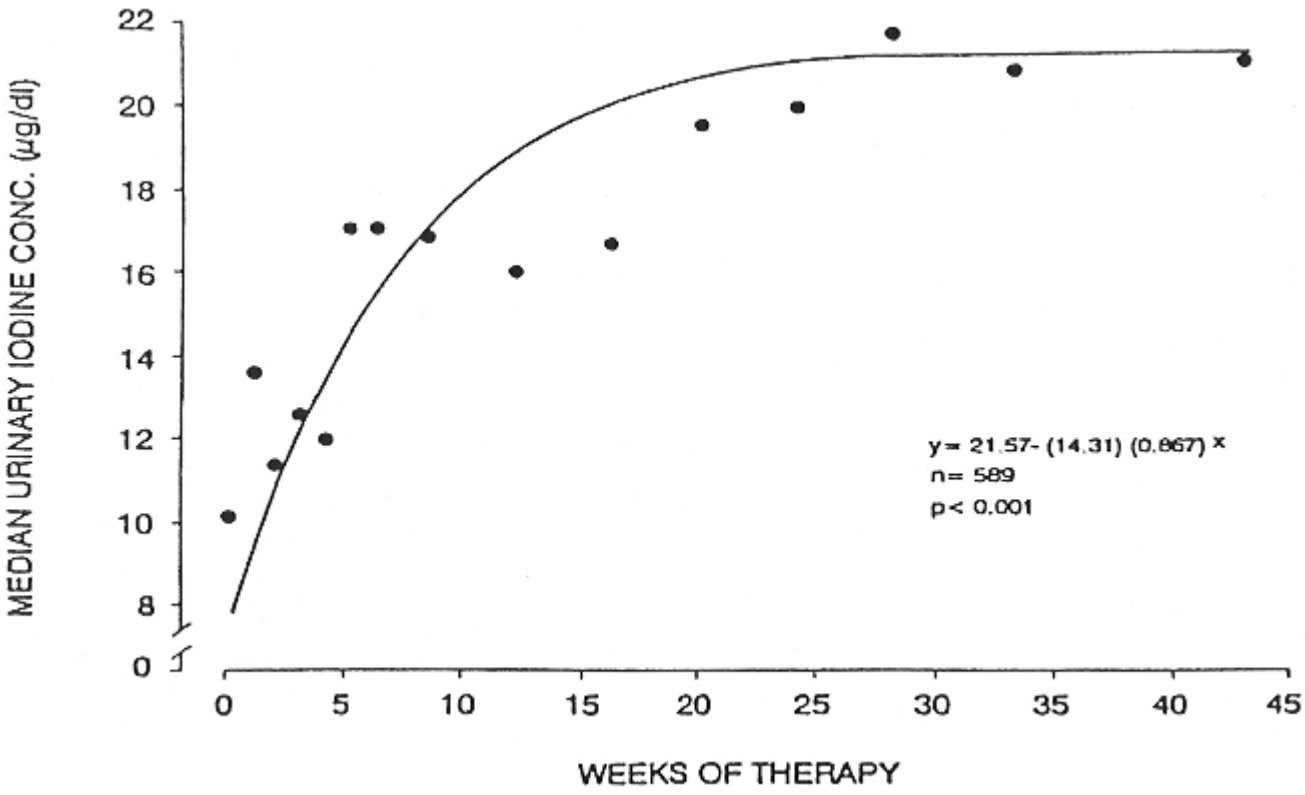
Iodine content of the inorganic world

Location	Iodine content
Terrestrial air	1.0 µg/l
Marine air	100.0 µg/l
Terrestrial water	5.0 µg/l
Sea water	50.0 µg/l
Igneous rocks	500.0 µg/kg
Soils from igneous rocks	9000.0 µg/kg
Sedimentary rocks	1500.0 µg/kg
Soils from sedimentary rocks	4000.0 µg/kg
Metamorphic rocks	1600.0 µg/kg
Soils from the metamorphic rocks	5000.0 µg/kg

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Figure 21

Changes over time for the median urinary concentration of iodine in healthy Belgian infants aged 6–36 months and supplemented with iodine at 90 µg/kg/day for 44 weeks



Each point represents 32–176 iodine determinations (13).

When the urinary iodine concentration in neonates and young infants is below a threshold of 50-60 µg/l (0.39-0.47µmol/l), corresponding to an intake of 25–35 µg/day, there is a sudden increase in the prevalence of neonatal serum TSH values in excess of 50 mU/ml, indicating sub-clinical hypothyroidism and eventually complicated by transient neonatal hypothyroidism (14). When the urinary iodine concentration is in the range of 10–20 µg/l (0.08-0.16µmol/l), as observed in severe endemic goitre regions, up to 10 percent of the neonates have overt severe hypothyroidism, with serum TSH levels above 100 mU/mL and serum T_3 values below 30 µg/l (39 nmol/L) (14). Untreated, these infants progress to myxedematous endemic cretinism (15).

Thus, the iodine requirement of the young infant approximates 15 µg/kg/day (30 µg/kg/day in pre-term infants). Hyperthyrotropinemia (high levels of serum TSH), indicating sub-clinical hypothyroidism with the risk of brain damage, occurs when the iodine intake is about one-third of this value, and dramatic neonatal hypothyroidism resulting in endemic cretinism occurs when the intake is about one-tenth of this value.

Iodine requirements in children

The daily iodine need on a body weight basis decreases progressively with age. A study by Tovar and colleagues (16) correlating 24-hour thyroid radioiodine uptake and urinary iodine excretion in 9–13-year-old schoolchildren in rural Mexico suggested that an iodine intake in excess of 60 µg/day is associated with a 24-hour thyroidal radioiodine uptake below 30 percent. Lower excretion values are associated with higher uptake values. This would approximate 3 µg/kg/day in an average size 10-year-old child (approximate body weight of 20 kg), so that an intake of 60–100 µg/day for child of 1–10 years seems appropriate. These requirements are based on the body weight of Mexican children who participated in this study. The average body weight of a 10-year-old child, as per the Food and Agriculture Organization references, is 25 kg. Thus, the iodine requirement for a 1–10-year-old child would be 90–120 µg/day.

Iodine requirements in adults

Iodine at 150 µg/day for adolescents and adults is justified by the fact that it corresponds to the daily urinary excretion of iodine and to the iodine content of food in non-endemic areas (areas where iodine intake is adequate) (5). It also provides the iodine intake necessary to maintain the plasma iodide level above the critical limit of 0.10 µg/dl, which is the average level likely to be associated with the onset of goitre (17). Moreover, this level of iodine intake is required to maintain the iodine stores of the thyroid above the critical threshold of 10 mg, below which an insufficient level of iodisation of thyroglobulin leads to disorders in thyroid hormone synthesis (18).

Data reflecting either iodine balance or its effect on thyroid physiology can help to define optimal iodine intake. In adults and adolescents in equilibrium with their nutritional environment, most dietary iodine eventually appears in the urine, so the urinary iodine concentration is a useful measure for assessing iodine intake. For this, casual samples are sufficient if enough are collected and if they accurately represent a community (19). A urinary iodine concentration of 100 µg/L corresponds to an intake of about 150 µg/day in the adult. Median urinary iodine concentrations below 100 µg/l in a population are associated with increases in median thyroid size and in serum TSH and thyroglobulin values. Correction of the iodine deficiency will bring all these measures back into the normal range. Recent data from the Thyro-Mobil project in Europe have confirmed these relations by showing that the largest thyroid sizes are associated with the lowest urinary iodine concentrations (20). Once a median urinary iodine excretion of about 100 µg/L is reached, the ratio of thyroid size to body size remains fairly constant. Moulopoulos *et al.* (21) reported that a urinary iodine excretion between 151 and 200 µg/g creatinine (1.18-1.57 µmol/g creatinine), corresponding to a concentration of about 200 µg/l (1.57 µmol/l), gave the lowest values for serum TSH in a non-goitrous population. Similar recent data from Australia show that the lowest serum TSH and thyroglobulin values were associated with urine containing 200–300 µg iodine/g creatinine (1.57-2.36µmol/g creatinine) (22).

Other investigations followed serum TSH levels in subjects without thyroid glands who were given graded doses of T_4 and found that euthyroidism established in adults with an average daily dose of 100 µg T_4 would require at least 65 µg of iodine with maximal efficiency of iodine use by the thyroid. In practice such maximal efficiency is never obtained and therefore considerably more iodine is necessary. Data from controlled observations associated a low urinary iodine concentration with a high goitre prevalence, high radioiodine uptake, and low thyroidal organic iodine content (23). Each of these measures reached a steady state once the urinary iodine excretion was 100 µg/l (0.78 µmol/l)or greater.

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Iodine requirements in pregnancy

The iodine requirement during pregnancy is increased to provide for the needs of the foetus and to compensate for the increased loss of iodine in the urine resulting from an increased renal clearance of iodine during pregnancy (24). These requirements have been derived from studies of thyroid function during pregnancy and in the neonate under conditions of moderate iodine deficiency. For example, in Belgium, where the iodine intake is estimated to be 50–70 µg/day (25), thyroid function during pregnancy is characterised by a progressive decrease of the serum concentrations of thyroid hormones and an increase in serum TSH and thyroglobulin. Thyroid volume progressively increases and is above the upper limit of normal in 10 percent of the women by the end of pregnancy. Serum TSH and thyroglobulin are still higher in the neonates than in the mothers (26). These abnormalities are prevented only when the mother receives a daily iodide supplementation of 161 µg/day during pregnancy (derived from 131 µg potassium iodide and 100 µg T_4 given daily) (27). T_4 with iodine was probably administered to the pregnant women to rapidly correct sub-clinical hypothyroidism, which would not have occurred if iodine had been administered alone. These data indicate that the iodine intake required to prevent the onset of sub-clinical hypothyroidism of mother and foetus during pregnancy, and thus to prevent the possible risk of brain damage of the foetus, is approximately 200 µg/day.

On the basis of the considerations reviewed above for the respective population groups to meet the daily iodine requirements, revisions of the current recommendations for daily iodine intake by WHO, UNICEF, and ICCIDD (10) are proposed; these proposed revisions are presented in ***Table 37***.

Table 37

Proposed revision for daily iodine intake recommendations of 1996 by the World Health Organization, United Nations Children's Fund, and International Council for the Control of Iodine Deficiency Disorders

Population sub-groups	Total iodine intake µg/day	Iodine µg/kg/day
Infants (first 12 months)	90[a]	15.0
Children (1–6 years)	90	6.0
Schoolchildren (7–12 years)	120	4.0
Adults (12+ years)	150	2.0
Pregnant and lactating women	200	3.5

[a] Revised to 90 µg from the earlier recommendation of 50 µg.

Upper limit of iodine intake for different age groups

An iodine excess also can be harmful to the thyroid of infants by inhibiting the process of synthesis and release of thyroid hormones (Wolff-Chaikoff effect) (28). The threshold upper limit of iodine intake (the intake beyond which thyroid function is inhibited) is not easy to define because it is affected by the level of iodine intake before exposure to iodine excess. Indeed, long-standing moderate iodine deficiency is accompanied by an accelerated trapping of iodide and by a decrease in the iodine stores within the thyroid (18). Under these conditions, the critical ratio between iodide and total iodine within the thyroid, which is the starting point of the Wolff-Chaikoff effect, is more easily reached during iodine depletion

than under normal conditions. In addition, the neonatal thyroid is particularly sensitive to the Wolff-Chaikoff effect because the immature thyroid gland is unable to reduce the uptake of iodine from the plasma to compensate for increased iodine ingestion (29). For these reasons transient neonatal hypothyroidism or transient hyperTSHemia after iodine overload of the mother, especially after the use of povidone iodine, has been reported more frequently in European countries such as in Belgium, France, and Germany, which have prevailing moderate iodine deficiency (30-33).

Iodine intake in areas of moderate iodine deficiency

In a study in Belgium, iodine overload of mothers (cutaneous povidone iodine) increased the milk iodine concentration and increased iodine excretion in the term newborns (mean weight about 3 kg). Mean milk iodine concentrations of 18 and 128 µg/dl were associated with average infant urinary iodine excretion levels of 280 and 1840 µg/l (2.20-14.48 µmol/l), respectively (30). Estimated average iodine intakes would be 112 and 736 µg/day, or 37 and 245 µg/kg/day, respectively. The lower dose significantly increased the peak TSH response to exogenous thyroid releasing hormone but did not increase the (secretory) area under the TSH response curve. The larger dose increased both the peak response and secretory area as well as the baseline TSH concentration. Serum T_4 concentrations were not altered, however. Thus, these infants had a mild and transient, compensated hypothyroid state. Non-contaminated mothers secreted milk containing 9.5 µg iodine/dl, and the mean urinary iodine concentration of their infants was 144 µg/l (1.13 µmol/l). These data indicate that modest iodine overloading of term infants in the neonatal period in an area of relative dietary iodine deficiency (Belgium) also can impair thyroid hormone formation.

Similarly, studies in France indicated that premature infants exposed to cutaneous povidone iodine or fluorescinated alcohol-iodine solutions and excreting iodine in urine in excess of 100 µg/day manifested decreased T_4 and increased TSH concentrations in serum (32). The extent of these changes was more marked in premature infants with less than 34 weeks gestation than in those with 35–37 weeks gestation. The full-term infants were not affected. These studies suggest that in Europe the upper limit of iodine intake, which predisposes to blockage of thyroid secretion in premature infants (about 200 µg/day) is 2 to 3 times the average intake from human milk and about equivalent to the upper range of intake.

Iodine intake in areas of iodine sufficiency

Similar studies have not been conducted in the United States, where transient hypothyroidism is rarely seen perhaps because iodine intake is much higher. For example, urinary concentrations of 50 µg/dl and above in neonates, which can correspond to a Wolff-Chaikoff effect in Europe, are frequently seen in healthy neonates in North America (11, 12).

The average iodine intake of infants in the United States in 1978, including infants fed whole cow milk, was estimated by the market-basket approach (34) to be 576 µg/day (standard deviation [SD] 196); that of toddlers was 728 µg/day (SD 315) and of adults was 952 µg/day (SD 589). The upper range for infants (968 µg/day) would provide a daily intake of 138 µg/kg for a 7-kg infant, and the upper range for toddlers (1358 µg/day) would provide a daily intake of 90 µg/kg for a 15-kg toddler.

Table 38 summarises the recommended dietary intake of iodine for age and approximate level of intake which appear not to impair thyroid function in the European studies of Delange in infants, in the loading studies of adults in the United States, or during ingestion of the highest estimates of dietary intake (just reviewed) in the United States (34). Except for the values for premature infants, these probably safe limits are 15–20 times more than the recommended intakes. These data refer to all sources of iodine intake. The average iodine content of infant formulas is approximately 5 µg/dl. The upper limit probably should

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be one that provides a daily iodine intake of no more than 100 µg/kg. For this limit and with the assumption that the total intake is from infant formula, with a daily intake of 150 ml/kg (100 kcal/kg), the upper limit of the iodine content of infant formula would be about 65 µg/dl. The current suggested upper limit of iodine in infant formulas of 75 µg/100 kcal (89µg/500 kJ or 50 µg/dl), therefore, seems reasonable.

Table 38

Recommended dietary intakes of iodine and probable safe upper limits

Group	Recommended µg/kg/day	Upper limit[a] µg/kg/day
Premature infants	30	100
Infants 0–6 months	15	150
Infants 7–12 months	15	140
Children 1–6 years	6	50
School children 7–12 years	4	50
Adolescents and adults (12+ years)	2	30
Pregnancy and lactation	3.5	40

[a] Probably safe.

Excess iodine intake

Excess iodine intake is more difficult to define. Many people regularly ingest huge amounts of iodine – in the range 10–200 mg/day – without apparent adverse effects. Common sources are medicines (e.g., amiodarone contains 75 mg iodine per 200-mg capsule), foods (particularly dairy products), kelp (eaten in large amounts in Japan), and iodine-containing dyes (for radiologic procedures). Excess consumption of salt has never been documented to be responsible for excess iodine intake. Occasionally each of these may have significant thyroid effects, but generally they are tolerated without difficulty. Braverman *et al*. (35) showed that people without evidence of underlying thyroid disease almost always remain euthyroid in the face of large amounts of excess iodine and escape the acute inhibitory effects of excess intra-thyroidal iodide on the organification (i.e., attachment of `oxidized iodine' species to throsyl residues in the thyroid gland for the synthesis of thyroid hormones) of iodide and on subsequent hormone synthesis (escape from or adaptation to the acute Wolff-Chaikoff effect). This adaptation most likely involves a decrease in thyroid iodide trapping, perhaps corresponding to a decrease in the thyroid sodium-iodide transporter recently cloned (36). Some people, especially those with long-standing nodular goitre who live in iodine-deficient regions and are generally ages 40 years or older, may develop iodine-induced hyperthyroidism after ingestion of excess iodine in a short period of time.

Iodine fortification

Iodine deficiency is present in almost all parts of the developed and developing world, and environmental iodine deficiency is the main cause of iodine deficiency disorders. Iodine is irregularly distributed over the earth's crust, resulting in acute deficiencies in areas such as mountainous regions and flood plains. The problem is aggravated by accelerated deforestation

and soil erosion. Thus, the food grown in iodine-deficient regions can never provide enough iodine for the people and livestock living there. The iodine deficiency results from geologic rather than social and economic conditions. It cannot be eliminated by changing dietary habits or by eating specific kinds of foods but must be corrected by supplying iodine from external sources. It has, therefore, been a common practice to use common salt as a vehicle for iodine fortification for the past 75 years. Salt is consumed at approximately the same level throughout the year by the entire population of a region. Universal salt iodisation is now a widely accepted strategy for preventing and correcting iodine deficiency disorders.

There are areas where consumption of goitrogens in the staple diet (e.g., cassava) affects the proper utilisation of iodine by the thyroid gland. For example, in Congo, Africa, as a result of cassava diets there is an overload of thiocyanate (37). To overcome this problem, appropriate increases in salt iodisation are required to ensure the recommended dietary intake. The iodisation of salt is done either by spraying potassium iodate or potassium iodide in amounts that ensure a minimum of 150 μg iodine/day. Both of these forms of iodine are absorbed as iodide ions and are completely bio-available. Other methods of iodine prophylaxis are also used: iodised oil (capsule and injections), iodised water, iodised bread, iodised soya sauce, iodoform compounds used in dairy and poultry, and certain food additives (38).

Iodine loss occurs as a result of improper packaging, Humidity and moisture, and transport in open trucks and railway wagons exposed to sunlight. To compensate for these losses, higher levels of iodine are used during the production of iodised salt. Losses during the cooking process vary from 20 percent to 40 percent depending on the type of cooking used (39).

To ensure the consumption of recommended levels of iodine, the iodine content of salt at the production level should be monitored with proper quality assurance programmes. Regular evaluation of the urinary iodine excretion pattern in the population consuming iodised salt or exposed to other iodine prophylactic measures would help the adjusting of iodine intake (40).

Recommendations

Recommendations for future research:

- elaborate the role of T_4 in brain development at the molecular level;
- investigate the relation between selenium and iodine deficiency, which has been reported in certain areas of Africa; and
- investigate the possible interference of infections and other systemic illnesses with iodine or thyroid hormone use (such interference has not been reported on a population basis).

Recommendations for future actions:

- establish quality assurance procedures at iodised salt production sites;
- track the progress of iodine deficiency disease elimination through the implementation of cyclic monitoring, which involves division of the country into five zones and carrying out the assessment in one zone each year; and
- develop and validate quantitative testing kits for iodised salt.

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Acknowledgments
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Chapter 13
Iron

The role of iron in human metabolic processes

Iron has several vital functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues. The physiology of iron has been extensively reviewed (*1-6*).

Most of the iron in the body is present in the erythrocytes as haemoglobin, a molecule composed of four units, each containing one heme group and one protein chain. The structure of haemoglobin allows it to be fully loaded with oxygen in the lungs and partially unloaded in the tissues (e.g., in the muscles). The iron-containing oxygen storage protein in the muscles, myoglobin, is similar in structure to haemoglobin but has only one heme unit and one globin chain. Several iron-containing enzymes, the cytochromes, also have one heme group and one globin protein chain. These enzymes act as electron carriers within the cell and their structures do not permit reversible loading and unloading of oxygen. Their role in the oxidative metabolism is to transfer energy within the cell and specifically in the mitochondria. Other key functions for the iron-containing enzymes (e.g., cytochrome P450) include the synthesis of steroid hormones and bile acids; detoxification of foreign substances in the liver; and signal controlling in some neurotransmitters, such as the dopamine and serotonin systems in the brain. Iron is reversibly stored within the liver as ferritin and hemosiderin whereas it is transported between different compartments in the body by the protein transferrin.

Iron requirements

Basal iron losses

Iron is not actively excreted from the body in urine or in the intestines. Iron is only lost with cells from the skin and the interior surfaces of the body – intestines, urinary tract, and airways. The total amount lost is estimated at 14 µg/kg body weight/day (*7*). In children, it is probably more correct to relate these losses to body surface. A non-menstruating 55-kg women loses about 0.8 mg Fe/day and a 70-kg man loses about 1 mg. The range of individual variation has been estimated to be ±15 percent (*8*).

Earlier studies suggested that sweat iron losses could be considerable, especially in a hot, Humid climate. However, new studies which took extensive precautions to avoid the interference of contamination of iron from the skin during the collection of total body sweat have shown that these sweat iron losses are negligible (*9*).

Growth

The newborn term infant has an iron content of about 250–300 mg (75 mg/kg body weight). During the first 2 months of life, haemoglobin concentration falls because of the improved oxygen situation in the newborn infant compared with the intrauterine foetus. This leads to a considerable redistribution of iron from catabolised erythrocytes to iron stores. This iron will cover the needs of the term infant during the first 4–6 months of life and is why iron requirements during this period can be provided by human milk, that contains very little iron. Because of the marked supply of iron to the foetus during the last trimester of pregnancy, the iron situation is much less favourable in the premature and low-birth-weight infant than in the

term infant. An extra supply of iron is therefore needed in these infants even during the first 6 months of life.

In the full-term infant, iron requirements will rise markedly after age 4–6 months and amount to about 0.7–0.9 mg/day during the remaining part of the first year. These requirements are therefore very high, especially in relation to body size and energy intake (***Table 39***) (*10*).

Table 39

Iron intakes required for growth under the age of 18 years, median basal iron losses, menstrual losses in women, and total absolute iron requirements

Group	Age	Body weight	Required Iron intakes for Growth	Basal Iron losses	Menstrual losses		Total Absolute Requirements †	
						95th		95th
	(Years)	Mean (kg)	(mg/day)	Median (mg/day)	Median (mg/day)	percentile (mg/day)	Median (mg/day)	percentile (mg/day)
Children	0.5–1	9	0.55	0.17			0.72	0.93
	1–3	13.3	0.27	0.19			0.46	0.58
	4–6	19.2	0.23	0.27			0.50	0.63
	7–10	28.1	0.32	0.39			0.71	0.89
Males	11–14	45	0.55	0.62			1.17	1.46
	15–17	64.4	0.60	0.90			1.50	1.88
	18+	75		1.05			1.05	1.37
Females	11–14 [b]	46.1	0.55	0.65			1.20	1.40
	11–14	46.1	0.55	0.65	0.48 [c]	1.90 [c]	1.68	3.27
	15–17	56.4	0.35	0.79	0.48 [c]	1.90 [c]	1.62	3.10
	18+	62		0.87	0.48 [c]	1.90 [c]	1.46	2.94
Post-menopausal		62		0.87			0.87	1.13
Lactating		62		1.15			1.15	1.50

† Total Absolute Requirements = Requirement for growth + basal losses + menstrual losses (females only)

[a]Based in part on a 1988 report from Food and Agriculture Organization of the United Nations (FAO)/World Health Organizaiton (WHO) (*8*) and in part on new calculations of the distribution of iron requirements in menstruating women. Because of the very skewed distribution of iron requirements in these women, dietary iron requirements are calculated for four levels of dietary iron bio-availability (***Table 40***).

[b]Non-menstruating.

[c]Effect of the normal variation in haemoglobin concentration not included in this figure.

In the first year of life, the full-term infant almost doubles its total iron stores and triple its body weight. The change in body iron during this period occurs mainly during the first 6–12 months of life. Between 1 and 6 years of age, the body iron content is again doubled. The requirements for absorbed iron in infants and children are very high in relation to their energy requirements. For example, in infants 6–12 months of age, about 1.5 mg of iron need to be absorbed per 4.184 MJ and about half of this amount is required up to age 4 years.

Table 40

The recommended nutrient intakes for iron based on varying dietary iron bio-availabilities

Group	Age (years)	Mean Body weight (kg)	Recommended Nutrient Intake[a] (mg/day) % Dietary Iron Bio-availability			
			15	**12**	**10**	**5**
Children	0.5–1	9	[6.2][b]	[7.7][b]	[9.3][b]	[18.6][b]
	1–3	13.3	3.9	4.8	5.8	11.6
	4–6	19.2	4.2	5.3	6.3	12.6
	7–10	28.1	5.9	7.4	8.9	17.8
Males	11–14	45	9.7	12.2	14.6	29.2
	15–17	64.4	12.5	15.7	18.8	37.6
	18+	75	9.1	11.4	13.7	27.4
Females	11–14[c]	46.1	9.3	11.7	14	28
	11–14	46.1	21.8	27.7	32.7	65.4
	15–17	56.4	20.7	25.8	31	62
	18+	62	19.6	24.5	29.4	58.8
Post-menopausal		62	7.5	9.4	11.3	22.6
Lactating		62	10	12.5	15	30

[a]Based in part on a 1988 report from the FAO/WHO (*8*) and in part on new calculations of the distribution of iron requirements in menstruating women. Because of the very skewed distribution of iron requirements in these women, dietary iron requirements are calculated for four levels of dietary iron bio-availability.
[b]Bio-availability of dietary iron during this period varies greatly.
[c]Non-menstruating.

In the weaning period, the iron requirements in relation to energy intake are the highest of the lifespan except for the last trimester of pregnancy, when iron requirements to a large extent have to be covered from the iron stores of the mother (see section on iron and pregnancy). The rapidly growing weaning infant has no iron stores and has to rely on dietary iron. It is possible to meet these high requirements if the diet has a consistently high content of meat and foods rich in ascorbic acid. In most developed countries today, infant cereal products are the staple foods for that period of life. Commercial products are regularly fortified with iron and ascorbic acid, and they are usually given together with fruit juices and solid foods containing meat, fish, and vegetables. The fortification of cereal products with iron and ascorbic acid is important in meeting the high dietary needs, especially considering the importance of an optimal iron nutrition during this phase of brain development.

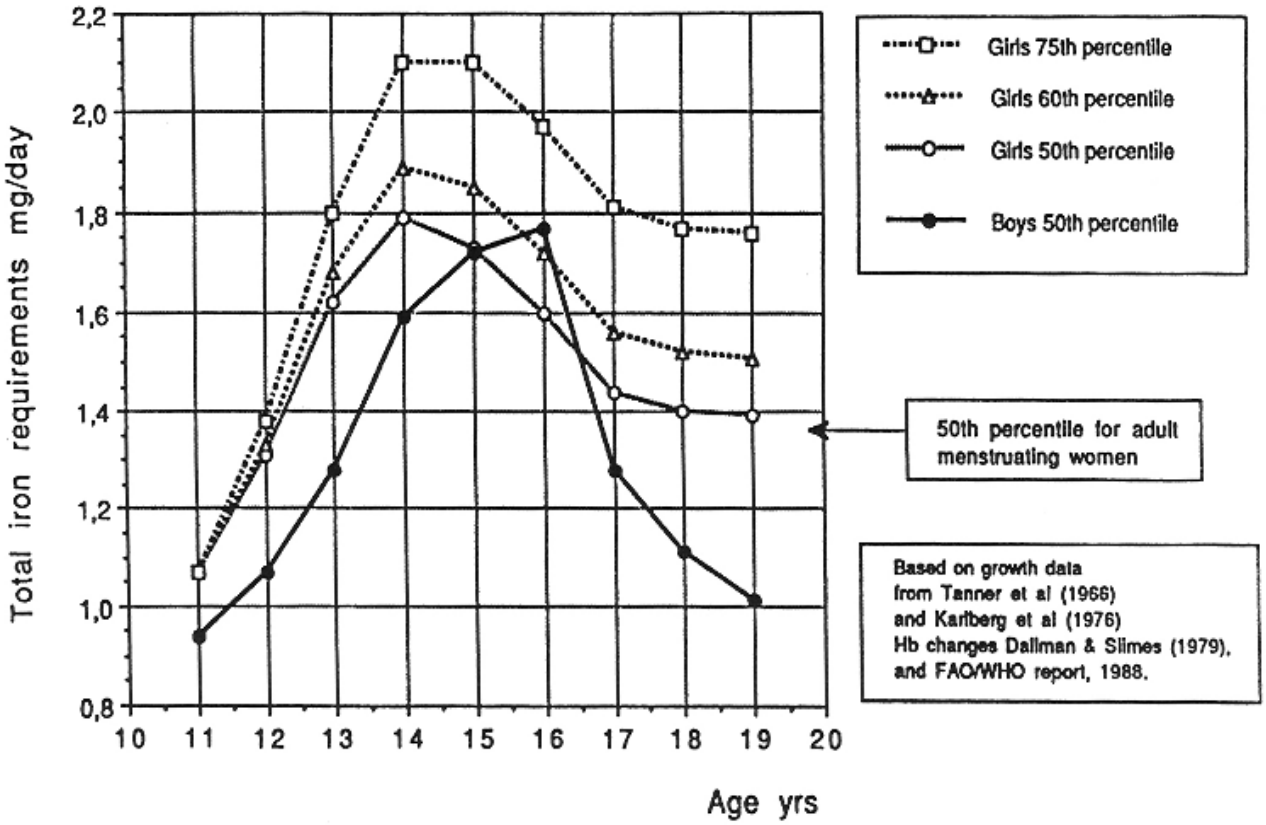
Iron requirements are also very high in adolescents, particularly during the period of rapid growth (*11*). There is a marked individual variation in growth rate and the requirements may be considerably higher than the calculated mean values given in ***Table 39***. Girls usually have their growth spurt before menarche, but growth is not finished at that time. Their total iron requirements are therefore considerable. In boys during puberty there is a marked increase in haemoglobin mass and concentration, further increasing iron requirements to a level above the average iron requirements in menstruating women (***Figure 22***). (The calculations in ***Figure 22*** are based on references *8* and *12–16.*)

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Figure 22

Iron requirements of boys and girls

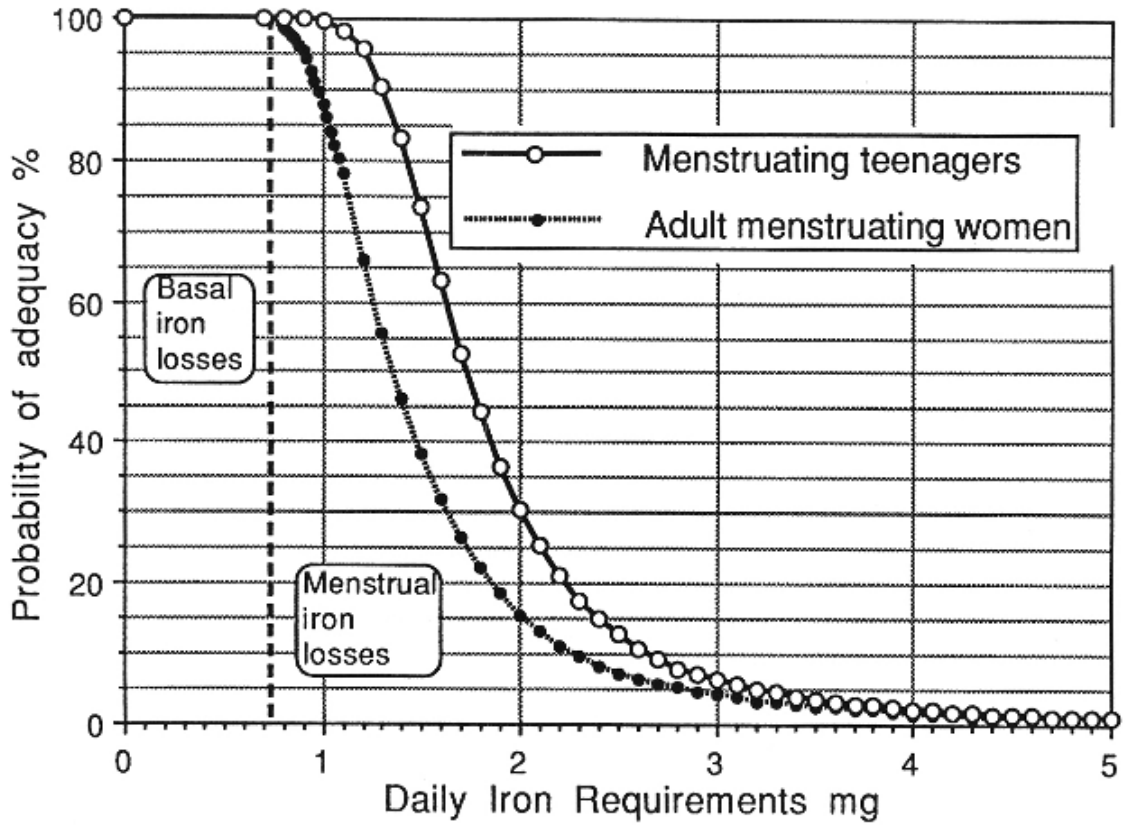
Menstrual iron losses



Menstrual blood losses are very constant from month to month for an individual but vary markedly from one woman to another (*16*).The main part of this variation is genetically controlled by the content of fibrinolytic activators in the uterine mucosa even in populations which are geographically widely separated (Burma, Canada, China, Egypt, England, and Sweden) (*17, 18*). These findings strongly suggest that the main source of variation in iron status in different populations is not related to a variation in iron requirements but to a variation in the absorption of iron from the diets. (This statement disregards infestations with hookworms and other parasites.) The mean menstrual iron loss, averaged over the entire menstrual cycle of 28 days, is about 0.56 mg/day. The frequency distribution of physiologic menstrual blood losses is highly skewed. Adding the average basal iron loss (0.8 mg) and its variation allows the distribution of the total iron requirements in adult women to be calculated as the convolution of the distributions of menstrual and basal iron losses (***Figure 23***). The mean daily total iron requirement is 1.36 mg. In 10 percent of women it exceeds 2.27 mg and in 5 percent it exceeds 2.84 mg (*19*). In 10 percent of menstruating (still-growing) teenagers, the corresponding daily total iron requirement exceeds 2.65 mg, and in 5 percent of the girls it exceeds 3.2 mg/day. The marked skewness of menstrual losses is a great nutritional problem because personal assessment of the losses is unreliable. This means that women with physiologic but heavy losses cannot be identified and reached by iron supplementation. The choice of contraceptive method greatly influences menstrual losses. The methods of calculating iron requirements in women and their variation were recently re-examined (*19*).

Figure 23

Distribution of daily iron requirements in menstruating adult women and teenagers: the probability of adequacy at different amounts of iron absorbed



Note: Left: basal obligatory losses that amount to 0.8 mg; right: varying menstrual iron losses. This graph illustrates that growth requirements in teenagers vary considerably at different age and between girls.

In postmenopausal women and in physically active elderly people, the iron requirements per unit of body weight are the same as in men. When physical activity decreases as a result of ageing, blood volume and haemoglobin mass also diminish, leading to a shift of iron from haemoglobin and muscle to iron stores. This implies a reduction of the daily iron requirements. Iron deficiency in the elderly is therefore seldom of nutritional origin but is usually caused by pathologic iron losses. The absorbed iron requirements in different groups are given in ***Table 39***. Dietary iron requirements will be discussed below. The iron situation during pregnancy and lactation are dealt with separately below.

Iron absorption

With respect to the mechanism of absorption, there are two kinds of dietary iron: heme iron and non-heme iron (*20*). In the human diet the primary sources of heme iron are the haemoglobin and myoglobin from consumption of meat, poultry, and fish whereas non-heme iron is obtained from cereals, pulses, legumes, fruits, and vegetables. The average absorption of heme iron from meat-containing meals is about 25 percent (*21*) The absorption of heme iron can vary from about 40 percent during iron deficiency to about 10 percent during iron repletion (*22*). Heme iron can be degraded and converted to non-heme iron if foods are cooked at a high temperature for too long. Calcium (see below) is the only dietary factor that

negatively influences the absorption of heme iron and does so to the same extent that it influences non-heme iron (***Table 41***) (*23*).

Table 41

Factors influencing dietary iron absorption

HEME IRON ABSORPTION	
Iron status of subject Amount of dietary heme iron, especially as meat Content of calcium in meal (e.g., milk, cheese) Food preparation (time, temperature)	
NON-HEME IRON ABSORPTION	
Iron status of subjects Amount of potentially available non-heme iron (adjustment for fortification iron and contamination iron) * Balance between enhancing and inhibiting factors	
Enhancing factors	**Inhibiting factors**
Ascorbic acid (e.g., certain fruit juices, fruits, potatoes, and certain vegetables) Meat, chicken, fish and other seafood Fermented vegetables (e.g., sauerkraut), fermented soy sauces, etc.	Phytates and other inositol phosphates (e.g., bran products, bread made from high-extraction flour, breakfast cereals, oats, rice [especially unpolished rice], pasta products, cocoa, nuts, soya beans, and peas) Iron-binding phenolic compounds (e.g., tea, coffee, cocoa, certain spices, certain vegetables, and most red wines) Calcium (e.g., milk, cheese) Soy proteins

Non-heme iron is the main form of dietary iron. The absorption of non-heme iron is influenced by individual iron status and by several factors in the diet. Dietary factors influencing iron absorption are outlined in ***Table 41***. Iron compounds used for the fortification of foods will only be partially available for absorption. Once iron is dissolved, its absorption from fortificants and food contaminants is influenced by the same factors as the iron native to the food substance (*24, 25*). Iron originating from the soil (e.g., from various forms of clay) is sometimes present in considerable amounts on the surface of foods as a contaminant originating from dust on air-dried foods or from water used in irrigation. Even if the fraction of iron that is available is often small, contamination iron may still be nutritionally important because of the great amounts present (*26, 27*).

Reducing substances (i.e., substances that keep iron in the ferrous form) must be present for iron to be absorbed (*28*). The presence of meat, poultry, and fish in the diet enhance iron absorption. Other foods contain factors (ligands) that strongly bind ferrous ions, that subsequently inhibit absorption. Examples are phytates and certain iron-binding polyphenols.

Inhibition of iron absorption

Phytates are found in all kinds of grains, seeds, nuts, vegetables, roots (e.g., potatoes), and fruits. Chemically, phytates are inositol hexaphosphate salts and are a storage form of phosphates and minerals. Other phosphates have not been shown to inhibit non-heme iron absorption. In North American and European diets, about 90 percent of phytates originate from cereals. Phytates strongly inhibit iron absorption in a dose-dependent fashion and even small amounts of phytates have a marked effect (*29, 30*).

Bran has a high content of phytate and strongly inhibits iron absorption. Whole-wheat flour, therefore, has a much higher content of phytates than does white wheat flour (*31*). In bread some of the phytates in bran are degraded during the fermentation of the dough. Fermentation for a couple of days (sourdough fermentation) can therefore almost completely degrade the phytate and increase the bio-availability of iron in bread made from whole-wheat flour (*32*). Oats strongly inhibit iron absorption because of their high phytate content, that results from native phytase in oats being destroyed by the normal heat process used to avoid rancidity (*33*). Sufficient amounts of ascorbic acid can counteract this inhibition (*34*). By contrast, non-phytate-containing dietary fibre components have almost no influence on iron absorption.

Almost all plants contain phenolic compounds as part of their defence system against insects, animals, and humans. Only some of the phenolic compounds (mainly those containing galloyl groups) seem to be responsible for the inhibition of iron absorption (*35*). Tea, coffee, and cocoa are common plant products that contain iron-binding polyphenols (*36-39*). Many vegetables, especially green leafy vegetables (e.g., spinach), and herbs and spices (e.g., oregano) contain appreciable amounts of galloyl groups, that strongly inhibit iron absorption. Consumption of betel leaves, common in areas of Asia, also has a marked negative effect on iron absorption.

Calcium, consumed as a salt or in dairy products interferes significantly with the absorption of both heme and non-heme iron *(40-42*). Because calcium and iron are both essential nutrients, calcium cannot be considered to be an inhibitor in the same way as phytates or phenolic compounds. The practical solution for this competition is to increase iron intake, increase its bio-availability, or avoid the intake of foods rich in calcium and foods rich in iron at the same meal (*43*).

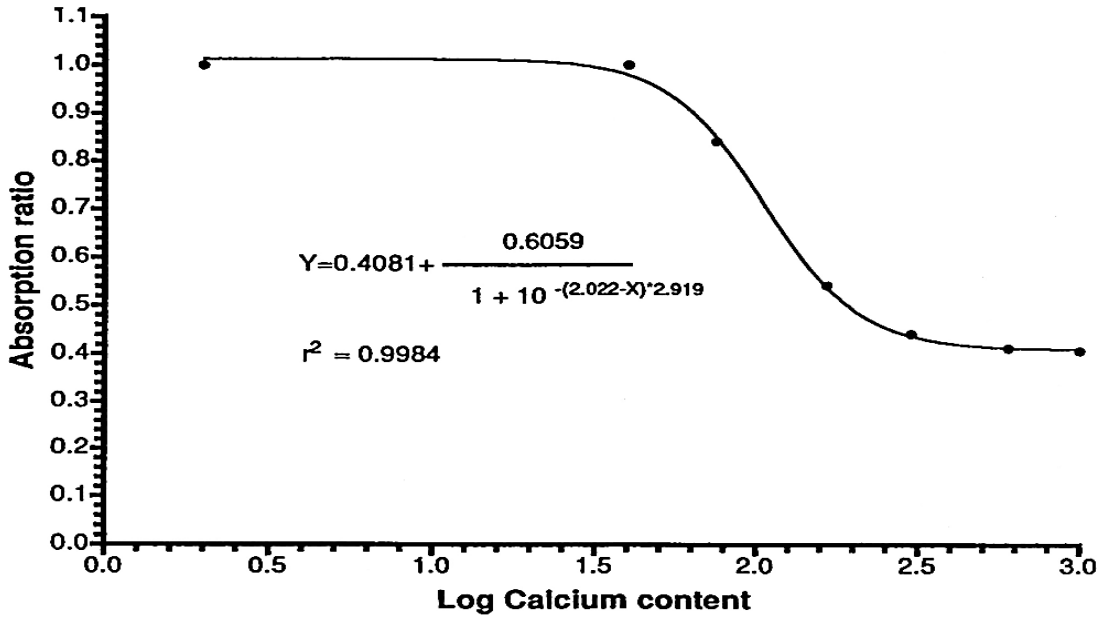
The mechanism of action for absorption inhibition is unknown, but the balance of evidence strongly suggest that the inhibition is located within the mucosal cell itself at the common final transfer step for heme and non-heme iron. Recent analyses of the dose-effect relationship show that no inhibition is seen from the first 40 mg of calcium in a meal. A sigmoid relationship is then seen, reaching a 60 percent maximal inhibition of iron absorption by 300–600 mg calcium. The form of this curve suggests a one-site competitive binding of iron and calcium (***Figure 24***). This relationship explains some of the seemingly conflicting results obtained in studies on the interaction between calcium and iron (*44*).

For unknown reasons, the addition of soy protein to a meal reduces the fraction of iron absorbed (*45-48*). This inhibition is not solely explained by the high phytate content of soy protein. However, because of the high iron content of soy proteins, the net effect on iron absorption of an addition of soy products to a meal is usually positive. In infant foods containing soy proteins, the inhibiting effect can be overcome by the addition of sufficient amounts of ascorbic acid. Some fermented soy sauces, however, have been found to enhance iron absorption (*49, 50*).

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Figure 24

Effect of different amounts of calcium on iron absorption



Enhancement of iron absorption

Ascorbic acid is the most potent enhancer of non-heme iron absorption (*34, 51-53*). Synthetic vitamin C increases the absorption of iron to the same extent as the native ascorbic acid in fruits, vegetables, and juices. The effect of ascorbic acid on iron absorption is so marked and essential that this effect could be considered as one of vitamin C's physiologic roles (*54*). Each meal should preferably contain at least 25 mg of ascorbic acid and possibly more if the meal contains many inhibitors of iron absorption. Therefore, a requirement of ascorbic acid for iron absorption should be taken into account when establishing the requirements for vitamin C, that are set only to prevent vitamin C deficiency (especially scurvy).

Meat, fish, and seafood all promote the absorption of non-heme iron *(55-58*). The mechanism for this effect has not been determined. It should be pointed out that meat also enhances the absorption of heme iron to about the same extent (*21*). Meat promotes iron nutrition in two ways: it stimulates the absorption of both heme and non-heme iron and it provides the well-absorbed heme iron. Epidemiologically, the intake of meat has been found to be associated with a lower prevalence of iron deficiency.

Organic acids, such as citric acid, have in some studies been found to enhance the absorption of non-heme iron (*29).* This effect is not observed as consistently as is the effect of ascorbic acid *(47, 52*). Sauerkraut (*59*) and other fermented vegetables and even some fermented soy sauces (*49, 50*) enhance iron absorption. The nature of this enhancement has not yet been determined.

Iron absorption from meals

The pool concept (see above) in iron absorption implies that there are two main pools in the gastrointestinal lumen – one pool of heme iron and another pool of non-heme iron – and that iron absorption takes place independently from these two pools (*24*). The pool concept also implies that the absorption of iron from the non-heme iron pool results from all ligands

present in the mixture of foods included in a meal. The absorption of non-heme iron from a certain meal not only depends on its iron content but also, and to a marked degree, on the composition of the meal (i.e., the balance among all factors enhancing and inhibiting the absorption of iron). The bio-availability can vary more than 10-fold among meals with a similar content of iron, energy, protein, fat, etc. (*20*). Just the addition of certain spices (e.g., oregano) or a cup of tea may reduce the bio-availability by one-half or more. However, the addition of certain vegetables or fruits containing ascorbic acid may double or even triple iron absorption, depending on the other properties of the meal and the amounts of ascorbic acid present.

Iron absorption from the whole diet

There is limited information about the total amounts of iron absorbed from the diet because no simple method is available to measure iron absorption from the whole diet. It has been measured by chemical balance studies using long balance periods or by determining the haemoglobin regeneration rate in subjects with induced iron deficiency anaemia and a well-controlled diet over a long period of time.

A method was recently developed to measure iron absorption from the whole diet. In the first studies all non-heme iron in all meals over periods of 5–10 days was homogeneously labelled to the same specific activity with an extrinsic inorganic radioiron tracer (*43*, *60*). Heme iron absorption was then estimated. In a further study, heme and non-heme iron were separately labelled with two radioiron tracers as biosynthetically labelled haemoglobin and as an inorganic iron salt (*22*). New information could be obtained, for example, about the average bio-availability of dietary iron in different types of diets, overall effects of certain factors (e.g., calcium) on iron nutrition, and regulation of iron absorption in relation to iron status. Iron absorption from the whole diet is the sum of the absorption of iron from the single meals included in the diet. It has been suggested that the iron absorption of single meals may exaggerate the absorption of iron from the diet (*61*, *62*). Iron absorption from single meals can never represent iron absorption from the whole diet, but iron absorption from a single meal was the same when the meal was served in the morning after an overnight fast or at lunch or supper (*63*). The same observation was made in another study when a hamburger meal was served in the morning or 2–4 hours after a breakfast (*42*).

Because energy expenditure and energy intake set the limit for the amount of food eaten and for meal size, it is practical to relate the bio-availability of iron in different meals to energy content (bio-available nutrient density). The use of bio-available nutrient density is a feasible way to compare different meals, construct menus, and calculate recommended intakes (*64*).

Intake of energy and essential nutrients such as iron was probably considerably higher for early humans than it is today (*65-67*). The present low iron intake associated with a low-energy lifestyle implies that the interaction between different factors influencing iron absorption, will be more critical. For example, the interaction between calcium and iron absorption probably had no importance in the nutrition of early humans, who had a diet with ample amounts of both iron and calcium.

Iron balance and regulation of iron absorption

The body has three unique mechanisms for maintaining iron balance and preventing iron deficiency and iron overload. The first is the continuous re-utilisation of iron from catabolised erythrocytes in the body. When an erythrocyte dies after about 120 days, it is usually degraded by the macrophages of the reticular endothelium. The iron is released and delivered to transferrin in the plasma, which brings the iron back to red blood cell precursors in the

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bone marrow or to other cells in different tissues. Uptake and distribution of iron in the body is regulated by the synthesis of transferrin receptors on the cell surface. This system for internal iron transport not only controls the rate of flow of iron to different tissues according to their needs but also effectively prevents the appearance of free iron and the formation of free radicals in the circulation.

The second mechanism is the access of the specific storage protein, ferritin, which can store and release iron to meet excessive iron demands. This iron reservoir is especially important in the third trimester of pregnancy.

The third mechanism involves the regulation of absorption of iron from the intestines, with an increased iron absorption in the presence of decreasing body iron stores and a decreased iron absorption when iron stores increase. Iron absorption decreases until an equilibrium is established between absorption and requirements. For a given diet this regulation of iron absorption, however, can only balance losses up to a certain critical point beyond which iron deficiency will develop (*68*). About half of the basal iron losses are from blood, primarily in the gastrointestinal tract. Both these losses and the menstrual iron losses are influenced by the haemoglobin level; during the development of an iron deficiency, menstrual and basal iron losses will successively decrease when the haemoglobin level decreases. In a state of more severe iron deficiency, skin iron losses may also decrease. Iron balance (absorption equals losses) may be present not only in normal subjects but also during iron deficiency and iron overload.

The three main factors that affect iron balance are absorption (intake and bio-availability of iron), losses, and amount in stores. The interrelationship among these factors was recently been described in mathematical terms, making it possible to predict, for example, the amount of stored iron when iron losses and bio-availability of dietary iron are known (69). With increasing iron requirements or decreasing bio-availability, the regulatory capacity to prevent iron deficiency is limited (68). However, to prevent iron overload with increasing dietary iron intake or bio-availability, the regulatory capacity seems to be extremely good (69).

Iron deficiency

Populations at risk for iron deficiency

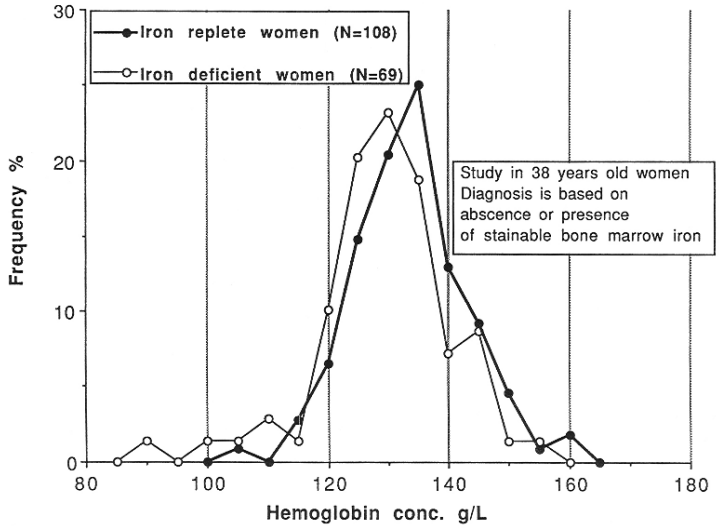
Worldwide, the highest prevalence of iron deficiency is found in infants, children, adolescents, and women of childbearing age, especially pregnant women. The weaning period in infants is especially critical because of the very high iron requirements in relation to energy requirements. Thanks to better information and access to fortified cereals for infants and children, the iron situation has markedly improved in these groups in most industrialized countries where the highest prevalences of iron deficiency today are observed in menstruating and pregnant women and adolescents of both sexes. In developing countries, however, the iron situation is very critical in many groups, especially in the weaning period. Iron nutrition is of great importance for the adequate development of the brain and other tissues such as muscles, which are finally differentiated early in life.

Iron deficiency and iron deficiency anaemia are often incorrectly used as synonyms. A definition of these terms may clarify some confusion about different prevalence figures given in the literature (*70*). The cause of the problem is the very wide distribution of the haemoglobin concentration in healthy, fully iron-replete subjects (in women, 120–160 g/l; in men, 140–180g/l [*71*]). During the development of a negative iron balance in subjects with no mobilisable iron from iron stores (no visible iron in technically perfect bone marrow smears or a serum ferritin concentration <15 µg/l), there will be an immediate impairment in the production of haemoglobin with a resulting decrease in haemoglobin and different erythrocyte

indexes (e.g., mean corpuscular haemoglobin and mean corpuscular volume). In turn this will lead to an overlap of the distributions of haemoglobin in iron-deficient and iron-replete women (***Figure 25***). The extent of overlap depends on the prevalence and severity of iron deficiency. In populations with more severe iron deficiency, for example, the overlap is much less marked.

Figure 25

Distribution of haemoglobin concentration in a sample of 38-year-old women with and without stainable bone marrow iron



Note: The main fraction (91 percent) of the iron-deficient women in this sample had haemoglobin levels above the lowest normal level for the population: 120 g/l (mean ± 2 SD) (*68*). The degree of overlap of the two distributions depends on the severity of anaemia in a population.

In women, anaemia is defined as a haemoglobin <120 g/l. For a woman who has her normal homeostatic value set at 150 g/l, haemoglobin level must decrease to 119 g/l (by 26 percent) before she is considered to be anaemic, whereas for a woman who has her normal haemoglobin set at 121 g/l, haemoglobin level must only decrease by 1.5 percent to 119 g/l. Iron deficiency anaemia is a rather imprecise concept for evaluating the single subject and has no immediate physiologic meaning. By definition, this implies that the prevalence of iron deficiency anaemia is less frequent than iron deficiency and that the presence of anaemia in a subject is a statistical rather than a functional concept. The main use of the cut-off value is in comparisons between population groups (*72*). In practical work, iron deficiency anaemia should be replaced by the functional concept of iron deficiency. Anaemia per se is mainly important when it becomes so severe that oxygen delivery to tissues is impaired. An iron deficiency anaemia which develops slowly in otherwise healthy subjects with moderately heavy work output will not give any symptoms until the haemoglobin level is about 80 g/l or lower (*71*). The reason for the continued use of the concept of iron deficiency anaemia is the ease of determining haemoglobin. Therefore, in clinical practice, knowledge of previous haemoglobin values in a subject is of great importance for evaluating the diagnosis.

Iron deficiency being defined as an absence of iron stores combined with signs of an iron-deficient erythropoiesis implies that in a state of iron deficiency there is an insufficient supply of iron to various tissues. This occurs at a serum ferritin level <15 µg/l. Iron can then no longer be mobilised from iron stores and insufficient amounts of iron will be delivered to

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transferrin, the circulating transport protein for iron. The binding sites for iron on transferrin will therefore contain less and less iron. This is usually described as a reduction in transferrin saturation. When transferrin saturation drops to a certain critical level, erythrocyte precursors, which continuously need iron for the formation of haemoglobin, will get an insufficient supply of iron. At the same time, the supply of iron by transferrin to other tissues will also be impaired. Liver cells will get less iron, more transferrin will be synthesised, and the concentration of transferrin in plasma will then suddenly increase. Cells with a high turnover rate are the first ones to be affected (e.g., intestinal mucosal cells with a short life span). The iron-transferrin complex is bound to transferrin receptors on cell surfaces and the whole complex is then taken up by special receptors on the surface of various cells and tissues. The uptake of iron seems to be related both to transferrin saturation and the number of transferrin receptors on the cell surface (*73*, *74*). There is a marked diurnal variation in the saturation of transferrin because the turnover rate of iron in plasma is very high. This fact makes it difficult to evaluate the iron status from single determinations of transferrin saturation.

Indicators of iron deficiency

The absence of iron stores (iron deficiency) can be diagnosed by showing that there is no stainable iron in the reticuloendothelial cells in bone marrow smears or more easily by a low concentration of ferritin in serum (≤15 µg/l). Even if an absence of iron stores per se may not necessarily be associated with any immediate adverse effects, it is a reliable and good indirect indicator of iron-deficient erythropoiesis and of an increased risk of a compromised supply of iron to different tissues.

Even before iron stores are completely exhausted, the supply of iron to the erythrocyte precursors in the bone marrow is compromised, leading to iron-deficient erythropoiesis (*70*). A possible explanation is that the rate of release of iron from stores is influenced by the amount of iron remaining. As mentioned above it can then be assumed that the supply of iron to other tissues needing iron is also insufficient because the identical transport system is used. During the development of iron deficiency haemoglobin concentration, transferrin concentration, transferrin saturation, transferrin receptors in plasma, erythrocyte protoporphyrin, and erythrocyte indexes are changed. All these methods, however, show a marked overlap between normal and iron-deficient subjects, that makes it impossible to identify the single subject with mild iron deficiency by using any of these methods. Therefore, these tests have been used in combination (e.g., for interpreting results from the second National Health and Nutrition Examination Survey in the United States of America [*75*, *76*]). The diagnostic specificity then increases but the sensitivity decreases, and thus the true prevalence of iron deficiency is markedly underestimated if multiple diagnostic criteria are used. By definition in screening for iron deficiency, the more tests that are used the higher is the diagnostic specificity but the lower is the sensitivity of the procedure. Fortunately, a low serum ferritin, ≤15µg/l is always associated with an iron-deficient erythropoiesis. The use of serum ferritin alone as a measure will also underestimate the true prevalence of iron deficiency but to a lesser degree than when the combined criteria are used.

A diagnosis of iron deficiency anaemia can be suspected if anaemia is present in subjects who are iron-deficient as described above. Preferably, to fully establish the diagnosis, the subjects should respond adequately to iron treatment. The pitfalls with this method are the random variation in haemoglobin concentrations over time and the effect of the regression towards the mean when a new measurement is made.

The use of serum ferritin has improved the diagnostic accuracy of iron deficiency. It is the only simple method available to detect early iron deficiency. Its practical value is somewhat reduced, however, by the fact that serum ferritin is a very sensitive acute-phase

reactant and may be increased for weeks after a simple infection with fever for a day or two (*77*). Several other conditions, such as use of alcohol (*78*, *79*), liver disease, and collagen diseases, may also increase serum ferritin concentrations. Determination of transferrin receptors in plasma has also been recommended in the diagnosis of iron deficiency. Its advantage is that it is not influenced by infections. Its main use is in subjects who are already anaemic and it is not sensitive for the early diagnosis of iron deficiency. The use of a combination of determinations of serum ferritin and serum transferrin receptors has also been suggested (*80*).

Causes of iron deficiency

Nutritional iron deficiency implies that the diet cannot cover physiologic iron requirements. Worldwide this is the most common cause of iron deficiency. In many tropical countries, infestations with hookworms lead to intestinal blood losses that may be considerable. The severity of the infestations with hookworms varies considerably between subjects and regions. The average blood loss can be well estimated by egg counts in stools. Usually the diet in these populations is also limited with respect to iron content and availability.

In clinical practice a diagnosis of iron deficiency must always lead to a search for pathologic causes of blood loss (e.g., tumours in the gastrointestinal tract or uterus, especially if uterine bleedings have increased or changed in regularity). Patients with achlorhydria absorb dietary iron less well (a reduction of about 50 percent) and patients who have undergone gastric surgery, especially if the surgery was extensive, may eventually develop iron deficiency because of impaired iron absorption. Gluten enteropathy is another possibility to consider, especially in young patients.

Prevalence of iron deficiency

Iron deficiency is probably the most frequent nutritional deficiency disorder in the world. A recent estimate based on World Health Organization (WHO) criteria indicated that around 600-700 million people worldwide have a marked iron deficiency anaemia (*81*). In industrialized countries, the prevalence of iron deficiency anaemia is much lower and usually varies between 2 percent and 8 percent. However, the prevalence of iron deficiency, including both anaemic and non-anaemic subjects (see definitions above), is much higher. In industrialized countries, for example, an absence of iron stores or subnormal serum ferritin values is found in about 20–30 percent of women of fertile age. In adolescent girls the prevalence is even higher.

It is difficult to determine the prevalence of iron deficiency more exactly because representative populations for clinical investigation are hard to obtain. Laboratory methods and techniques for blood sampling need careful standardization. One often neglected source of error, for example, when materials in different regions or at different times are compared, is the fact that there are still reagent kits on the market for determining serum ferritin which are not adequately calibrated at different concentrations against the international WHO standards. In addition, seasonal variations in infection rates influence the sensitivity and specificity of most methods used.

Worldwide, the highest prevalence figures for iron deficiency are found in infants, children, teenagers, and women of childbearing age. Thanks to better information and access to fortified cereals for infants and children, the iron situation has markedly improved in these groups in most industrialized countries, where the highest prevalence today is observed in menstruating women and adolescents of both sexes.

In developing countries, where the prevalence of iron deficiency is very high and the severity of anaemia is marked, studies on the distribution of haemoglobin in different

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population groups can provide important information as a valuable basis for action programmes (*72*). A more detailed analysis of subsamples may then give excellent information for the planning of more extensive programmes.

Effects of iron deficiency

Studies in animals have clearly shown that iron deficiency has several negative effects on important functions in the body (*3*). Physical working capacity in rats has been shown to be significantly reduced in iron deficiency, that is especially valid for endurance activities (*82*, *83*). This negative effect seems to be less related to the degree of anaemia than to impaired oxidative metabolism in the muscles with an increased formation of lactic acid, that in turn is due to a lack of iron-containing enzymes which are rate limiting for the oxidative metabolism (*84*).

The relationship between iron deficiency and brain function is of great importance for the choice of strategy in combating iron deficiency (*85*-*88*). Several structures in the brain have a high iron content of the same magnitude as observed in the liver. Of great importance is the observation that the lower iron content of the brain in iron-deficient growing rats cannot be increased by giving iron later on. This fact strongly suggests that the supply of iron to brain cells takes place during an early phase of brain development and that, as such, early iron deficiency may lead to irreparable damage to brain cells.

In humans about 10 percent of brain iron is present at birth; at the age of 10 years the brain has only reached half its normal iron content, and optimal amounts are first reached at the age of 20-30 years.

In populations with long-standing iron deficiency, a reduction of physical working capacity has been demonstrated by several groups with improvement in working capacity after iron administration (*84*).

Iron deficiency also negatively influences the normal defence systems against infections. The cell-mediated immunologic response by the action of T lymphocytes is impaired as a result of a reduced formation of these cells. This in turn is due to a reduced DNA synthesis depending on the function of ribonucleotide reductase, which requires a continuous supply of iron for its function. The phagocytosis and killing of bacteria by the neutrophil leukocytes is an important component of the defence mechanism against infections. These functions are impaired in iron deficiency. The killing function is based on the formation of free hydroxyl radicals within the leukocytes, the respiratory burst, and results from the activation of the iron-sulphur enzyme NADPH oxidase and probably also cytochrome b (a heme enzyme) (*89*).

The impairment of the immunologic defence against infections that was found in animals is also regularly found in humans. Administration of iron normalises these changes within 4–7 days. It has been difficult to demonstrate, however, that the prevalence of infections is higher or that their severity is more marked in iron-deficient subjects than in control subjects. This may well be ascribed to the difficulty in studying this problem with an adequate experimental design.

A relationship between iron deficiency and behaviour such as attention, memory, and learning, has been demonstrated in infants and small children by several groups. In the most recent well-controlled studies, no effect was noted from the administration of iron. This finding is consistent with the observations in animals. Therapy-resistant behavioural impairment and the fact that there is an accumulation of iron during the whole period of brain growth should be considered strong arguments for the more active and effective combating of iron deficiency. This is valid for women, especially during pregnancy, for infants and children, and up through the period of adolescence and early adulthood. In a recent well-

controlled study, administration of iron to non-anaemic but iron-deficient adolescent girls improved verbal learning and memory (*90*).

Well-controlled studies in adolescent girls show that iron-deficiency without anaemia is associated with reduced physical endurance (*91*) and changes in mood and ability to concentrate (*92*). A recent careful study showed that there was a reduction in maximum oxygen consumption in non-anaemic women with iron deficiency that was unrelated to a decreased oxygen-transport capacity of the blood (*93*).

Iron during pregnancy and lactation

Iron requirements during pregnancy are well established (***Table 42***). Most of the iron required during pregnancy is used to increase the haemoglobin mass of the mother, which occurs in all healthy pregnant women who have sufficiently large iron stores or who are adequately supplemented with iron. The increased haemoglobin mass is directly proportional to the increased need for oxygen transport during pregnancy and is one of the important physiologic adaptations that occurs in pregnancy (*94*, *95*). A major problem for iron balance in pregnancy is that iron requirements are not equally distributed over its duration. The exponential growth of the foetus implies that iron needs are almost negligible in the first trimester and that more than 80 percent relates to the last trimester. The total daily iron requirements, including the basal iron losses (0.8 mg), increase during pregnancy from 0.8 mg to about 10 mg during the last 6 weeks of pregnancy.

Iron absorption during pregnancy is determined by the amount of iron in the diet, its bio-availability (meal composition), and the changes in iron absorption that occur during pregnancy. There are marked changes in the fraction of iron absorbed during pregnancy. In the first trimester there is a marked, somewhat paradoxical, decrease in the absorption of iron, which is closely related to the reduction in iron requirements during this period as compared with the non-pregnant state (see below). In the second trimester iron absorption is increased by about 50 percent, and in the last trimester it may increase by up to about four times. Even considering the marked increase in iron absorption, it is impossible for the mother to cover her iron requirements from diet alone, even if its iron content and bio-availability are very high. It can be calculated that with diets prevailing in most industrialized countries, there will be a deficit of about 400–500 mg in the amount of iron absorbed during pregnancy (***Figure 26***).

An adequate iron balance can be achieved if iron stores of 500 mg are available. However, it is uncommon for women today to have iron stores of this size. It is therefore recommended that iron supplements in tablet form, preferably together with folic acid, be given to all pregnant women because of the difficulties in correctly evaluating iron status in pregnancy with routine laboratory methods. In the non-anaemic pregnant woman, daily supplements of 100 mg of iron (e.g., as ferrous sulphate) given during the second half of pregnancy are adequate. In anaemic women higher doses are usually required.

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Table 42

Iron requirements during pregnancy

	Iron requirements (mg)
IRON REQUIREMENTS DURING PREGNANCY	
Foetus	300
Placenta	50
Expansion of maternal erythrocyte mass	450
Basal iron losses	240
Total iron requirement	1040
NET IRON BALANCE AFTER DELIVERY	
Contraction of maternal erythrocyte mass	+450
Maternal blood loss	-250
Net iron balance	+200
Net iron requirements for pregnancy if sufficient maternal iron stores are present (1040 - 200 = 840)	840

During the birth process, the average blood loss corresponds to about 250 mg iron. At the same time, however, the haemoglobin mass of the mother is gradually normalised, which implies that about 200 mg iron from the expanded haemoglobin mass (150–250 mg) is returned to the mother. To cover the needs of a woman after pregnancy, a further 300 mg of iron must be accumulated in the iron stores in order for the woman to start her next pregnancy with about 500 mg of stored iron. Such a restitution is not possible with present types of diets.

There is an association between low haemoglobin values and prematurity. An extensive study (*96*) showed that a woman with a hematocrit of 37 percent had twice the risk of having a premature birth as did a woman with a hematocrit between 41 percent and 44 percent ($P \leq 0.01$). A similar observation was reported in another extensive study in the United States of America (*97*). These materials were examined retrospectively and the cause of the lower hematocrit was not examined.

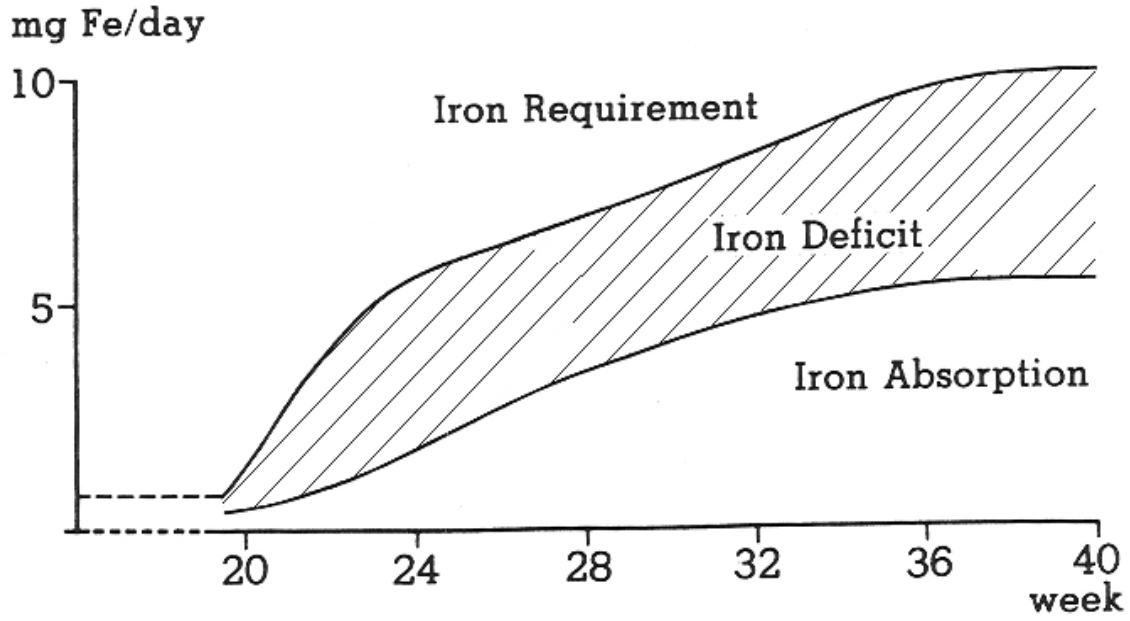
In lactating women, the daily iron loss in milk is about 0.3 mg. Together with the basal iron losses of 0.8 mg, the total iron requirements during the lactation period amount to 1.1 mg/day.

Early in pregnancy there are marked hormonal, haemodynamic, and haematologic changes. There is, for example, a very early increase in the plasma volume, which has been used to explain the physiologic anaemia of pregnancy observed also in iron-replete women. The primary cause of this phenomenon, however, is more probably an increased ability of the haemoglobin to deliver oxygen to the tissues (foetus). This change is induced early in pregnancy by increasing the content of 2, 3-diphospho-D-glycerate in the erythrocytes, which shifts the hemoglobin-oxygen dissociation curve to the right. The anaemia is a consequence of

this important adaptation and is not primarily a desirable change, for example, to improve placental blood flow by reducing blood viscosity.

Figure 26

Daily iron requirements and daily dietary iron absorption in pregnancy



Note: The hatched area represents the deficit of iron that has to be covered by iron from stores or iron supplementation.

Another observation has likewise caused some confusion about the rationale of giving extra iron routinely in pregnancy. In extensive studies of pregnant women, there is a U-shaped relationship between various pregnancy complications and the haemoglobin level (i.e., there are more complications at both low and high levels). There is nothing to indicate, however, that high haemoglobin levels (within the normal non-pregnant range) per se have any negative effects. The haemoglobin increase is caused by pathologic hormonal and hemodynamic changes induced by an increased sensitivity to angiotensin II that occurs in some pregnant women, leading to a reduction in plasma volume, hypertension, and toxaemia of pregnancy.

Pregnancy in adolescents presents a special problem because iron is needed to cover the requirements of growth. In countries with very early marriage, a girl may get pregnant before menstruating. The additional iron requirements for growth of the mother are then very high and the iron situation is very serious.

In summary, the marked physiologic adjustments occurring in pregnancy are not sufficient to balance its very marked iron requirements, and the pregnant woman has to rely on her iron stores, if present. The composition of the diet has not been adjusted to the present low-energy-demanding lifestyle in industrialized countries. This is probably the main cause of the critical iron-balance situation in pregnancy today, that is due to absent or insufficient iron stores in women before they get pregnant. The unnatural necessity to give extra nutrients such as iron and folate to otherwise healthy pregnant women should be considered in this perspective.

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Iron supplementation and fortification

The prevention of iron deficiency has become even more urgent in recent years with the accumulation of evidence strongly suggesting a relationship between even mild iron deficiency and brain development and especially with the observation that functional defects affecting learning and behaviour cannot be reversed by giving iron later on. As mentioned, iron deficiency is common both in developed and in developing countries. Great efforts have been made by WHO to develop methods to combat iron deficiency.

Iron deficiency can generally be combated by one or more of the following three strategies: 1) iron supplementation (i.e., giving iron tablets to certain target groups such as pregnant women and preschool children); 2) iron fortification of certain foods, such as flour; and 3) food and nutrition education to improve the amount of iron absorbed from the diet by increasing the intake of iron and especially by improving the bio-availability of the dietary iron.

Several factors determine the feasibility and effectiveness of different strategies, such as the health infrastructure of a society, the economy, access to suitable vehicles for iron fortification, etc. The solutions are therefore often quite different in developing and developed countries. There is an urgency to obtain knowledge about the feasibility of different methods to improve iron nutrition and to apply present knowledge. In addition, initiation of local activities should be stimulated while actions from governments are awaited.

The evidence for estimating the recommended nutrient intake for iron

To translate physiologic iron requirements, given in ***Table 30***, into dietary iron requirements, the bio-availability of iron in different diets must be calculated. It is therefore necessary to choose an iron status where the supply of iron to the erythrocyte precursors and other tissues starts to be compromised. A state of iron-deficient erythropoiesis occurs when iron can no longer be mobilised from iron stores; iron can no longer be mobilised when stores are almost completely empty. A reduction then occurs, for example, in the concentration of haemoglobin and in the average content of haemoglobin in the erythrocytes (a reduction in mean corpuscular haemoglobin). At the same time the concentration of transferrin in the plasma increases because of an insufficient supply of iron to liver cells. These changes were recently shown to occur rather suddenly at a level of serum ferritin of ≤15 µg/l (*68*, *70*). A continued negative iron balance will further reduce the level of haemoglobin. Symptoms related to iron deficiency are less related to the haemoglobin level and more to the fact that there is a compromised supply of iron to tissues.

The bio-availability of iron in meals consumed in countries with a Western-type diet has been measured by using different methods. Numerous single-meal studies have shown absorption of non-heme iron ranging from 5 percent to 40 percent (*59*, *98*, *99*). Attempts have also been made to estimate the bio-availability of dietary iron in populations consuming Western-type diets by using indirect methods (e.g., calculation of the coverage of iron requirements in groups of subjects with known dietary intake). Such estimations suggest that in borderline iron-deficient subjects the bio-availability from good diets may reach a level around 14–16 percent (15 percent relates to subjects who have a serum ferritin value of <15µg/l or a reference dose absorption of 56.5 percent) (*19*).

Recently, direct measurements were made of the average bio-availability of iron in different Western-type diets (*22*, *43*, *60*). Expressed as total amounts of iron absorbed from the whole diet, it was found that 53.2 µg Fe/kg/day could be absorbed daily from each of the two main meals of an experimental diet which included ample amounts of meat or fish. For a body weight of 55 kg and an iron intake of 14 mg/day, this corresponds to a bio-availability of 21 percent in subjects with no iron stores and an iron-deficient erythropoiesis. A diet common

among women in Sweden contained smaller portions of meat and fish, higher phytates, and some vegetarian meals each week was found to have a bio-availability of 12 percent. Reducing the intake of meat and fish further will reduce the bio-availability to about 10 percent (25µg Fe/kg/day). In vegetarians the bio-availability is usually low because of the absence of meat and fish and a high intake of phytate and polyphenols. An average good Western-type whole diet has a bio-availability of about 15 percent but for common diets, especially among women, the bio-availability is around 12 percent or even 10 percent. In countries or for certain groups in a population with a very high meat intake, the bio-availability may rather be around 18 percent. In Western countries, a high bio-availability is mainly associated with a high meat intake, a high intake of ascorbic acid with meals, a low intake of phytate-rich cereals, and no coffee or tea within 2 hours of the main meals (*38*). ***Table 43*** shows examples of diets with different iron bio-availability.

Table 43

Examples of diet with different iron bio-availability

Type of diet	Bio-availability µg/kg/day
Preagricultural ancestors Plant/animal subsistence: 65/35	
Very high meat and ascorbic acid	150
Very high meat in 2 main meals daily and high ascorbic acid (theoretical)	75
High meat/fish in 2 main meals daily	66.7
Moderate meat/fish in 2 main meals daily	53.2
Moderate meat/fish in 2 main meals daily, low phytate and calcium	42.3
Meat/fish in 60% of 2 main meals daily, high phytate and calcium	31.4
Lower meat intake, high phytate. Often one main meal.	25
Meat/fish negligible, high phytate. High tannin, low ascorbic acid.	15

Table 44

Translation of bio-availability expressed as amount of iron absorbed into percent absorbed for two levels of iron intake

Bio-availability, µg/kg/day	Absorption as mg Fe at no iron stores in women of 55 kg body weight	Bio-availability %	
		15 mg	17 mg
150	8.25	55	48.8
75	4.13	27.5	24.4
66.7	3.67	24.5	21.8
53.2	2.93	19.5	17
42.3	2.32	15.5	13.5
31.4	1.73	11.5	10
25	1.38	9.2	8.2
15	0.83	5.5	4.7

Iron absorption data (***Table 44***) are also available from several population groups in Africa (*100*), South America (*101*), India, and Southeast Asia. The bio-availability of different Indian diets after an adjustment to a reference dose absorption of 56.5 percent was 1.7–1.8 percent for millet-based diets, 3.5–4.0 percent for wheat-based diets, and 8.3-10.3 percent for rice-based diets (*102*). In Southeast Asia, iron absorption data has been reported from Burma and Thailand. In Burma, iron absorption from a basal rice-based meal was 1.7

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percent, when the meal contained 15 g of fish the bio-availability of iron was 5.5 percent, and with 40 g of fish it was 10.1 percent (*103*). In Thailand, iron absorption from a basal rice-based meal was 1.9 percent; adding 100 g of fresh fruit increased absorption to 4.8 percent and adding 80 g of lean meat increased non-heme iron absorption to 5.4 percent (*104*, *105*). In three other studies serving basal meals with vegetables rich in ascorbic acid, the absorption figures were 5.9 percent, 10 percent, and 10.8 percent, respectively (*106*). In a further study in Thailand, 60 g of fish were added to the same meal, which increased absorption to 21.6 percent. An even more realistic field study was done in Central Thailand to examine the reproducibility of dietary iron absorption measurements under optimal field conditions for 20 farmers and labourers (16 men, 4 women). The subjects had a free choice of foods (rice, vegetables, soup, a curry, and fish). All foods consumed were weighed and the rice was labelled with an extrinsic radioiron tracer. The mean absorption was 20.3 percent (adjusted to reference dose absorption of 56.5 percent) (*107*).

It is obvious that absorbed iron requirements need to be adjusted to different types of diets, especially in vulnerable groups. The Food and Agriculture Organization of the United Nations (FAO) and WHO recommended, for didactic reasons, three bio-availability levels of 5 percent, 10 percent, and 15 percent (8). For developing countries, it may be realistic to use the figures of 5 percent and 10 percent. In populations consuming more Western-type diets, two levels would be adequate – 12 percent and 15 percent – mainly depending on meat intake.

The amount of dietary iron absorbed is mainly determined by the amount of body stores of iron and by the properties of the diet (iron content and bio-availability). In anaemic subjects the rate of erythrocyte production also influences iron absorption. In a 55-kg woman with average iron losses who consumes a diet with an iron bio-availability of 15 percent, the mean iron stores would be about 120 mg. Under these circumstances approximately 10–15 percent of women would have no iron stores. When a diet with a bio-availability of 12 percent is consumed by a 55-kg woman, iron stores would be approximately 75 mg and about 25–30 percent of women would have no iron stores at all. When the bio-availability of iron decreases to 10 percent, mean iron stores are reduced to about 25 mg and about 40–50 percent of women consuming this diet would have no iron stores. Those consuming diets with an iron bio-availability of 5 percent have no iron stores and they are iron deficient. These calculations are based on a recent study (*69*).

Recommendations for iron intake for infants, children, younger and older adults, and pregnant and lactating women

Tables 39 and 40 showed both the physiologic absorbed iron requirements and the dietary iron requirements. All these figures are for the 95th percentile of iron requirements. The figures are given for women with a body weight of 55 kg and men with a body weight of 70 kg. For example, women with a body weight of 45 kg and men with a body weight of 55 kg have iron requirements that are 20 percent lower than those given in ***Table 39***.

No figures are given for dietary iron requirements in pregnant women because the iron balance in pregnancy depends not only on the properties of the diet but also and especially on the amounts of stored iron.

Future research

- Acquire knowledge of the content of phytate and iron-binding polyphenols in food, condiments, and spices. Produce new food tables, which include such data.
- Acquire knowledge about detailed composition of common meals and their usual variation in composition to examine the feasibility to make realistic recommendations

about changes in meal composition, taking into consideration the effect of such changes on other nutrients (e.g., vitamin A).

- Give high priority to systematic research. The very high iron requirements, especially in relation to energy requirements, in the weaning period make it difficult to develop foods and give recommendations that are effective and realistic. Alternatives such as home fortification of weaning foods should also be considered.
- Critically analyse the effectiveness of iron compounds used for fortification.
- Study models for improving iron supplementation, from the distribution of iron tablets to the increase of motivation to take iron supplements, especially during pregnancy.

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Chapter 14
Magnesium

Tissue distribution and functions of magnesium

The human body contains about 760 mg of magnesium at birth, approximately 5 g at age 4–5 months, and 25 g when adult (*1–3*). Of the body's magnesium, 30–40 percent is found in muscles and soft tissues, 1 percent is found in extracellular fluid, and the remainder is in the skeleton, where it accounts for up to 1 percent of bone ash (*4, 5*).

Soft tissue magnesium functions as a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes. Of particular importance with respect to the pathologic effects of magnesium depletion is the role of this element in regulating potassium fluxes and its involvement in the metabolism of calcium (*6–8*). Magnesium depletion depresses both cellular and extracellular potassium and exacerbates the effects of low-potassium diets on cellular potassium content. Muscle potassium becomes depleted as magnesium deficiency develops, and tissue repletion of potassium is virtually impossible unless magnesium status is restored to normal. Low plasma calcium develops frequently as magnesium status declines. It is not clear whether this occurs because parathyroid hormone release is inhibited or, more probably, because of a reduced sensitivity of the bone to parathyroid hormone, thus restricting withdrawal of calcium from the skeletal matrix.

Between 50 percent and 60 percent of body magnesium is located within bone, where it is thought to form a surface constituent of the hydroxyapatite (calcium phosphate) mineral component. Initially much of this magnesium is readily exchangeable with serum and therefore represents a moderately accessible magnesium store, which can be drawn on in times of deficiency. However, the proportion of bone magnesium in this exchangeable form declines significantly with increasing age (*9*).

Significant increases in bone mineral density of the femur have been associated positively with rises in erythrocyte magnesium when the diets of subjects with gluten-sensitive enteropathy were fortified with magnesium (*10*). Little is known of other roles for magnesium in skeletal tissues.

Origins and effects of magnesium deficiency

Pathologic effects of primary nutritional deficiency of magnesium occur infrequently in infants (*11*) but are even less common in adults unless a relatively low magnesium intake is accompanied by prolonged diarrhoea or excessive urinary magnesium losses (*12*). Susceptibility to the effects of magnesium deficiency rises when demands for magnesium increase markedly with the resumption of tissue growth during rehabilitation from general malnutrition (*6, 13*). Studies have shown that a decline in urinary magnesium excretion during protein-energy malnutrition (PEM) is accompanied by a reduced intestinal absorption of magnesium. The catch-up growth associated with recovery from PEM is achieved only if magnesium supply is increased substantially (*6, 14*).

Most of the early pathologic consequences of magnesium depletion are neurologic or neuromuscular defects (*12, 15*), some of which probably reflect the influence of the element on potassium flux within tissues. Thus, a decline in magnesium status produces anorexia,

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nausea, muscular weakness, lethargy, staggering, and, if deficiency is prolonged, weight loss. Progressively increasing with the severity and duration of depletion are manifestations of hyperirritability, hyperexcitability, muscular spasms, and tetany, leading ultimately to convulsions. An increased susceptibility to audiogenic shock is common in experimental animals. Cardiac arrhythmia and pulmonary oedema frequently have fatal consequences (*12*). It has been suggested that a sub-optimal magnesium status may be a factor in the aetiology of coronary heart disease and hypertension but additional evidence is needed (*16*).

Dietary sources, absorption, and excretion of magnesium

Dietary deficiency of magnesium of a severity sufficient to provoke pathologic changes is rare. Magnesium is widely distributed in plant and animal foods, and geochemical and other environmental variables rarely have a major influence on its content in foods. Most green vegetables, legume seeds, peas, beans, and nuts are rich in magnesium, as are some shellfish, spices, and soya flour, all of which usually contain more than 500 mg/kg fresh weight. Although most unrefined cereal grains are reasonable sources, many highly refined flours, tubers, fruits, and fungi and most oils and fats contribute little dietary magnesium (<100 mg/kg fresh weight) (*17–19*). Corn flour, cassava and sago flour, and polished rice flour have an extremely low magnesium content. ***Table 45*** presents representative data for the dietary magnesium intakes of infants and adults.

Table 45

Typical daily intakes of magnesium by infants (6 kg) and adults (65 kg)

Group and source of intake (reference)	Magnesium intake, mg/day [a]
Infants: 750 ml liquid milk or formula as sole food source	
Human milk	
Finland (*17*)	24 (23-25)
United States (*11, 20*)	23 (18-30)
United Kingdom (*21, 22*)	21 (20-23)
India (*23*)	24 ± 0.9
Formula	
United States (*11, 20*)	30–52
United Kingdom (whey based) (*24*)	30–52
United Kingdom (soya based) (*24*)	38–60
Adults: conventional diets	
France, males (*25*)	369 ± 106
France, females (*25*)	280 ± 84
United Kingdom, males (*26*)	323
United Kingdom, females (*26*)	237
United States, males (*27, 28*)	329
United States, females (*27, 28*)	207
India (*29*)	300–680
China, females (*30*)	190 ± 59
	232 ± 62
	333 ± 103

[a] Mean ± SD or mean (range).

Stable isotope studies with ^{25}Mg and ^{26}Mg indicate that between 50 percent and 90 percent of the labelled magnesium from maternal milk and infant formula can be absorbed by infants (*11, 20*). Studies with adults consuming conventional diets show that the efficiency of magnesium absorption can vary greatly depending on magnesium intake (*31, 32*). In one study 25 percent of magnesium was absorbed when magnesium intake was high compared with 75 percent when intake was low (*33*). During a 14-day balance study a net absorption of 52 ± 8 percent was recorded for 26 adolescent females consuming 176 mg magnesium daily (*34*). Although this intake is far below the US recommended dietary allowance (RDA) for this age group (280 mg/day), magnesium balance was still positive and averaged 21 mg/day. This provided one of several sets of data illustrating the homeostatic capacity of the body to adapt to a wide variety of ranges in magnesium intake (*35, 36*). Magnesium absorption appears to be greatest within the duodenum and ileum and occurs by both passive and active processes (*37*).

High intakes of dietary fibre (40–50 g/day) lower magnesium absorption. This is probably attributable to the magnesium-binding action of phytate phosphorus associated with the fibre (*38-40*). However, consumption of phytate- and cellulose-rich products (usually containing high concentrations of magnesium) increases magnesium intake, which often compensates for the decrease in absorption. The effects of dietary components such as phytate on magnesium absorption are probably critically important only at low magnesium intake. There is no consistent evidence that modest increases in the intake of calcium (*34-36*), iron, or manganese (*22*) affect magnesium balance. In contrast, high intakes of zinc (142 mg/day) decrease magnesium absorption and contribute to a shift toward negative balance in adult males (*41*).

The kidney has a very significant role in magnesium homeostasis. Active reabsorption of magnesium takes place in the loop of Henle in the proximal convoluted tubule and is influenced by both the urinary concentration of sodium and probably by acid-base balance (*42*). The latter relationship may well account for the observation from Chinese studies that dietary changes which result in increased urinary pH and decreased titratable acidity also reduce urinary magnesium output by 35 percent despite marked increases in dietary magnesium input for vegetable protein diets (*30*). Several studies have now shown that dietary calcium intakes in excess of 2600 mg/day (*37*), particularly if associated with high sodium intakes, contribute to a shift toward negative magnesium balance or enhance its urinary output (*42, 43*).

Criteria for assessing magnesium requirements and allowances

In 1996 Shils and Rude (*44*) published a constructive review of past procedures used to derive estimates of magnesium requirements. They questioned the arguments of many authors that metabolic balance studies are probably the only practicable, non-invasive techniques for assessing the relationships of magnesium intake to magnesium status. At the same time, they emphasised the great scarcity of data on variations in urinary magnesium output and on magnesium levels in serum, erythrocytes, lymphocytes, bone, and soft tissues. Such data are needed to verify current assumptions that pathologic responses to a decline in magnesium supply are not likely occur to if magnesium balance remains relatively constant.

In view of the recent conclusion that many estimates of dietary requirements for magnesium were "based upon questionable and insufficient data" (*44*), a closer examination is needed of the value of biochemical criteria for defining the adequacy of magnesium status (*13*). Attention could be paid to the effects of changes in magnesium intake on urinary magnesium-creatinine ratios (*45*), the relationships between serum magnesium-calcium and magnesium-potassium concentrations (*7, 8*), and other functional indicators of magnesium status.

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Estimated allowances of magnesium

The scarcity of studies from which to derive estimates of dietary allowances for magnesium has been emphasised by virtually all the agencies faced with this task. One United Kingdom agency commented particularly on the scarcity of studies with young subjects, and circumvented the problem of discordant data from work with adolescents and adults by restricting the range of studies considered (*21*). Using experimental data virtually identical to those used for a detailed critique of the basis for US estimates (27), the Scientific Committee for Food of the European Communities (*46*) did not propose magnesium allowances (or population reference intakes, PRIs) because of inadequate data. Instead, they offered an acceptable range of intakes for adults of 150–500 mg/day and described a series of quasi-PRI values for specific age groups, including an increment of 30 percent to allow for individual variations in growth. Statements of acceptable intakes leave uncertainty as to the extent of overestimation of derived recommended intakes.

It is questionable whether more reliable estimates of magnesium requirements can be made until data from balance studies are supported by the use of biochemical indexes of adequacy that could reveal the development of manifestations of sub-optimal status. Such indexes have been examined, for example, by Nichols *et al*. (*14*) in their studies of the metabolic significance of magnesium depletion during PEM. A loss of muscle and serum magnesium resulted if total body magnesium retention fell below 2 mg/kg/day and was followed by a fall in the myofibrillar nitrogen-collagen ratio of muscle and a fall in muscle potassium content. Repletion of tissue magnesium status preceded a threefold increase in muscle potassium content. It accelerated by 7–10 days at the rate of recovery of muscle mass and composition initiated by restitution of nitrogen and energy supplies to infants previously deficient.

Neurologic signs such as hyper-irritability, apathy, tremors, and occasional ataxia accompanied by low concentrations of potassium and magnesium in skeletal muscle and strongly negative magnesium balances were reported by many other studies of protein calorie deficiency in infants (*47–49*). Particularly noteworthy is evidence that all these effects are ameliorated or eliminated by increased oral magnesium, as were specific anomalies in the electrocardiographic T-wave profiles of such malnourished subjects (*49*). Evidence that the initial rate of growth at rehabilitation is influenced by dietary magnesium intake indicates the significance of this element for those involved in the aetiology of the PEM syndromes (*31, 50*).

Regrettably, detailed studies have yet to be carried out to define the nature of changes resulting from a primary deficiency of dietary magnesium. Definition of magnesium requirements must continue to be based on the limited information provided by balance techniques, which give little or no indications of responses to inadequacy in magnesium supply which may induce covert pathologic changes. Reassurance must thus be sought from the application of dietary standards for magnesium in communities consuming diets differing widely in magnesium content (*29*). The inadequate definition of lower acceptable limits of magnesium intakes raises concern in communities or individuals suffering malnutrition or from a wider variety of nutritional or other diseases which influence magnesium metabolism adversely (*12, 51, 52*).

Derivation of allowances for magnesium

The infrequency with which magnesium deficiency develops in human-milk-fed infants implies that the content and physiologic availability of magnesium in human milk meets the infants' requirements. The intake of maternal milk from exclusively human-milk-fed infants 1–10 months of age ranges from 700 to 900 g/day in both industrialized and developing

countries (*53*). If the magnesium content of milk is assumed to be 29 mg/l (*11, 54, 55*), the intake from milk is 20–26 mg/day, or approximately 0.04 mg/kcal.

The magnesium in human milk is absorbed with substantially greater efficiency (about 80–90 percent) than that of formula milks (about 55–75 percent) or solid foods (about 50 percent) (*56*), and such differences must be taken into account when comparing differing dietary sources. For example, a daily intake of 23 mg from maternal milk probably yields 18 mg available magnesium, a quantity similar to that of the 36 mg or more suggested as meeting the requirements of young infants given formula or other foods (***Table 46***).

An indication of a likely requirement for magnesium at other ages can be derived from studies of magnesium-potassium relationships in muscle (*58*) and the clinical recovery of young children rehabilitated from malnutrition with or without magnesium fortification of therapeutic diets. Nichols *et al.* (*14*) showed that 12 mg magnesium/day was not sufficient to restore positive magnesium balances, serum magnesium content, or the magnesium and potassium contents of muscle of children undergoing PEM rehabilitation. Muscle potassium was restored to normal by 42 mg magnesium/day but higher intakes of dietary magnesium, up to 160 mg/day, were needed to restore muscle magnesium to normal. Although these studies show clearly that magnesium synergized growth responses resulting from nutritional rehabilitation, they also indicated that rectification of earlier deficits of protein and energy was a pre-requisite to initiation of this effect of magnesium.

Similar studies by Caddell *et al.* (*49, 50*) also illustrate the secondary significance of magnesium accelerating clinical recovery from PEM. They indicate that prolonged consumption of diets low in protein and energy and with a low ratio (<0.02) of magnesium (in milligrams) to energy (in kilocalories) can induce pathologic changes which respond to increases in dietary magnesium supply. It is noteworthy that of the balance trials intended to investigate magnesium requirements, none has yet included treatments with magnesium-energy ratios of <0.04 or induced pathologic responses.

The relationship Mg = (kcal x 0.0099) - 0.0117 (SE ± 0.0029) holds for many conventional diets (*59*). Some staple foods in common use have very low magnesium contents; cassava, sago, corn flour or cornstarch, and polished rice all have low magnesium-energy ratios (0.003–0.02) (*18*). Their use in bulk merits appraisal of total dietary magnesium content.

It has been reported, with increasing frequency, that high percents (e.g., <70 percent) (*25*) of individuals from some communities in Europe have magnesium intakes substantially lower than estimates of magnesium requirements derived principally from US and UK sources (*21, 27*). Such reports emphasise the need for reappraisal of estimates for reasons previously discussed (*44*).

The estimates submitted by this Consultation must be regarded as provisional. Until additional data become available, these estimates reflect consideration of anxieties that previous recommendations for magnesium are overestimates. They make greater allowance for developmental changes in growth rate and in protein and energy requirements. In reconsidering data cited in previous reports (*21, 27, 46*), particular attention has been paid to balance data suggesting that the experimental conditions established have provided reasonable opportunity for the development of equilibrium during the investigation (*34, 60-62*).

Recommended magnesium intakes are presented in ***Table 46*** together with indications of the relationships of each recommendation to relevant estimates of the average requirements for dietary protein, and energy (*19*).

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Table 46

Recommended nutrient intakes for magnesium (Mg) in milligrams (mg)

Age Group[a]	Assumed body weight kg[b]	RNI mg/day	Relative intake ratios Mg/kg	Mg/g protein	Mg/kcal/day
Infants and children					
0–6 months					
Human-milk fed	6	26		2.5	0.05
Formula fed	6	36	6.0	2.9	0.06
7–12 months	9	54	6.0	3.9	0.06
1–3 years	12	60	5.5	4.0	0.05
4–6 years	19	76	4.0	3.9	0.04
7–9 years	25	100	4.0	3.7	0.05
Adolescents, 10–18 years					
Females	49	220	4.5	5.2	0.10
Males	51	230	3.5	5.2	0.09
Adults, 19–65 years					
Females	55	220	4.0	4.8	0.10
Males	65	260	4.0	4.6	0.10
65+ years					
Females	54	190	3.5	4.1	0.10
Males	64	224	3.5	4.1	0.09

[a] No increment for pregnancy; 50 mg/day increment for lactation.
[b] Assumed body weights of age groups derived by interpolation (*57*).
[c] Intake per gram of recommended protein intake for age of subject (*21*).
[d] Intake per kilocalorie estimated average requirement (*21*).

The detailed studies of magnesium economy during malnutrition and subsequent therapy, with or without magnesium supplementation, provide reasonable grounds that the dietary magnesium recommendations derived herein for young children are realistic. Data for other ages are more scarce and are confined to magnesium balance studies. Some have paid little attention to the influence of variations in dietary magnesium content and of the effects of growth rate before and after puberty on the normality of magnesium-dependent functions.

It is assumed that during pregnancy the foetus accumulates 8 mg and foetal adnexa accumulate 5 mg magnesium. If it is assumed that this dietary magnesium is absorbed with 50 percent efficiency, the 26 mg required over a pregnancy of 40 weeks (0.09 mg/day) can probably be accommodated by adaptation. A lactation allowance of 50–55 mg/day for dietary magnesium is made for the secretion of milk containing 25–28 mg magnesium (*21 ,64*). An absorption efficiency of 50 percent is assumed for all solid diets; data are not sufficient to allow for the adverse influence of phytic acid on magnesium absorption from high-fibre diets or from diets with a high content of pulses. Not surprisingly, few of the representative dietary analyses presented in ***Table 45*** fail to meet these allowances. The few exceptions, deliberately selected for inclusion, are the marginal intakes (232 ± 62 mg) of the 168 women of Changle county and the lower intake (190 ± 59 mg) of 147 women surveyed from Tuoli county China (*30*).

Upper tolerable limits of magnesium intake

Magnesium from dietary sources is relatively innocuous. Contamination of food or water supplies with magnesium salt has been known to cause hypermagnesemia, nausea, hypotension, and diarrhoea. Intakes of 380 mg magnesium as magnesium chloride have

produced such signs in women. Upper limits of 65 mg for children ages 1–3 years, 110 mg for 4–10 years, and 350 mg for adolescents and adults are suggested as tolerable limits for the content of soluble magnesium in foods and drinking water (*63*).

Relationships to previous estimates

The recommended intakes for infants ages 0–6 months take account of differences in the physiologic availability of magnesium from maternal milk as compared with infant formulas or solid foods. With the exception of the Canadian RNI estimates, which are 20 mg/day for ages 0–4 months and 32 mg/day for ages 5–12 months (*64*), other national estimates recommend intakes as RDAs or RNIs which substantially exceed the capacity of the lactating mother to supply magnesium for her offspring.

Recommendations for other ages are based subjectively on the absence of any evidence that magnesium deficiency of nutritional origin has occurred after consumption of a range of diets sometimes supplying considerably less than the US RDA or the UK RNI recommendations based on estimates of average magnesium requirements of 3.4–7 mg/kg body weight. The recommendations submitted herewith assume that demands for magnesium plus a margin of approximately 20 percent (to allow for methodologic variability) are probably met by allowing approximately 3.5–5 mg/kg from pre-adolescence to maturity. This assumption yields estimates virtually identical to those for Canada. Expressed as magnesium allowance (in milligrams) divided by energy allowance (in kilocalories) (the latter based upon energy recommendations from UK estimates (*21*), all the recommendations of ***Table 46*** exceed the provisionally estimated critical minimum ratio of 0.02.

It is appreciated that magnesium demand probably declines in late adulthood as requirements for growth diminish. However, it is reasonable to expect that the efficiency with which magnesium is absorbed declines in elderly subjects. It may well be that the recommendations are overgenerous for elderly subjects, but data are not sufficient to support a more extensive reduction than that indicated.

Future research

There is need for closer investigation of the biochemical changes that develop as magnesium status declines. The responses to magnesium intake which influence the pathologic effects resulting from disturbances in potassium utilisation caused by low magnesium should be studied. They may well provide an understanding of the influence of magnesium status on growth rate and neurologic integrity.

Closer investigation of the influence of magnesium status on the effectiveness of therapeutic measures during rehabilitation from PEM is needed. The significance of magnesium in the aetiology and consequences of PEM in children needs to be clarified. Claims that restoration of protein and energy supply aggravates the neurologic features of PEM if magnesium status is not improved merit priority of investigation. Failure to clarify these aspects may continue to obscure some of the most important pathologic features of a nutritional disorder in which evidence already exists for the involvement of a magnesium deficit.

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Chapter 15
Selenium

The role of selenium in human metabolic processes

Our understanding of the significance of selenium in the nutrition of human subjects has grown rapidly during the past 20 years (*1, 2*). Demonstrations of its essentiality to rats and farm animals were followed by appreciation that the development of selenium-responsive diseases often reflected the distribution of geochemical variables which restricted the entry of the element from soils into food chains. Such findings were the stimulus to in-depth investigations of the regional relevance of selenium in human nutrition (*3*). These studies have now yielded an increased understanding of the complex metabolic role of this trace nutrient. Selenium has been implicated in the protection of body tissues against oxidative stress, maintenance of defences against infection, and modulation of growth and development.

The selenium content of normal adult humans can vary widely. Values from 3 mg in New Zealanders to 14 mg in some Americans reflect the profound influence of the natural environment on the selenium contents of soils, crops, and human tissues. Approximately 30 percent of tissue selenium is contained in the liver, 15 percent in kidney, 30 percent in muscle, and 10 percent in blood plasma. Much of tissue selenium is found in proteins as seleno-analogues of sulphur amino acids; other metabolically active forms include selenotrisulphides and other acid-labile selenium compounds. At least 15 selenoproteins have now been characterised. Examples are given in ***Table 47***.

Table 47

A selection of characterised selenoproteins

Protein	Selenocysteine residues	Tissue distribution
Cytosolic glutathione peroxidase (GSHPx)	1	All, including thyroid
Phospholipid hydroperoxide GSHPx	1	All, including thyroid
Gastrointestinal GSHPx	1	Gastrointestinal tract
Extracellular GSHPx	1	Plasma, thyroid
Thioredoxin reductase	1 or 2	All including thyroid
Iodothyronine-deiodinase (type 1)	1	Liver, kidneys, and thyroid
Iodothyronine-deiodinase (type 2)	1	Central nervous system (CNS), and pituitary
Iodothyronine-deiodinase (type 3)	1	Brown adipose tissue, CNS, and placenta
Selenoprotein P	10	Plasma
Selenoprotein W	1	Muscle
Sperm capsule selenoprotein	3	Sperm tail

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Functionally, there appear to be at least two distinct families of selenium-containing enzymes. The first includes glutathione peroxidases (*4*) and thioredoxin reductase (*5*), which are involved in controlling tissue concentrations of highly reactive oxygen-containing metabolites. These metabolites are essential at low concentrations for maintaining cell-mediated immunity against infections but highly toxic if produced in excess. The role of selenium in the cytosolic enzyme glutathione peroxidase (GSHPx) was first illustrated in 1973. During stress, infection, or tissue injury, selenoenzymes may protect against the damaging effects of hydrogen peroxide or oxygen-rich free radicals. This family of enzymes catalyses the destruction of hydrogen peroxide or lipid hydroperoxides according to the following general reactions:

$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$$

$$ROOH + 2GSH \rightarrow ROH + H_2O + GSSG$$

where GSH is glutathione and GSSG is its oxidized form. At least four forms of GSHPx exist; they differ both in their tissue distribution and in their sensitivity to selenium depletion (*4*). The GSHPx enzymes of liver and blood plasma fall in activity rapidly at early stages of selenium deficiency. In contrast, a form of GSHPx associated specifically with phospholipid-rich tissue membranes is preserved against selenium deficiency and is believed to have broader metabolic roles (e.g., in prostaglandin synthesis) (*6*). In concert with vitamin E, selenium is also involved in the protection of cell membranes against oxidative damage (see ***Chapter 6***, ***Chapter 9***, and ***Chapter 17***).

The selenoenzyme thioredoxin reductase is involved in disposal of the products of oxidative metabolism (*5*). It contains two selenocysteine groups per molecule and is a major component of a redox system with a multiplicity of functions, among which is the capacity to degrade locally excessive and potentially toxic concentrations of peroxide and hydroperoxides likely to induce cell death and tissue atrophy (*6*).

Another group of selenoproteins is essential in the conversion of thyroxin, or tetraiodothyronine (T_4), to its physiologically active form, triiodothyronine (T_3) (*7*). Three types of these iodothyronine deiodinases, differing both in tissue distribution and sensitivity to selenium deficiency, have been characterised. The consequences of a low selenium status on physiologic responses to a shortage of iodine are complex. The influence of a loss of selenium-dependent iodothyronine deiodinase differs in its severity depending on whether a target tissue needs a preformed supply of T_3 (e.g., via plasma) or whether, as with the brain, pituitary gland, and placenta, it can rely upon local synthesis of T_3 from T_4. Despite this, marked changes in the T_3-T_4 ratio as a consequence of a reduced selenium status (when iodine supplies are also marginal) indicate the modifying influence of selenium on thyroid hormone balance in both animal models and human subjects. Their possible significance can be anticipated from the fact that whereas thyroid weights increase typically by 50 percent in rats offered an iodine-deficient diet, thyroid weight is increased 154 percent by diets concurrently deficient in both selenium and iodine.

Between 60 percent and 80 percent of selenium in human plasma is accounted for by a well-characterised fraction designated selenoprotein P, the function of which has yet to be determined. It is thought to be a selenium storage protein because there is limited evidence that it also has an antioxidant role. At least 10 other selenoproteins exist, including one which is a component of the mitochondrial capsule of sperm cells, damage to which may account for the development of sperm abnormalities during selenium deficiency. Other aspects of the function and metabolism of selenium are reviewed elsewhere (*8*, *9*).

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Selenium deficiency

Non-endemic deficiencies of selenium

Biochemical evidence of selenium depletion (e.g., a decline in blood GSHPx activity) is not uncommon in subjects maintained on parenteral or enteral feeding for long periods. Blood selenium values declining to one-tenth of normal values have been reported when the selenium content of such preparations has not been maintained by fortification (*10, 11*). Low selenium contents of some commercial formulas for infants resulting in a fall in daily selenium intake to approximately 0.5 µg/day have been shown to strongly exacerbate the fall in serum selenium and GSHPx activity normally experienced from 2 to 8 months of age even in human-milk-fed infants typically receiving threefold higher selenium intakes (*12, 13*). The importance of maintaining trace element levels in such preparations was reviewed elsewhere (*14*).

Clinical manifestations of deficiency arising from such situations are uncommon and poorly defined. They include muscular weakness and myalgia with, in several instances, the development of congestive heart failure. In at least one instance such pathologic signs have developed as a consequence of a generally inadequate diet providing selenium at less than 10 µg/day. The 2-year-old subject recovered rapidly after selenium administration (*15*). With this last exception, virtually all of the above reports describe observations with subjects under close medical supervision. This may well be relevant to the scarcity of consistent pathologic findings (*16*).

Keshan disease

Keshan disease was first described in Chinese medical literature more than 100 years ago, but not until 40 years after its widespread occurrence in 1935 was it discovered that selenium deficiency was an important factor in its aetiology (*3*). Endemic in children aged 2–10 years and in women of childbearing age, this disease has a geographic distribution covering localities from northeast to southwest China. Typical manifestations are fatigue after even mild exercise, cardiac arrhythmia and palpitations, loss of appetite, cardiac insufficiency, cardiomegaly, and congestive heart failure. Pathologic changes include a multifocal myocardial necrosis and fibrosis. The coronary arteries are essentially unaffected. Ultrastructural studies show that membranous organelles, such as mitochondria or sarcolemma, are affected earliest. The disease has a marked seasonal fluctuation in incidence (*3*) and may appear after only 3 months exposure to conditions in localities known to be associated with a high risk of myocarditis (*3, 8*). Once the disease is established, selenium is of little or no therapeutic value. Prophylaxis consisting of oral administration of selenium 3 months before the periods of highest anticipated risk is highly effective.

Although geographic similarities in the distribution of Keshan disease and the selenium and vitamin E–responsive white muscle disease in animals first prompted successful investigation of the relevance of a low selenium status, evidence has grown steadily that the disease is multifactorial in origin. The strongest suspicions have fallen on the development of a viral myocarditis probably attributable to enhancement of the virulence of a coxsackievirus during its passage through selenium-deficient host tissues (*17*). Although other nutritional variables such as a marginal vitamin E status may also be involved, the finding of extremely low selenium contents in staple crops of affected areas and convincing demonstrations of the prophylactic effectiveness of selenium administration leave no doubt that selenium deficiency is the primary factor (*3*, *18*).

Recent studies indicate that geochemical variables have an important influence on the distribution of Keshan disease. Acid soils high in organic matter and iron oxide content appear to be responsible for fixing selenium in forms which are poorly absorbed by staple

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crops which, in the instance of cereal grains, typically have a selenium content of less than 0.01 μg/g (*19*). A similar geochemical background is believed to be associated with reports of selenium-responsive disorders resembling Keshan disease in the Transbaikalia region of south Siberia. Dietary intakes of selenium are inadequate to maintain blood GSHPx activity; biochemical indicators of tissue peroxidative damage are elevated until selenium therapy is initiated (*8*).

Kaschin-beck disease

A selenium-responsive bone and joint disease (osteoarthropathy) has been detected in children aged 5–13 years in China and less extensively in south-east Siberia. The disease is characterised by joint necrosis – epiphyseal degeneration of the arm and leg joints resulting in structural shortening of the fingers and long bones with consequent growth retardation and stunting (*3*, *20*). Although not identical to Keshan disease, Kaschin-Beck disease also occurs in areas where the availability of soil selenium for crop growth is low. The selenium contents of hair and of whole blood are abnormally low and the blood content of GSHPx is reduced. Although it is ameliorated by selenium therapy, other factors such as the frequent presence of mycotoxins in cereal grains grown in the area may be involved. A spontaneous decrease in incidence from 1970 (44 percent) to 1980 (14 percent) to 1986 (1 percent) has been attributed to general improvements in the nutritional status of Chinese rural communities (*20*).

Selenium status and susceptibility to infection

As stated earlier, the expressions of the cardiac lesions of Keshan disease probably involve not only the development of selenium deficiency but also the presence of a Coxsackie virus (BA) infection. Animal studies have confirmed that selenium-deficient mice infected with Coxsackie virus (CVB/0) were particularly susceptible to the virus. These studies also illustrated that passage of the virus through selenium-deficient subjects enhanced its virulence (*17*). Myocarditic virulence developed even in strains such as CVB/0 which normally were not myopathogenic. The enhancement of virulence in this RNA virus involves modifications to the nucleotide sequence of the phenotype. These modifications were maintained and expressed even during subsequent passage through animals with normal selenium status (*21*).

Enhancing the virulence of a virus with a selenium deficiency (resulting either from a nutritional challenge or an increased metabolic demand on tissue selenium depots) appears not to be unique to the Coxsackie viruses. The early pre-clinical stages of development of human immunodeficiency virus (HIV) infection are accompanied by a very marked decline in plasma selenium. Sub-clinical malnutrition assumes increased significance during the development of acquired immune deficiency syndrome (AIDS). However, for the nutrients affected, there are strong indications that only the extent of the decline in selenium status has predictive value with respect to both the rate of development of AIDS and its resulting mortality (*22-25*). The virulence of other RNA viruses such as hepatitis B and those associated with the development of haemolytic anaemias are enhanced similarly by a decline in selenium status. The mechanisms underlying these effects are not yet resolved. There are indications that the loss of protective antioxidant functions dependent on selenium and vitamin E are both involved and that the resulting structural changes in viral nucleotide sequences are reproducible and appear to provoke additional selenoprotein synthesis (*26*). It is suspected that this further depletes previously diminished pools of physiologically available selenium and accelerates pathologic responses (*27-29*).

Whatever mechanisms are involved, further understanding is needed of the influence of selenium status on susceptibility to viral diseases ranging from cardiomyopathies to haemolytic anaemias. The relationship already illustrates the difficulty of defining nutritional essentiality for nutrients which may primarily maintain defences against infection. Studies of

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the effects of selenium deficiency in several experimental animal species have shown that the microbicidal activity of blood neutrophils is severely impaired even though phagocytic activity remains unimpaired (*30, 31*). The complexity of species differences in the influence of selenium status on the effectiveness of cell-mediated immune processes is summarised elsewhere (*8*).

The possibility that increased intakes of selenium might protect against the development of cancer in humans has generated great interest (*32*). However, a number of epidemiologic studies have now been reported which show no relationship between selenium and cancer risk (*33*). Moreover, an analysis of the relationship between selenium and cancer suggests that "the question of whether selenium protects against cancer is still wide open" (*34*). An increased intake of selenium appears to stimulate tumorigenesis in some animal models of pancreatic and skin cancer. In contrast, the protective effect of higher exposures to selenium observed in several animal studies, together with small but statistically significant differences in selenium blood plasma levels detected in some retrospective-prospective studies of subgroups of people developing cancer, explains the continuing interest in the anticarcinogenic potential of selenium. However, the results of prospective-retrospective studies had no predictive value for individuals and could have reflected non-specific influences on groups. The association between low selenium intake and high cancer risk, although clearly of some interest, is in need of further investigation before a conclusion can be reached.

Although a biochemical mechanism can be postulated whereby selenium could protect against heart disease by influencing platelet aggregation (through an effect on the prostacyclin-thromboxane ratio), the epidemiologic evidence linking selenium status and risk of cardiovascular disease is still equivocal (*33*).

Selenium and thyroid hormones

The importance of selenium for thyroid hormone metabolism (*35, 36*) is evident from changes in the T_3-T_4 ratio which develop after relatively mild selenium depletion in infants and elderly (65+ years) subjects. Decreases in the T_3-T_4 ratio indicative of decreased thyroid hormone balance have been detected when serum selenium falls below 0.9 μmol/l (*37*). In a recent Scottish study these decreases were correlated with a decline in dietary and plasma selenium after the replacement of selenium-rich wheat from Canada and the United States with selenium-deficient wheat from European sources (*38*).

Communities noted for a high incidence of myxedematous cretinism have been found to have low plasma selenium status and GSHPx activity in addition to having low iodine status (*39*) and being exposed to high thiocyanate intakes from cassava. Restoration of iodine supply, particularly if excessive, tends to induce a high peroxidative stress through the action of iodide peroxidase, the first step in iodine utilisation by the thyroid. It is postulated that necrosis and thyroid fibrosis leading to irreversible hypothyroidism result if a concurrent deficiency of selenium limits peroxide destruction by the protective action of the selenium-dependent enzymes, GSHPx and, more probably, thioredoxin reductase (*40*). In areas where myxedematous cretinism is endemic and characterised by persistent hypothyroidism, dwarfism, and stunting, it has been recommended that attempts to introduce iodine therapy for mildly affected individuals should be preceded by an assessment of selenium status and rectification of any observed deficit (*39*). Although this suggestion is compatible with pathologic observations on hypothyroid rats differing in selenium status, its validity has yet to be assessed adequately in humans (*41*, *42*).

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The influence of diet on selenium status

Environmental conditions and agricultural practices have a profound influence on the selenium content of many foods. ***Tables 48, 49 and 50*** illustrate the wide range of selenium content of the principal food groups and the variability in the selenium content of individual foods. This variability is exceeded only by that found in the iodine content of foods. Geographic differences in the content and availability of selenium from soils to food crops and animal products have a marked effect on the selenium status of entire communities. For example, the distribution of Keshan disease and Kaschin-Beck disease in China reflects the distribution of soils from which selenium is poorly available to rice, maize, wheat, and pasture grasses (***Table 48***). Cereal crop selenium contents of 3–7 ng/g are not uncommon (*3*) and it has been suggested that <10 ng/g for grain selenium and <3 ng/g for water-soluble soil selenium could be used as indexes to define deficient areas (*19*). Fluctuations in the selenium status of many communities in northern Europe reflect the intrinsically low selenium content of its glacial soils and the extent to which selenium supplementation of fertilisers has been successful in increasing the selenium content of cereal grains, milk, and other animal products. Deliberate importation of cereals from areas with relatively high available selenium in soil has also occurred or been recommended in some areas of Finland, New Zealand, and the United Kingdom after steady declines in the selenium status of some communities were noted. Conversely, low-selenium grains are being selected in China, India, and Venezuela to reduce the risks of selenosis.

Comprehensive data summarising the selenium contents of staple foods are available elsewhere (e.g., *45*). Reports from the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) provide representative data on daily total selenium intakes for more than 40 countries (see reference *8*, p 215–217). The great influence of dietary and geographic variables on selenium status is evident from recent summaries of data describing national and regional differences for the selenium content of human and formula milks, diets, and human serum (***Tables 48, 49, 50 and 51***).

Absorption and bio-availability

Selenium compounds are generally very efficiently absorbed by humans, and selenium absorption does not appear to be under homeostatic control (*80*). For example, absorption of the selenite form of selenium is greater than 80 percent whereas that of selenium as selenomethionine or as selenate may be greater than 90 percent (*80, 81*). Therefore, the rate-limiting step determining the overall availability of dietary selenium is not likely to be its absorption but rather its conversion within tissues to its metabolically active forms (e.g., its incorporation into GSHPx or 5'-deiodinase) (*40*). A number of depletion-repletion experiments have been carried out on animals to estimate the bio-availability of selenium in human foods (*82*). Based on the restoration of GSHPx activity in depleted rats, the bio-availability of selenium in wheat is quite good, usually 80 percent or better. The selenium in Brazil nuts and beef kidney also appears readily available (90 percent or more by most criteria). The selenium in tuna seems of lesser availability (perhaps only 20–60 percent of that from selenite) whereas the availability of selenium from certain other seafoods (shrimp, crab, and Baltic herring) is high. The selenium in a variety of mushrooms appears to be of uniformly low availability to rats.

Data on the nutritional bio-availability of selenium to humans are sparse. A supplementation study carried out on Finnish men of relatively low selenium status showed that selenate selenium was as effective as the selenium in seleniferous wheat in increasing platelet GSHPx activity (*83*). The wheat selenium, however, increased plasma selenium levels more than did selenate selenium and once the supplements were withdrawn, platelet GSHPx activity declined less in the group given wheat. This study showed the importance of

estimating not only short-term availability but also long-term retention and the convertibility of tissue selenium stores into biologically active forms.

Table 48

The selenium contents of foods and diets

A. Typical ranges of selenium concentrations (ng/g fresh wt) in food groups.

Food group	India (*43*)	United States (*44*)	International compilation (*8*)
Cereals and cereal products	5–95	10–370	10–550
Meat, meat products, and eggs	40–120	100–810	10–360
Fish and marine	280–1080	400–1500	110–970
Fish and freshwater	—	—	180–680
Pulses	10–138	—	—
Dairy products	5–15	10–130	1–170
Fruits and vegetables	1–7	1–60	1–20

B. Typical distribution of selenium in dietary constituents (µg/day) in selected countries.

	China Keshan disease area (*18*)	China Disease-free area (*18*)	India Low-income vegetarian diets (*43*)	India Low-income conventional diets (*43*)	Finland (*45*)	United Kingdom (*46*)
Total diet	7.7	16.4	27.4	52.5	30	31
Food group						
Cereals and cereal products	5.4	11.6	15.7	21.1	2.8	7
Pulses	—	—	3.9	3.6	1.1	—
Meat and eggs			—	3.7	9.2	10
Fish	>0.6	> 2.2	—	18.4	9.5	4
Dairy products			6.9	4.8	6.5	3
Fruits and vegetables	1.7	2.6	0.9	0.9	0.5	6
Other	—	—	—	—	1.1	3

Table 49

Geographic differences in the selenium intakes of infants

Region or country	Selenium intake (μg/day)[a]	Reference
Human milk		
China: Keshan disease area	2.0	*18*
Burundi	4.7 ± 0.8	*48*
New Zealand: south island	5.3	*49*
Former Yugoslavia	6.0 ± 1.3	*50*
Finland	4.0–7.6	*51*
New Zealand: north island	8.1–10.2	*52*
Belgium	8.4	*53*
Austria	8.8–9.8	*13*
Australia	9.4 ± 3.6	*54*
Hungary	9.6 ± 3.7	*50*
Sweden	10.6 ± 2.3	*50*
United States, east coast	8.8–11.4	*55*
United States, unspecified	12.3	*56*
Zaire	12.3 ± 3.6	*50*
Chile	14.1 ± 2.6	*50*
India	14.1 ± 3.6	*50*
Germany	19.3	*57*
Philippines	22.9 ± 4.1	*50*
China (seleniferous area)	199	*18*
International reference value	13.0 ± 18.2	*58*
Infant formula		
Belgium	2.0	*53*
New Zealand	3.3	*59*
Austria	3.6	*13*
United Kingdom	4.9 (2.3–8.2)	*53*
Spain	6.6	*53*
United States, 1982	5.9 (4.2–8.1)	*60*
Germany	6.5-6.8	*57*
New Zealand (selenium fortified)	11.3	*59*
United States, 1997	11.7–18.3	*61*

[a] Mean ± standard deviation (SD) or range.
Assumed age 6 months; assumed human milk or infant formula intake 750 ml (*47*).

Table 50

Geographic differences in the selenium intakes (µg/day) of adults

Region or country	Selenium intake [a]	Reference
China, Keshan disease area	3–11	*62, 63*
China, Kaschin -Beck disease area	2.6–5.0	*20*
Sweden, vegans	10	*64*
New Zealand, low-selenium area	11 ± 3	*64, 65*
China, disease-free area	13.3 ± 3.1	*18*
South Sweden, conventional diets	40 ± 4	*66*
India,vegan low income	27	*43*
Finland, before selenium fertilization	26	*67–69*
Finland, after selenium fertilization	56	*67–69*
Slovakia	27 ± 8	*70*
United Kingdom, 1974	60	*38*
United Kingdom, 1985	43	*38*
United Kingdom, 1994	32	*38*
United Kingdom, 1995	33	*46*
Italy	41	*64*
Germany	38–48	*71*
France	47	*72*
India, conventional diet	48	*43*
United States, all	80 ± 37	*73*
Males	90 ± 14	*73*
Females	74 ± 12	*73*
Canada	98–224	*74*
United States, seleniferous area	216	*64*
Venezuela	80–500	*75*
China, seleniferous area	1338	*64*

[a] Mean ± standard error or range.

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Table 51

Representative mean serum selenium concentration (µmol/l) in specific studies[a]

Pathologic investigations	Sample Serum Selenium Concentrations[b]
Keshan disease (China)	0.15–0.25
Kaschin-Beck disease (China)	0.22 ± 0.03
Myxoedematous cretins (Zaire)	0.26 ± 0.12
HIV and AIDS[c]	0.36–0.54
Data for normal subjects	
Serbia and Croatia	0.63–0.85
Bulgaria	0.66–0.72
New Zealand	0.69
Hungary	0.71 ± 0.13
Norway	1.52–1.69
United States, Maryland	1.69–2.15
United States, South Dakota	2.17–2.50
Proposed reference ranges for healthy subjects	0.5–2.5 0.67– 2.04

[a] References 8, 18, 23, 25, 44, 76–79.
[b] Ranges of means or mean ± standard error.
[c] HIV, Human immunodeficiency virus; AIDS, acquired immune deficiency syndrome.

Criteria for assessing selenium requirements

Levander (*84*) convincingly illustrated the impracticability of assessing selenium requirements from input-output balance data because the history of selenium nutrition influences the proportion of dietary selenium absorbed, retained, or excreted. The changing equilibria when selenium intake is varied experimentally yield data which are of limited value for estimating minimal requirements. Examples are cited of estimates of selenium requirement for adults of 7.4 and 80 µg/day derived from Chinese and United States studies, respectively. Such discrepancies reflect differences in the usual daily selenium intakes of the experimental subjects and the extent to which this was changed experimentally. This situation, not unique to selenium, emphasises the importance of basing requirement estimates on functional criteria derived from evidence describing the minimum levels of intake which, directly or indirectly, reflect the normality of selenium-dependent processes.

New opportunities for the development of biochemical indexes of selenium adequacy such as those listed in ***Table 47*** have yet to be exploited. Until this is done, the most suitable alternative is to monitor changes in the relationship of serum selenium to dietary selenium supply, taking advantage of its relatively constant proportionality to the fraction of serum selenium in functionally significant GSHPx (*85*).

A detailed review of 36 reports describing serum selenium values in healthy subjects indicated that they ranged from a low of 0.52 µmol/l in Serbia to a high of 2.5 µmol/l in Wyoming and South Dakota in the United States (*76*). It was suggested that mean values within this range derived from 7502 apparently healthy individuals should be regarded tentatively as a standard for normal reference. This survey clearly illustrated the influence of crop management on serum selenium level; in Finland and New Zealand, selenium fortification of fertilisers for cereals increased serum selenium from 0.6 to 1.5 µmol/l. A summary of these data in ***Table 51*** also includes representative mean serum selenium values within the range of 0.15–0.55 µmol/l reported for specific diseases known to be associated

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with disturbances in selenium nutrition or metabolism. These include reports from studies of Keshan disease, Kaschin-Beck disease, and specific studies of cretinism, hypothyroidism, and HIV and AIDS where clinical outcome or prognosis has been related to selenium status.

This report and the report by the World Health Organization (WHO), FAO, and IAEA (*86*) use virtually identical approaches to derive their estimates of basal requirements for selenium (Se_R^{basal}). As yet there are no published reports suggesting that these basal estimates using Se or GSHPx activity as criteria of adequacy are invalid. Some modification is necessary however to estimate population minimum intakes with adequate allowance for the variability (CV) associated with estimates of the average selenium intakes from the typical diets of many communities. In the WHO-FAO-IAEA report (*86*) a CV of 16 percent was assumed for the selenium conventional diets and 12.5 percent for the milk-based diets of infants to limit the risks of inadequacy arising from unexpectedly low selenium contents. More recent studies suggest that the variability of selenium intake from diets for which the selenium content has been predicted rather than measured may be substantially greater than estimated previously (**Table *49*** [*47*] and ***Table 50***).

Recommended selenium intakes

Because balance techniques were shown to be inappropriate for determining selenium requirements, the WHO-FAO-IAEA report (*86*) presented requirement estimates based on of epidemiologic evidence derived from areas of China endemic or non-endemic for Keshan disease (*18*). These comprehensive biochemical and clinical studies showed that Keshan disease did not occur in regions where the mean intake of selenium by adult males or females was greater than 19.1 or greater than 13.3 µg/day, respectively. Although these intakes were sufficient to eliminate clinical evidence of myocarditis and other signs of Keshan disease, other studies showed that they were inadequate to restore erythrocyte or plasma selenium concentrations or GSHPx activities to levels indicative of reserves.

Studies with adult male subjects initially of low selenium status given a carefully monitored diet providing selenium at 11 µg/day together with supplements of selenomethionine given orally which provided 0, 10, 30, 60, or 90 µg/day. Starting at frankly deficient levels, total daily selenium intakes of above 41 µg/day were found sufficient increase plasma GSHPx substantially and to saturate plasma activity in 60-kg male subjects within 5–8 months. It was estimated that satisfactory levels of plasma selenium (>80 µmol/l) and of GSHPx (>0.3 mmol NADPH oxidized/min/l; approximately two-thirds of plasma saturation activity) indicative of adequate selenium reserves would be attained after intakes of approximately 27 µg/day by 65-kg male subjects (*86*). Such criteria satisfying the definition of average normative requirements for selenium ($Se_R^{normative}$) have been used as the basis for calculating recommended nutrient intake (RNI) values in this report after interpolating estimates of average requirements by allowing for differences in weight and basal metabolic rate of age groups to up to 65 years and adding a 25 percent increase (2 x assumed SD) to allow for individual variability in the estimates of RNI (***Table 52***).

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Table 52

Recommended nutrient intakes of selenium (µg/day)

Age Group	Assumed Weight	Average normative requirement $Se_R^{normative}$ (kg/day)	Average normative requirement $Se_R^{normative}$ (total/day)	RNI[c], µg/day
Infants and children				
0–6 months	6	0.85	5.1	6
7–12 months	9	0.91	8.2	10
1–3 years	12	1.13	13.6	17
4–6 years	19	0.92	17.5	22
7–9 years	25	0.68	17.0	21
Adolescents				
Female,10–18 years	49	0.42	20.6	26
Male, 10–18 years	51	0.50	22.5	32
Adults				
Female,19–65 years	55	0.37	20.4	26
Male, 19–65 years	65	0.42	27.3	34
Female, 65+ years	54	0.37	20.2	25
Male, 65+ years	64	0.41	26.2	33
Pregnancy				
2nd trimester				28
3rd trimester				30
Lactation				
0–6 months post-partum				35
7–12 months post-partum				42

[a] Weight (kg) interpolated from FAO/WHO (reference *86*, page 8, Table 2.1).
[b] Derived from WHO-FAO-IAEA values (reference *86*, page 116, Table 6.1, by interpolation).
[c] Recommended nutrient intake (RNI) derived from average $Se_R^{normative}$ + 2 x assumed standard error (of 12.5 percent)

The estimates of RNI for infants (***Table 52***) are compatible with estimates of the international reference range of the selenium content of breast milk (18.5 µg /l; ***Table 49***), with data from an extensive international survey of breast milk selenium (WHO-IAEA [*50*]) and with WHO data (*47*) on the milk consumption of exclusively human-milk-fed infants in developed and developing countries. Data from the WHO-IAEA (*50*) survey from six countries suggest that the human milk from all countries met the RNI for infants aged 0–6 months. In two of six countries, Hungary and Sweden, the human milk selenium was marginal with respect to the RNI for infants aged 7–12 months.

Data from Germany (*13, 88*), Austria (*12*), the United States (*89*), and elsewhere suggest that infant formula may contain selenium in amounts insufficient to meet the RNI or recommended dietary allowance for infants. Lombeck *et al.* (*13*) in an extensive study showed that cow-milk-based formula could provide less than one-third of the selenium of human milk. Estimates of selenium intake by 2-month-old infants were 7.8 µg/day from formula compared with 22.4 µg/day from human milk. Levander (*89*) has suggested that infant formulas should provide a minimum of 10 µg/day and not more than 45 µg/day. This recommendation may well have been implemented judging from recent increases in the selenium content of infant formulas (*61*).

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Selenium requirements during pregnancy and lactation

Data from balance experiments are not sufficiently consistent for defining the increase of selenium needed to support foetal growth and development during pregnancy. For this reason the European Union Scientific Committee for Food (*90*), the UK Committee on Medical Aspects of Food Policy (*91*), and the Netherlands Food and Nutrition Council (*92*) have suggested that the component of selenium needed for human pregnancy is obtained by an adaptive increase in the efficiency of absorption of dietary selenium rather than by an increased dietary demand.

Others, contesting this view, have attempted to predict the increase of dietary selenium needed for pregnancy by factorial estimation of the likely quantity of selenium incorporated into the tissues of the foetus (*47, 86*). Such estimates have assumed that the total products of conception amount to 4.6–6 kg lean tissue with a protein content of approximately 18.5–20 percent. If, as appears to be a reasonable assumption, the selenium content of this protein resembles that of a skeletal muscle, growth of these tissues could account for between 1.0 and 4.5 µg/day of selenium depending on whether the analyses reflect consumption of diets from a low-selenium (but non-pathogenic) environment (e.g., New Zealand [*49, 52*]) or from a region with relatively high selenium intakes (e.g., United States, ***Table 50*** [73]). Typically such estimates have assumed an 80 percent absorption and utilisation of dietary selenium from which it would appear reasonable to estimate that allowing for a variability of estimates (CV 12.5 percent) an increase of 2 µg/day would be appropriate for the second trimester and 4 µg/day would be appropriate for the third trimester of pregnancy (***Table 52***).

As is evident from ***Table 49*** the selenium content of human milk is sensitive to changes in maternal dietary selenium. The increase of maternal dietary selenium needed to meet requirements for lactation has been estimated from the estimated RNI for infants aged 0–6 months and 7–12 months. It is assumed that the selenium of maternal milk is used with an efficiency of 80 percent, and a SD of 12.5 percent is assumed. For the period 0–6 months it is estimated that the infant must receive 6 µg/day from human milk. The increase of maternal dietary selenium required to produce this will be $6 \times \frac{100}{80} + (2 \times SD) = 9$ µg/day. The corresponding increase needed to meet the infant RNI of 10 µg/day for ages 7–12 months will be 16 µg/day. Added to the non-pregnancy maternal RNI of 26 µg/day, the total RNI for lactation during the first 6 months post-partum will be 35 µg/day and for months 7–12 will be 42 µg/day (***Table 52***).

As implied by the data in ***Tables 48, 49*** *and* ***50***, agricultural growing practices, geologic factors, and social deprivation enforcing the use of an abnormally wide range of dietary constituents may significantly modify the variability of dietary selenium intakes. If accumulated experience suggests that the CV of selenium intake may be 40 percent or more and tabulated rather than analysed data are used to predict the dietary content of selenium, the selenium allowance may have to be increased accordingly or assessed by using the WHO-FAO-IAEA technique (*86*).

Upper tolerable nutrient level for selenium

A comprehensive account of the clinically significant biochemical manifestations of chronic and acute intoxication from selenium arising from high concentrations in food, drinking water, and the environment were published jointly by WHO and the United Nations Environment Programme and the International Labour Organisation (*80*). This report (*44*) stresses that the signs and symptoms of human overexposure to selenium are not well defined. Common clinical features are hair loss and structural changes in the keratin of hair and of nails, the development of icteroid skin, and gastrointestinal disturbances (*93, 94*). An increased incidence of nail dystrophy has been associated with consumption of high-selenium

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foods supplying more than 900 µg/day. These foods were grown in selenium-rich (seleniferous) soil from specific areas in China (*95*). A positive association between dental caries and urinary selenium output under similar circumstances was reported (*96*, *97*).

Sensitive biochemical markers of impending selenium intoxication have yet to be developed. In their absence it is suggested that the upper tolerable nutrient intake level (UL) for selenium should be set, provisionally, at 400 µg/day for adults. It is noteworthy that a maximum tolerable dietary concentration of 2 mg/kg dry diet was suggested for all classes of domesticated livestock and has proved satisfactory in use (*98*). This suggests that the proposed UL of 400 µg/day for human subjects provides a fully adequate margin of safety. The UL for children and for pregnant or lactating women has yet to be determined.

Comparison with previous estimates

Compared with WHO-FAO-IAEA (*86*), US (*87*), UK (*91*), and European Union (*90*) recommendations, the present proposals represent a significant decrease in the suggested need for selenium. Reasons for this are:

- the need to derive recommendations which are applicable for a proportionally lower weight range than for most Western and developed communities;
- the decision, also accepted by WHO, FAO, and IAEA (*86*), that it is neither essential nor desirable to maintain selenium status at a level which fully saturates blood GSHPx activity when, on current evidence, this is not an advantage for health; and
- the decision to present estimates as RNIs which, although including an allowance for individual variability, do not provide for the possibility that foods may often differ widely in selenium content according to their geographic sources.

The reduced estimates presented in this report are physiologically justifiable and will only give rise to concern if there are grounds for serious uncertainty as to the predictability of dietary selenium intake.

Food commodity inputs are changing rapidly and in some instances, unpredictably. Under most circumstances it will be unreasonable to expect that the often marked influence of geographic variability on the supply of selenium from cereals and meats can be taken into account. Changes in trade patterns with respect to the sources of cereals and meats are already having significant influences on the selenium nutrition of consumer communities (*38, 66*). Such evidence fully justifies the warning to allow for a high intrinsic variability of dietary selenium content when estimating selenium requirements of populations for which the principal sources of this microelement are unknown.

Future research

Relationships between selenium status and pathologically relevant biochemical indexes of deficiency merit much closer study with the object of providing more reliable and earlier means of detecting a suboptimal status.

Indications that a suboptimal selenium status may have much wider significance in influencing disease susceptibility must be pursued. Such studies must cover both the impact of selenium deficiency on protection against oxidative damage during tissue trauma and its genetic implication for viral virulence.

We lack knowledge of the influence of soil composition on the selenium content of cereals and animal tissues. Chinese experience with respect to the dramatic influence of soil iron and low pH on selenium availability may well be relevant to extensive tracts of lateritic soils in Africa and elsewhere. There are grounds for the belief that factors in common for

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selenium and iodine may influence their supply and availability from soils into the human food chain. FAO should be encouraged to develop studies relevant to the influence of soil conditions on the supply of these two metabolically interdependent elements which affect human health.

The early detection of selenium toxicity (selenosis) is hindered by a lack of suitable biochemical indicators. Effective detection and control of selenosis in many developing countries awaits the development of improved specific diagnostic techniques.

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Chapter 16
Zinc

Role of zinc in human metabolic processes

Zinc is present in all body tissues and fluids. The total body zinc content has been estimated to be 30 mmol (2 g). Skeletal muscle accounts for approximately 60 percent of the total body content and bone mass, with a zinc concentration of 1.5–3 μmol/g (100-200 μg/g), for approximately 30 percent. Zinc concentration of lean body mass is approximately 0.46 μmol/g (30 μg/g). Plasma zinc has a rapid turnover rate and it represents only about 0.l percent of total body zinc content. This level appears to be under close homeostatic control. High concentrations of zinc are found in the choroid of the eye 4.2 μmol/g (274 μg/g) and in prostatic fluids 4.6-7.7 mmol/l (300-500 mg/l) (*1*).

Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. Zinc stabilises the molecular structure of cellular components and membranes and contributes in this way to the maintenance of cell and organ integrity. Furthermore, zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms.

Zinc plays a central role in the immune system, affecting a number of aspects of cellular and Humoral immunity (*2*). The role of zinc in immunity was reviewed extensively by Shanglar *et al*. (*2*).

The clinical features of severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioural changes (*1*). The effects of marginal or mild zinc deficiency are less clear. A reduced growth rate and impairments of immune defence are so far the only clearly demonstrated signs of mild zinc deficiency in humans. Other effects, such as impaired taste and wound healing, which have been claimed to result from a low zinc intake, are less consistently observed.

Zinc metabolism and homeostasis

Zinc absorption is concentration dependent and occurs throughout the small intestine. Under normal physiologic conditions, transport processes of uptake are not saturated. Zinc administered in aqueous solutions to fasting subjects is absorbed efficiently (60–70 percent), whereas absorption from solid diets is less efficient and varies depending on zinc content and diet composition (*3*).

Zinc is lost from the body through the kidneys, skin, and intestine. The endogenous intestinal losses can vary from 7 μmol/day (0.5 mg/day) to more than 45 μmol/day (3 mg/day), depending on zinc intake (*4*). Urinary and skin losses are of the order of 7-10 μmol/day (0.5–0.7 mg/day) each and depend less on normal variations in zinc intake (*4*). Starvation and muscle catabolism increase zinc losses in urine. Strenuous exercise and elevated ambient temperatures could lead to losses by perspiration.

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The body has no zinc stores in the conventional sense. In conditions of bone resorption and tissue catabolism, zinc is released and may be re-utilised to some extent. Human experimental studies with low-zinc diets 2.6–3.6 mg/day (40-55 μmol/day) have shown that circulating zinc levels and activities of zinc-containing enzymes can be maintained within normal range over several months (*5*, *6*), which highlights the efficiency of the zinc homeostasis mechanism. Controlled depletion-repletion studies in humans have shown that changes in the endogenous excretion of zinc through the kidneys, intestine, and skin and changes in absorptive efficiency are how body zinc content is maintained (*7-10*). The underlying mechanisms are poorly understood.

Sensitive indexes for assessing zinc status are unknown at present. Static indexes, such as zinc concentration in plasma, blood cells, and hair, and urinary zinc excretion are decreased in severe zinc deficiency. A number of conditions that are unrelated to zinc status can affect all these indexes, especially zinc plasma levels. Infection, stress situations such as fever, food intake, and pregnancy lower plasma zinc concentrations whereas, for example, long-term fasting increases it (*11*). However, on a population basis, reduced plasma zinc concentrations seem to be a marker for zinc-responsive growth reductions (*12*, *13*). Experimental zinc depletion studies suggest that changes in immune response occur before reductions in plasma zinc concentrations are apparent (*14*). So far, it has not been possible to identify zinc-dependent enzymes which could serve as early markers for zinc status.

A number of functional indexes of zinc status have been suggested, for example, wound healing, taste acuity, and dark adaptation (*11*). Changes in these functions are, however, not specific to zinc and these indexes have so far not been proven useful for identifying marginal zinc deficiency in humans.

The introduction of stable isotope techniques in zinc research (*15*) has created possibilities for evaluating the relationship between diet and zinc status and is likely to lead to a better understanding of the mechanisms underlying the homeostatic regulations of zinc. Estimations of turnover rates of administered isotopes in plasma or urine have revealed the existence of a relatively small rapidly exchangeable body pool of zinc of about 1.5-3 mmol (100-200 mg) (*16-19*). The size of the pool seems to be correlated to habitual dietary intake and it is reduced in controlled depletion studies (*18*). The exchangeable zinc pool was also found to be correlated to endogenous faecal excretion of zinc (*19*) and to total daily absorption of zinc. These data suggest that the size of the exchangeable pool depends on recently absorbed zinc and that a larger exchangeable pool results in larger endogenous excretion. Changes in endogenous intestinal excretion of zinc seem to be more important than changes in absorptive efficiency for maintenance of zinc homeostasis (*19*).

Dietary sources and availability of zinc

Lean red meat, whole-grain cereals, pulses, and legumes provide the highest concentrations of zinc 25-50 mg/kg (380-760 μmol/kg) raw weight. Processed cereals with low extraction rates, polished rice, and lean meat or meat with high fat content have a moderate zinc content 10-25 mg/kg (150-380 μmol/kg). Fish, roots and tubers, green leafy vegetables, and fruits are only modest sources of zinc <10 mg/kg (<150 μmol/kg) (*20*). Separated fats and oils, sugar, and alcohol have a very low zinc content.

The utilisation of zinc depends on the overall composition of the diet. Experimental studies have identified a number of dietary factors as potential promoters or antagonists of zinc absorption (*21)*. Soluble low-molecular-weight organic substances, such as amino and hydroxy acids, facilitate zinc absorption. In contrast, organic compounds forming stable and poorly soluble complexes with zinc can impair absorption. In addition, competitive interactions between zinc and other ions with similar physicochemical properties can affect

the uptake and intestinal absorption of zinc. The risk for competitive interactions seems mainly to be related to high doses in the form of supplements or in aqueous solutions. However, at levels present in food and at realistic fortification levels, zinc absorption appears not to be affected, for example, by iron and copper (*21*).

Isotope studies with human subjects have identified two factors which together with the total zinc content of the diet are major determinants of absorption and utilisation of dietary zinc. The first is the content of inositol hexaphosphate (phytate) and the second is the level and source of dietary protein. Phytates are present in whole-grain cereals and legumes and in smaller amounts in other vegetables. They have a strong potential for binding divalent cations and their depressive effect on zinc absorption has been demonstrated in humans (*21)*. The molar ratio between phytates and zinc in meals or diets is a useful indicator of the effect of phytates in depressing zinc absorption. At molar ratios above the range of 6–10, zinc absorption starts to decline; at ratios above 15 absorption is typically less than 15 percent (*20*). The effect of phytate is, however, modified by the source and amount of dietary proteins consumed. Animal proteins improve zinc absorption from a phytate-containing diet. Zinc absorption from some legume-based diets is comparable with that from animal-protein-based diets despite a higher phytate content in the former. High dietary calcium potentiated the antagonistic effects of phytates on zinc absorption in experimental studies. The results from human studies are less consistent and any effects seem to depend on the source of calcium and the composition of the diet (*22*).

Some examples of recently published absorption studies illustrate the effect of zinc content and diet composition on fractional zinc absorption (***Table 53***) (*19, 23- 25*). The results from the total diet studies, where all main meals of a day's intake have been extrinsically labelled, show a remarkable consistency in fractional absorption despite relatively large variations in meal composition and zinc content. Thus, approximately twice as much zinc was absorbed from a non-vegetarian or high-meat diet (*24*, *25*) than from a diet in rural China based on rice and wheat flour (*20*). Data are lacking on zinc absorption from typical diets of developing countries, which usually have a high phytate content.

The availability of zinc from the diet can be improved by reductions in the phytate content and inclusion of animal protein sources. Lower extraction rates of cereal grains will result in lower phytate content but at the same time the zinc content is reduced, so that the net effect on zinc supply is limited. The phytate content can be reduced by activating the phytase present in most phytate-containing foods or through the addition of microbial or fungal phytases. Phytases hydrolyse the phytate to lower inositol phosphates, resulting in an improved zinc absorption (*26*, *27*). The activity of phytases in tropical cereals such as maize and sorghum is lower than that in wheat and rye (*28*). Germination of cereals and legumes increases phytase activity and addition of some germinated flour to ungerminated maize or sorghum followed by soaking at ambient temperature for 12–24 hours can reduce the phytate content substantially (*28*). Additional reduction can be achieved by the fermentation of porridge for weaning foods or doughs for bread making. Commercially available phytase preparations could also be used but may not be economically accessible in many populations.

Populations at risk for zinc deficiency

The central role of zinc in cell division, protein synthesis, and growth makes infants, children, adolescents, and pregnant women especially at risk for an inadequate zinc intake. Zinc-responsive stunting has been identified in several studies (*29*), and a more rapid body weight gain in malnourished children supplemented with zinc was reported. Other studies have failed to show a growth-promoting effect of zinc supplementation (*13*). A recent meta-analysis of 25 intervention trials comprising 1834 children under 13 years of age, with a mean duration of approximately 7 months and a mean dose of zinc of 14 mg/day (214 μmol/day),

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showed a small but significant positive effect of zinc supplementation on height and weight increases (*13*). The initial presence of stunting was significantly associated with an effect of zinc supplementation on height, whereas initial low plasma zinc concentrations were associated with a more pronounced effect on weight gain.

Results from zinc supplementation studies suggest that a low zinc status in children not only affects growth but is also associated with an increased risk of severe infectious diseases (*30*). Episodes of acute diarrhoea with shorter duration and less severity and reductions in incidence of diarrhoea in zinc-supplemented groups have been reported. Other studies indicate that the incidence of acute lower respiratory tract infections and malaria may also be reduced by zinc supplementation. Prevention of sub-optimal zinc status and zinc deficiency in children by an increased intake and availability of zinc could consequently have a significant effect on child health in developing countries.

The role of maternal zinc status on pregnancy outcome is still unclear. Positive as well as negative associations between plasma zinc concentration and foetal growth or labour and delivery complications have been reported (*31*). Results of zinc supplementation studies also remain inconclusive (*31*). Interpretation of plasma zinc concentrations in pregnancy is complicated by the effect of hemodilution, and low plasma zinc levels may reflect other metabolic disturbances (*11*). Zinc supplementation studies of pregnant women have been performed mainly in relatively well-nourished populations, which may be one of the reasons for the mixed results (*31*). A recent study in low-income American women with plasma zinc concentrations below the mean at enrolment in prenatal care showed that a zinc intake of 25 mg/day resulted in greater infant birth weights and head circumferences and a reduction in very low birth weights among non-obese women compared with the placebo group (*12*).

Table 53

Examples of fractional zinc absorption from total diets measured by isotope techniques

Subject characteristics *(ref.)*	Diet/meal characteristics	Isotope technique	Zinc content μmol (mg)	Phytate-zinc molar ratio	Zinc absorption, %(x±SD)[a]
Young adults (n=8) *(22)*	High-fibre diet	Radioisotopes	163 (10.7)	7	27±6
Young women (n=10) *(19)*	Habitual diet	Stable isotopes	124 (8.1)	10	34±9
Women (20-42years) (n=21) *(24)*	Lacto-ovo vegetarian	Radioisotopes	139 (9.1)	14	26 [b]
	Non-vegetarian	Radioisotopes	169 (11.1)	5	33 [b]
Women (20-42years) (n=21) *(24)*	Low meat	Radioisotopes	102 (6.7)	—	30 [c]
Postmenopausal women (n-14) *(25)*	High meat	Radioisotopes	198 (13.0)	—	28 [c]

[a] SD, standard deviation.
[b] Pooled SD=5.[c] Pooled SD=4.6.
Source: Adapted from FAO/WHO Trace Mineral Report(*32*)

Zinc requirements

The lack of specific and sensitive indexes for zinc status limits the possibilities for evaluating zinc requirements from epidemiologic observations. In the FAO/IAEA/WHO 1996 report (*32*), zinc requirements were estimated by using the factorial technique (i.e., by adding the requirements for tissue growth, maintenance, metabolism, and endogenous losses). Experimental zinc repletion studies with low zinc intakes have clearly shown that the body has a pronounced ability to adapt to different levels of zinc intakes by changing the endogenous zinc losses through the kidneys, intestine, and skin (*5-9,33*). The normative requirement for absorbed zinc was defined as the obligatory loss during the early phase of zinc depletion before adaptive reductions in excretion take place and was set at 1.4 mg/day for men and 1.0 mg/day for women. To estimate the normative maintenance requirements for other age groups, the respective basal metabolic rates were used for extrapolation. In growing individuals the rate of accretion and zinc content of newly formed tissues were used to obtain the data required for tissue growth. Similarly, the retention of zinc during pregnancy and the zinc concentration in milk at different stages of lactation were used to estimate the physiologic requirements in pregnancy and lactation (*32*).

The translation of these estimates of absorbed zinc to requirements for dietary zinc involves several considerations. First, the nature of the diet (i.e., its content of promoters and inhibitors of zinc absorption) determines the fraction of the dietary zinc that is potentially absorbable. Second, the efficiency of absorption of potentially available zinc is inversely related to the content of zinc in the diet. The review of available data from experimental zinc absorption studies of single meals or total diets resulted in a division of diets into three categories – high, moderate, and low zinc bio-availability – as detailed in ***Table 54*** (*32*). It was then discovered that the relationship between efficiency of absorption and zinc content differed for these diets (*32*). Algorithms were developed (*32*) and applied to the estimates of requirements for absorbed zinc to achieve a set of figures for the average individual dietary zinc requirements (***Table 55***). The fractional absorption figures applied for the three diet categories were 50 percent, 30 percent, and 15 percent, respectively. From these estimates and from the evaluation of data from dietary intake studies, mean population intakes were identified which were deemed sufficient to ensure a low prevalence of individuals at risk of inadequate zinc intake (*32*).

Infants, children, and adolescents

Endogenous losses of zinc in human-milk-fed infants were assumed to be 20 μg/kg/day (0.31μmol/kg/day) whereas 40 μg/kg/day (0.6 μmol/kg/day) was assumed for infants fed formula or weaning foods (*32*). For other age groups an average loss of 0.002 μmol/basal kJ (0.57 μg/basal kcal) was derived from the estimates in adults. Estimated zinc increases for infant growth were set at 120 and 140 μg/kg/day (1.83–2.14 μmol/kg/day) for female and male infants, respectively, for the first 3 months (*32*). These values decrease to 33 μg/kg/day (0.50 μmol/kg/day) for ages 6–12 months. For ages 1–10 years the requirements for growth were based on the assumption that new tissue contains 30 μg/g (0.46 μmol zinc/g) (*32*). For adolescent growth, a zinc content of 23 μg/g (0.35 μmol/g) increase in body weight was assumed. Pubertal growth spurts increase physiologic zinc requirements substantially. Growth of adolescent males corresponds to an increase in body zinc requirement of about 0.5 mg/day (7.6 μmol/day) (*32*).

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Pregnancy

The total amount of zinc retained during pregnancy has been estimated to be 1.5 mmol (100 mg) (*34)*. During the third trimester the physiologic requirement of zinc is approximately twice as high as that in women who are not pregnant (*32*).

Lactation

Zinc concentrations in human milk are high in early lactation, 2-3 mg/l (31-46 μmol/l) in the first month, and fall to 0.9 mg/l (14 μmol/l) after 3 months (*35*). From data on maternal milk volume and zinc content, it was estimated that the daily output of zinc in milk during the first 3 months of lactation could amount to 1.4 mg/day (21.4 μmol/l), which would theoretically triple the physiologic zinc requirements in lactating women compared with non-lactating, non-pregnant women. In setting the estimated requirements for early lactation it was assumed that part of this requirement was covered by postnatal involution of the uterus and from skeletal resorption (*32*).

Elderly

Requirements for the elderly are estimated to be the same as those for other adults. A lower absorptive efficiency has been reported in the elderly, which could justify a higher dietary requirement. On the other hand, endogenous losses seem to be lower in the elderly. Because of the suggested role of zinc in infectious diseases, an optimal zinc status in the elderly could have a significant public health effect and is an area of zinc metabolism requiring further research.

Inter-individual variations in zinc requirements and recommended nutrient intakes

The studies (*6-10*) used to estimate the average physiologic requirements with the factorial technique have considered a relatively small number of subjects and do not allow any estimate of inter-individual variations in obligatory losses of zinc at different intakes. Because zinc requirements are related to tissue turnover rate and growth, it is reasonable to assume that variations in physiologic zinc requirements are of the same magnitude as variations in protein requirements (*36*) and that the same figure (12.5 percent) for the inter-individual coefficient of variation (CV) could be adopted. However, the estimates of dietary zinc requirements involve an estimate of absorption. Consequently, variations in absorptive efficiency, not relevant in relation to estimates of protein requirements, may have to be taken into account in the estimates of the total inter-individual variation in zinc requirements. Systematic studies of the inter-individual variations in zinc absorption under different conditions are few. In small groups of healthy well-nourished subjects, the reported variations in zinc absorption from a defined meal or diet are of the order of 20–40 percent and seem to be independent of age, sex, or diet characteristics. How much these variations, besides being attributable to methodologic imprecision, reflect variations in physiologic requirement, effects of preceding zinc intake, *etc*. is not known. From the available data from zinc absorption studies (*19, 20, 23-27*) it is tentatively suggested that the variation in dietary zinc requirements, which covers variation in requirement for absorbed zinc (i.e., variations in metabolism and turnover rate of zinc) and variation in absorptive efficiency, corresponds to a CV of 25 percent. The recommended nutrient intakes derived from the estimates of average individual dietary requirements (***Table 55***) with the addition of 50 percent (2 standard deviations) are given in ***Table 56***.

Table 54

Criteria for categorising diets according to the potential availability of their zinc

Nominal category	Principal dietary characteristics
High availability	Refined diets low in cereal fibre, low in phytic acid content, and with phytate-zinc (molar) ratio <5; adequate protein content principally from non-vegetable sources, such as meats, fish. Includes semisynthetic formula diets based on animal protein.
Moderate availability	Mixed diets containing animal or fish protein. Lacto-ovo, ovovegetarian, or vegan diets not based primarily on unrefined cereal grains or high-extraction-rate flours. Phytate-zinc molar ration of total diet within the range 5–15 or not exceeding 10 if more than 50% of the energy intake is accounted for by unfermented, unrefined cereal grains and flours whereas the diet is fortified with inorganic calcium salts (>1 g Ca^{2+}/ day). Availability of zinc improves when the diet includes animal or protein sources or milks.
Low availability	Diets high in unrefined, unfermented, and ungerminated cereal grain,[a] especially when fortified with inorganic calcium salts and when intake of animal protein is negligible. Phytate-zinc molar ration of total diet exceeds 15.[b] High-phytate soya-protein products constitute the primary protein source. Diets in which, singly or collectively, approximately 50% of the energy intake is accounted for by the following high-phytate foods: high-extraction-rate (90% +) wheat, rice, maize, grains and flours, oatmeal, and millet; chapatti flours and *tanok*; and sorghum, cowpeas, pigeon peas, grams, kidney beans, blackeye beans, and groundnut flours. High intakes of inorganic calcium salts (>1 g Ca^{2+}/ day), either as supplements or as adventitious contaminants (e.g., from calcareous geophagia), potentiate the inhibitory effects; low intakes of animal protein exacerbate these effects.

[a]Germination of such grains or fermentation (e.g., leavening) of many flours can reduce antagonistic potency; the diet should then be classified as moderate availability.

[b]Vegetable diets with phytate-zinc ratios exceeding 30 are not unknown; for such diets, an assumption of 10 percent availability of zinc or less may be justified, especially if the intake of protein is low, calcium salts is excessive, or both (e.g., calcium salts providing >1.5 g Ca^{2+}/day).

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Table 55

Average individual normative requirements for zinc (μg/kg body weight/day) from diets differing in zinc bio-availability[a]

Age range years	High bio-availability[b]	Moderate bio-availability[c]	Low bio-availability[d]
	μg/kg body weight/day		
Infants and Children			
Females, 0–0.25	175[e]	457[f]	1067[g]
Males, 0–0.25	200[e]	514[f]	1200[g]
0.25–0.5	79[e]	204[f]	477[g]
0.5–1	66[e]	—	—
0.5–1	186	311	621
1–3	138	230	459
3-6	114	190	380
6-10	90	149	299
Adolescents			
Females, 10–12	68	113	227
Males, 10–12	80	133	267
Females, 12–15	64	107	215
Males, 12–15	76	126	253
Females, 15–18	56	93	187
Males, 15–18	61	102	205
Adults			
Females, 18–60+	36	59	119
Males, 18–60+	43	72	144

Source: Adapted from FAO/IAEA/WHO (*32*).

[a] For information on diets, see ***Table 54***

[b] Assumed bio-availability of dietary zinc 50 percent.

[c] Assumed bio-availability of dietary zinc 30 percent.

[d] Assumed bio-availability of dietary zinc 15 percent.

[e] Applicable exclusively to infants fed maternal milk alone for which the bio-availability of zinc is with no allowance for storage.

[f] Applicable to infants partly human-milk-fed or fed whey-adjusted cow milk formula or milk plus low-phytate solids. No allowance for storage.

[g] Applicable to infants receiving phytate-rich vegetable-protein-based infant formula with or without whole-grain cereals. No allowance for storage.

Upper limits of zinc intake

Only a few occurrences of acute zinc poisoning have been reported. The toxicity signs are nausea, vomiting, diarrhoea, fever, and lethargy and have been observed after ingestion of 4-8 g (60-120 mmol) zinc. Long-term zinc intakes higher than the requirements could, however, interact with the metabolism of other trace elements. Copper seems to be especially sensitive to high zinc doses. A zinc intake of 50 mg/day (760 μmol) affects copper status indexes, such as CuZn-superoxide dismutase in erythrocytes (*37, 38*). Low copper and ceruloplasmin levels and anaemia have been observed after higher zinc intakes 450-660 mg/day (6.9-10 mmol/day) (*39, 40*). Changes in serum lipid pattern and in immune response have also been observed in zinc supplementation studies (*41, 42*). Because copper also has a central role in immune defence, these observations call for caution before large-scale zinc supplementation programmes are undertaken. Any positive effects of zinc supplementation on growth or

infectious diseases could be disguised or counterbalanced by negative effects on copper-related functions.

The upper level of zinc intake for an adult man is set at 45 mg/day (690 μmol/day) and extrapolated to other groups in relation to basal metabolic rate. For children this extrapolation means an upper limit of intake of 23–28 mg/day (350–430 μmol/day), which is close to what has been used in some of the zinc supplementation studies. Except for excessive intakes of some types of seafood, such intakes are unlikely to be attained with most diets. Adventitious zinc in water from contaminated wells and from galvanized cooking utensils could also lead to high zinc intakes.

Table 56

Recommended nutrient intakes (RNIs) for dietary zinc (mg/day) to meet the normative storage requirements from diets differing in zinc bio-availability[a]

Age group	Assumed body weight, kg	High bio-availability	Moderate bio-availability	Low bio-availability
Infants and children				
0–6 months	6	1.1[b]	2.8[c]	6.6[d]
7–12 months	9	0.8[b]	–	–
7–12 months	9	2.5[e]	4.1	8.4
1–3 years	12	2.4	4.1	8.3
4–6 years	17	2.9	4.8	9.6
7–9 years	25	3.3	5.6	11.2
Adolescents				
Females, 10–18 years,	47	4.3	7.2	14.4
Males, 10–18 years	49	5.1	8.6	17.1
Adults				
Females, 19– 65 years	55	3.0	4.9	9.8
Males, 19–65 years	65	4.2	7.0	14.0
Females, 65+ years	55	3.0	4.9	9.8
Males, 65+ years	65	4.2	7.0	14.0
Pregnant Women				
First trimester	–	3.4	5.5	11.0
Second trimester	–	4.2	7.0	14.0
Third trimester	–	6.0	10.0	20.0
Lactating women				
0–3 months	–	5.8	9.5	19.0
3–6 months	–	5.3	8.8	17.5
6–12 months	–	4.3	7.2	14.4

[a]For information on diets, see ***Table 54***. Unless otherwise specified, the intra-individual variation of zinc requirements is assumed to be 25 percent. Weight data interpolated from FAO/WHO, 1988 (*36*).

[b]Human-milk-fed infants receiving maternal milk only; assumed coefficient of variation (CV) 12.5 percent; assumed availability 80 percent.

[c]Formula-fed infants: moderate bio-availability for whey-adjusted milk formula and for infants partly human-milk-fed or given low-phytate feeds supplemented with other liquid milks; assumed CV 12.5 percent.

[d]Formula-fed infants; low bio-availability applicable to phytate-rich vegetable-protein-based formula with or without whole-grain cereals; CV 12.5 percent.

[e]Not applicable to infants consuming human milk only.

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Adequacy of zinc intakes in relation to requirement estimates

The risk for inadequate zinc intakes in children has been evaluated by using the suggested estimates of zinc requirements (*32*) and by using data available on food composition and dietary intake in different parts of the world (*43*). For this assessment it was assumed that the distribution of zinc requirements is Gaussian with a CV of 15 percent and that the correlation between intake and requirement is very low. Zinc absorption from diets in Malawi, Kenya, Mexico, and Guatemala was estimated to be 15 percent based on the high phytate-zinc molar ratio (*37-42*) in these diets, whereas an absorption of 30 percent was assumed for diets in Ghana, Guatemala, Egypt, and Papua New Guinea. Fermented maize and cassava products (*kenkey*, *banku*, and *gari*) in Ghana, yeast leavened wheat-based bread in Egypt, and the use of sago with a low phytate content as the staple in the New Guinean diets were assumed to result in a lower phytate-zinc molar ratio and a better availability. With this approach 68–94 percent of the children were estimated to be at risk for zinc deficiency in these populations, with the exception of Egypt where the estimate was 36 percent (*43*). The average daily zinc intakes of these children were 3.7-6.9 mg (56-105 μmol). Most of the zinc supplementation studies have not provided dietary intake data, which could be used to identify the zinc intake critical for growth effects. In a recent study in Chile, positive effects on height gain in boys after 14 months of zinc supplementation was noted (*44*). The intake in the placebo group at the start was 6.3 ± 1.3 mg/day (96 ± 20 μmol/day) (n=49). Because only 15 percent of the zinc intake of the Chilean children was derived from flesh foods, availability was assumed to be relatively low.

Krebs et al (*45*) observed no effect of zinc supplementation on human-milk zinc content or on maternal status of a group of lactating women and judged their intake sufficient to maintain adequate zinc status through 7 months or more of lactation. The mean zinc intake of the non-supplemented women was 13.0 ± 3.4 mg/day (199 ± 52 μmol/day).

The efficient homeostatic mechanisms for maintaining body zinc content at low intakes, which formed the basis for the estimates of physiologic requirements in the FAO/IAEA/WHO 1996 report (*32*), as well as the presumed negative impact of a high-phytate diet on zinc status, were confirmed in recent experimental studies (*10, 44, 46, 47*). Reductions in urinary and faecal losses maintained normal plasma zinc concentrations over 5 weeks in 11 men with intakes of 2.45 mg zinc/day (37 μmol/day) or higher in a diet with a presumably high availability (*10*). In a similar repletion-depletion study with 15 men, an intake of 4 mg/day (61 μmol/day) from a diet with a molar phytate-zinc ratio of 58 for 7 weeks resulted in a reduction of urinary zinc excretion from 0.52 ± 0.18 to 0.28 ± 0.15 mg/day (7.9 ± 2.8 μmol/day to 4.3 ± 2.3 μmol/day) (*46*). A significant reduction of plasma zinc concentrations and changes in cellular immune response were observed. Effects on immunity were also observed when a zinc-restricted diet with a high phytate content (molar ratio approximately 20) was consumed by five young male volunteers for 20–24 weeks (*14*). Sub-optimal zinc status has also been documented in pregnant women consuming diets with high phytate-zinc ratios (>17) (*47*). Frequent reproductive cycling and high malaria prevalence seemed to contribute to the impairment of zinc status.

Conclusion

In conclusion, the approach used for derivation of average individual requirements of zinc used in the FAO/IAEA/WHO 1996 report (32) and the resulting estimates still seem valid and useful for assessment of the adequacy of zinc intakes in population groups and for planning diets for defined population groups.

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Future research

As already indicated in the FAO/IAEA/WHO 1996 report (*32*), there is an urgent need to characterise the early functional effects of zinc deficiency and to define their relation to pathologic changes. This knowledge is especially needed for understanding the role of zinc deficiency in the aetiology of stunting and impaired immunocompetence.

For a better understanding of the relationship between diet and zinc supply, there is a need for further research to carefully evaluate the availability of zinc from diets typical of developing countries. The research should include an assessment of the effect of availability of adopting realistic and culturally accepted food preparation practises such as fermentation, germination, soaking, and inclusion of inexpensive and available animal protein sources in plant-food-based diets.

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Chapter 17

Dietary antioxidants: a consideration of factors influencing requirements

Nutrients with an antioxidant role

The potential beneficial effects from antioxidants in protecting against disease have been used as an argument for recommending increasing intakes of several nutrients above those derived by conventional methods. If it is possible to quantify such claims, antioxidant properties should be considered in decisions concerning the daily requirements of these nutrients. This section examines metabolic aspects of the most important dietary antioxidants – vitamins C and E, the carotenoids, and several minerals – and tries to define the populations which may be at risk of inadequacy to determine whether antioxidant properties *per se* should be and can be considered in setting a requirement. In addition, pro-oxidant metabolism and the importance of iron are also considered.

Members of the Food and Nutrition Board of the National Research Council in the United States, recently defined a dietary antioxidant as a substance in foods which significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiologic function in humans (*1*). It is recognised that this definition is somewhat narrow because maintenance of membrane stability is also a feature of antioxidant function (*2*) and an important antioxidant function of both vitamin A (*3*) and zinc (*4*). However, it was decided to restrict consideration of antioxidant function in this document to nutrients which were likely to interact more directly with reactive species.

The need for biologic antioxidants

It is now well established that free radicals, especially superoxide ($O_2^{\cdot -}$), nitric oxide ($NO^{\cdot}$), and other reactive species such as H_2O_2, are continuously produced *in vivo* (*5-7*). Superoxide in particular is produced by leakage from the electron transport chains within the mitochondria and microsomal P450 systems (*8*) or formed more deliberately, for example, by activated phagocytes as part of the primary immune defence in response to foreign substances or to combat infection by micro-organisms (*9*). Nitric oxide is produced from L-arginine by nitric oxide synthases, and these enzymes are found in virtually every tissue of the mammalian body, albeit at widely different levels (*7*). Nitric oxide is a free radical but is believed to be essentially a beneficial metabolite and indeed it may react with lipid peroxides and function as an antioxidant (*10*). Nitric oxide also serves as a mediator whereby macrophages express cytotoxic activity against micro-organisms and neoplastic cells (*11*). If nitric oxide is at a sufficiently high concentration, it can react rapidly with superoxide in the absence of a catalyst to form peroxynitrite. Peroxynitrite is a potentially damaging nitrogen species which can react through several different mechanisms, including the formation of an intermediate with the reactivity of the hydroxyl radical (*12*).

To cope with potentially damaging reactive oxidant species (ROS), aerobic tissues contain endogenously produced antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase and several exogenously acquired radical-scavenging substances such as vitamins E and C and the carotenoids (*13*). Under normal conditions the high concentrations of SOD maintains superoxide concentrations too low to

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allow the formation of peroxynitrite. It is also important to mention the antioxidant reduced glutathione (GSH). GSH is ubiquitous in aerobic tissues, and although it is not a nutrient, it is synthesised from sulfhydryl-containing amino acids and is highly important in intermediary antioxidant metabolism (*14*).

Integrated antioxidant defences protect tissues and are presumably in equilibrium with continuously generated ROS to maintain tissues metabolically intact most of the time. Disturbances to the system occur when production of ROS is rapidly increased, for example, by excessive exercise, high exposure to a xenobiotic compounds (such as an anaesthetic, pollutants, or unusual food), infection, or trauma. Superoxide production is increased by activation of NADPH oxidases in inflammatory cells or after the production of xanthine oxidase, which follows ischaemia. The degree of damage resulting from the temporary imbalance depends on the ability of the antioxidant systems to respond to the oxidant or pro-oxidant load. Fruits and vegetables are good sources of many antioxidants, and it is reported that diets rich in these foods are associated with a lower risk of the chronic diseases of cancer (*15*) and heart disease (*16*). Hence, it is believed that a healthful diet maintains the exogenous antioxidants at or near optimal levels thus reducing the risk of tissue damage. The most prominent representatives of dietary antioxidants are vitamin C, tocopherols, carotenoids, and flavonoids (*17-19*). Requirements for flavonoids are not being considered at this time and work on this subject is still very much in its infancy. In contrast, several intervention studies have been carried out to determine whether supplements of the other nutrients can provide additional benefits against such diseases.

The components in biologic tissues make an ideal mixture of substrates for oxidation. Polyunsaturated fatty acids (PUFAs), oxygen, and transition metals are present in abundance but are prevented from reaction by cellular organisation and structure. PUFAs are present in membranes but are always found with vitamin E. Transition metals, particularly iron, are bound to both transport and storage proteins; abundant binding sites on such proteins prevent overloading the protein molecule with metal ions. Tissue structures, however, break down during inflammation and disease, and free iron and other transition metals have been detected (*20, 21*). Potentially damaging metabolites can arise from interactions between transition metals and the ROS described above. In particular the highly reactive hydroxyl radical can be formed by the Fenton (*reaction 1*) and Haber-Weiss reactions (*reaction 2*; with an iron-salt catalyst) (*22*). Pathologic conditions greatly increase the concentrations of both superoxide and nitric oxide, and the formation of peroxynitrite has been demonstrated in macrophages, neutrophils, and cultured endothelium (*reaction 3*) (*12, 23*). Peroxynitrite can react through several different mechanisms, including the formation of an intermediate with the reactivity of the hydroxyl radical (12).

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH^{\cdot} + OH^{-} \qquad 1$$

$$O_2^{-\cdot} + H_2O_2 = O_2 + OH^{\cdot} + OH^{-} \qquad 2$$

$$NO + O_2^{-\cdot} = ONOO^{\cdot} \qquad 3$$

During inflammation or other forms of stress and disease, new measures are adopted by the body to counter potential pro-oxidant damage. The body alters the transport and distribution of iron by blocking iron mobilisation and absorption and stimulating iron uptake from plasma by liver, spleen, and macrophages (*3, 24, 25*). Nitric oxide has been shown to play a role in the coordination of iron traffic by mimicking the consequences of iron starvation and leading to the cellular uptake of iron (*26*). The changes accompanying disease are generally termed the acute-phase response and are, generally, protective (*27*). Some of the changes in plasma acute-phase reactants which affect iron at the onset of disease or trauma are shown in ***Table 57***.

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Table 57

Systems altered in disease which reduce risk of autoxidation

System	Changes in plasma	Physiologic objectives
Mobilisation and metabolism of iron	Decrease in transferrin Increase in ferritin Increase in lactoferrin Increase in haptoglobin Decrease in iron absorption Movement of plasma iron from blood to storage sites.	Reduce levels of circulating and tissue iron to reduce risk of free radical production and pro-oxidant damage. Reduce level of circulating iron available for microbial growth.
Positive acute phase proteins	Increase in antiproteinases Increase in fibrinogen	Restriction of inflammatory damage to diseased area.
White blood cells	Variable increase in white blood cells of which 70% are granulocytes.	Production of reactive oxygen species to combat infection. Scavenge vitamin C to prevent interaction of vitamin C with free iron.
Vitamin C metabolism	Uptake of vitamin C from plasma by stimulated granulocytes. Reduction of plasma vitamin C in acute and chronic illness or stress-associated conditions. Temporary fall in leukocyte vitamin C associated with acute stress.	Reduce levels of vitamin C in the circulation – because it is a potential pro-oxidant in inflamed tissue – or where free iron may be present. Facilitate movement of vitamin C to tissues affected by disease (e.g., lungs in smokers). Protect granulocytes and macrophages from oxidative damage.

Source: Modified from Koj (*28*) and Thurnham (*3, 29, 30*).

Pro-oxidant activity of biologic antioxidants

Most biologic antioxidants are antioxidants because when they accept an unpaired electron, the free radical intermediate formed has a relatively long half-life in the normal biologic environment. The long half-life means that these intermediates remain stable for long enough to interact in a controlled fashion with intermediates which prevent autoxidation, and the excess energy of the surplus electron is dissipated without damage to the tissues. Thus it is believed that the tocopheroxyl radical formed by oxidation of α-tocopherol is sufficiently stable to enable its reduction by vitamin C or GSH to regenerate the quinol (*31, 32*) rather than oxidizing surrounding PUFAs. Likewise the oxidized forms of vitamin C, the ascorbyl free radical and dehydroascorbate, may be recycled back to ascorbate by GSH or the enzyme dehydroascorbate reductase (*13*). The ability to recycle these dietary antioxidants may be an indication of their physiologic essentiality to function as antioxidants.

Carotenoids are also biologic antioxidants but their antioxidant properties very much depend on oxygen tension and concentration (*33, 34*). At low oxygen tension β-carotene acts as a chain-breaking antioxidant whereas at high oxygen tension it readily autoxidizes and exhibits pro-oxidant behaviour (*33*). Palozza (*34*) reviewed much of the evidence and suggests that β-carotene has antioxidant activity between 2 and 20 mmHg of oxygen tension, but at the oxygen tension in air or above (>150 mmHg) it is much less effective as an antioxidant and can show pro-oxidant activity as the oxygen tension increases. Palozza (*34*) also suggests that autoxidation reactions of β-carotene may be controlled by the presence of other antioxidants (e.g., vitamins E and C) or other carotenoids. There is some evidence that large supplements of fat-soluble nutrients such as β-carotene and other carotenoids may compete with each other during absorption and lower plasma concentrations of other nutrients derived from the diet. However, a lack of other antioxidants is unlikely to explain the increased incidence of lung cancer in the α-tocopherol β-carotene intervention study, because there was no difference in cancer incidence between the group which received both β-carotene and α-tocopherol and the groups which received one treatment only (*35*).

The free radical formed from a dietary antioxidant is potentially a pro-oxidant as is any other free radical. In biologic conditions which might deviate from the norm, there is always the potential for an antioxidant free radical to become a pro-oxidant if a suitable receptor molecule is present to accept the electron and promote the autoxidation (*36*). Mineral ions are particularly important pro-oxidants. For example, vitamin C will interact with both copper and iron to generate cuprous or ferrous ions, respectively, both of which are potent pro-oxidants (*29, 37*). Fortunately, mineral ions are tightly bound to proteins and are usually unable to react with tissue components unless there is a breakdown in tissue integrity. Such circumstances can occur in association with disease and excessive phagocyte activation, but even under these circumstances there is rapid metabolic accommodation in the form of the acute-phase response to minimise the potentially damaging effects of an increase in free mineral ions in extra-cellular fluids (***Table 57***).

Nutrients associated with endogenous antioxidant mechanisms

Both zinc and selenium are intimately involved in protecting the body against oxidant stress. Zinc combined with copper is found in the cytoplasmic form of SOD whereas zinc and magnesium occur in the mitochondrial enzyme. SOD occurs in all aerobic cells and is responsible for the dismutation of superoxide (*reaction 4*):

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ = H_2O_2 + O_2 \qquad 4$$

Hydrogen peroxide produced as a product of dismutation reaction is removed by GPx of which selenium is an integral component (*reaction 5*). To function effectively, this enzyme also needs a supply of hydrogen, which it obtains from GSH. Cellular concentrations of GSH are maintained by the riboflavin-dependent enzyme glutathione reductase.

$$H_2O_2 + 2GSH = GSSG + 2H_2O \qquad 5$$

Four forms of selenium-dependent GPx have been described which have different activities in different parts of the cell (*38*). In addition, a selenium-dependent thyrodoxin reductase was recently characterised in human thyrocytes. Thyrodoxin reductase may be particularly important to the thyroid gland because it can cope with higher concentrations of peroxide and hydroperoxides generated in the course of thyroid hormone synthesis better than can GPx (*39*). It is suggested that in combination with iodine deficiency, the inability to remove high concentrations of hydrogen peroxide may cause atrophy in the thyroid gland, resulting in myxedematous cretinism (*39*).

SOD and GPx are widely distributed in aerobic tissues and, if no catalytic metal ions are available, endogenously produced superoxide and hydrogen peroxide at physiologic concentrations may have limited, if any, damaging effects (*36*). SOD and GPx are of fundamental importance to the life of the cell, and their activity is not readily reduced by deficiencies in dietary intake of these nutrients. In contrast, enzyme activity can be stimulated by increased oxidant stress (e.g., ozone) (*40*). Activities of zinc-dependent enzymes have been shown to be particularly resistant to the influence of dietary zinc (*41*), and although erythrocyte GPx activity correlates with selenium when the intake is below 60–80 μg/day (*42*), there is no evidence of impaired clinical function at low GPx activities found in humans. Nevertheless, one selenium intervention study reported remarkably lower risks of several cancers after 4.5 years of selenium at 200 μg/day (*43*). The effects were so strong on total cancer mortality that the study was stopped prematurely. However, the subjects were patients with a history of basal or squamous cell carcinomas and were not typical of the general population. In addition, a prospective analysis of serum selenium in cancer patients (*44*) (1.72 μmol/L) found very little difference from concentrations in matched controls (1.63 μmol/L) although the difference was significant (*45*). Furthermore, areas with high selenium intakes have a lower cancer incidence than do those with low intakes, but the high selenium areas were the least industrialized (*45*).

Nutrients with radical-quenching properties

Vitamins C and E are the principal nutrients which possess radical-quenching properties. Both are powerful antioxidants, and the most important difference between these two compounds stems from their different solubility in biologic fluids. Vitamin C is water soluble and is therefore especially found in the aqueous fractions of the cell and in body fluids whereas vitamin E is highly lipophilic and is found in membranes and lipoproteins.

Vitamin E

Vitamin E falls into the class of conventional antioxidants which are generally phenols or aromatic amines (see ***Chapter 9***). In the case of the four tocopherols that together constitute vitamin E, the initial step involves a very rapid transfer of phenolic hydrogen to the recipient free radical with the formation of a phenoxyl radical from vitamin E. The phenoxyl radical is resonance stabilised and is relatively unreactive towards lipid or oxygen. It does not therefore continue the chain (*33, 46*). However, the phenoxyl radical is no longer an antioxidant and to maintain the antioxidant properties of membranes, it must be recycled or repaired – that is,

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reconverted to vitamin E – because the amount of vitamin E present in membranes can be several thousand-fold less than the amount of potentially oxidizable substrate (*47*). Water-soluble vitamin C is the popular candidate for this role (*31*), but thiols and particularly GSH can also function *in vitro* (*32, 48-50*).

There are eight possible isomers of vitamin E, but α-tocopherol (5,7,8 trimethyl tocol) is the most biologically important antioxidant *in vivo* (*46*). In plasma samples, more than 90 percent is present as α-tocopherol but there may be approximately 10 percent of γ-tocopherol. In foods such as margarine and soy products the γ form may be predominant and palm oil products are rich in the tocotrienols.

Vitamin E is found throughout the body in both cell and sub-cellular membranes. It is believed to be orientated with the quinol ring structure on the outer surface (i.e., in contact with the aqueous phase) to enable it to be maintained in its active reduced form by circulating reductants such as vitamin C (*31*). Within biologic membranes, vitamin E is believed to intercalate with phospholipids and provide protection to PUFAs. PUFAs are particularly susceptible to free radical–mediated oxidation because of their methylene-interrupted double-bond structure. The amount of PUFAs in the membrane far exceeds the amount of vitamin E, and the tocopherol-PUFAs ratios are highest in tissues where oxygen exposure is greatest and not necessarily where the PUFAs content is highest (*47*).

Oxidation of PUFAs leads to disturbances in membrane structure and function and is damaging to cell function. Vitamin E is highly efficient at preventing the autoxidation of lipid and it appears as if the primary, and possibly only, role in biologic tissues is to provide this function (*46*). Autoxidation of lipid is initiated by a free radical abstracting hydrogen from PUFA to form a lipid radical (*reaction 6*) which is followed by a rearrangement of the double-bond structure to form a conjugated diene. *In vitro* the presence of minute amounts of peroxides and transition metals will stimulate the formation of the initial radical. Oxygen adds to the lipid radical to form a lipid peroxide (*reaction 7*) which then reacts with another lipid molecule to form a hydroperoxide and a new lipid radical (*reaction 8*). This process is shown in general terms below for the autoxidation of any organic molecule (RH), where the initial abstraction is caused by a hydroxyl radical ($OH^{\cdot}$).

$$RH + OH^{\cdot} = R^{\cdot} + H_2O \qquad 6$$

$$R^{\cdot} + O_2 = ROO^{\cdot} \qquad 7$$

$$ROO^{\cdot} + RH = ROOH + R^{\cdot} \qquad 8$$

Autoxidation or lipid peroxidation is represented by reactions 6 and 7. The process stops naturally when reaction between two radicals (*reaction 9*) occurs more frequently than does reaction 8.

$$ROO^{\cdot} + ROO^{\cdot} = \text{non-radical products} \qquad 9$$

The presence of the chain-breaking antioxidant, vitamin E (ArOH), reacts in place of RH shown in reaction 8 and donates the hydrogen from the chromanol ring to form the hydroperoxide (*reaction 10*). The vitamin E radical ($ArO^{\cdot}$, tocopheroxyl radical) which is formed is fairly stable and therefore stops autoxidation. Hydroperoxides formed by lipid peroxidation can be released from membrane phospholipids by phospholipase A2 and then degraded by GPx in the cell cytoplasm (*see* ***Chapter 15***).

$$ROO^{\cdot} + ArOH = ArO^{\cdot} + ROOH \qquad 10$$

Vitamin C

Many, if not all of the biologic properties of vitamin C are linked to its redox properties (see ***Chapter 6***). For example, essential defects in scurvy such as the breakdown of connective tissue fibres (*51*) and muscular weakness (*52*) are both linked to hydroxylation reactions in which ascorbate maintains loosely bound iron in the ferrous form to prevent its oxidation to the ferric form, which makes the hydroxylase enzymes inactive (*53*). Ascorbate exhibits similar redox functions in catecholamine biosynthesis (*53*) and in microsomal cytochrome P450 enzyme activity, although the latter may only be important in young animals (*54*). In the eye, vitamin C concentrations may be 50 times higher than in the plasma and may protect against the oxidative damage of light (*55*). Vitamin C is also present in the gonads, where it may play a critical role in sperm maturation (*56*). Spermatogenesis involves many more cell divisions than does oogenesis, resulting in an increased risk of mutation. Fraga *et al.* (57) reported that levels of sperm oxidized nucleoside 8-OH-2'-deoxyguanosine (an indicator of oxidative damage to DNA) varied inversely with the intake of vitamin C (5-250 mg/day). No apparent effects on sperm quality were noted. Frei (*58*) also showed that vitamin C was superior to all other biologic antioxidants in plasma in protecting lipids exposed *ex vivo* to a variety of sources of oxidative stress. The importance of vitamin C in stabilising various plasma components such as folate, homo-cysteine, proteins, other micronutrients, *etc*. has not been properly evaluated. When blood plasma is separated from erythrocytes, vitamin C is the first antioxidant to disappear.

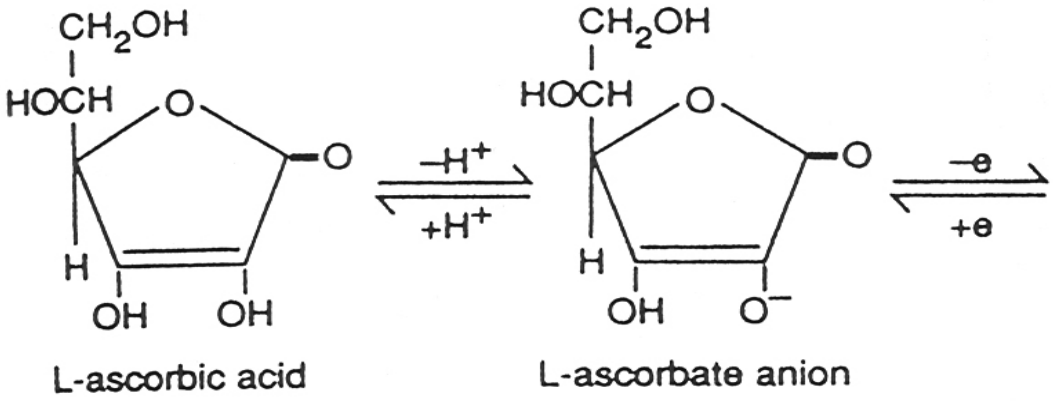
Vitamin C is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical (***Figure 27***). As a scavenger of ROS, ascorbate has been shown to be effective against the superoxide radical anion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen (*59, 60*). Vitamin C also scavenges reactive nitrogen oxide species to prevent nitrosation of target molecules (*61*). The ascorbyl free radical can be converted back to reduced ascorbate by accepting another hydrogen atom or it can undergo further oxidation to dehydroascorbate. Dehydroascorbate is unstable but is more fat soluble than ascorbate and is taken up 10–20 times more rapidly by erythrocytes, where it will be reduced back to ascorbate by GSH or NADPH from the hexose monophosphate shunt (*56*).

Thus, mechanisms exist to recycle vitamin C similarly to those for vitamin E. The existence of a mechanism to maintain plasma ascorbate in the reduced state means that the level of vitamin C necessary for optimal antioxidant activity is not absolute because the turnover will change in response to oxidant pressure. Recycling of vitamin C will depend on the reducing environment which exists in metabolically active cells. In atrophic tissues or tissues exposed to inflammation, cell viability may fail and with it the ability to recycle vitamin C. In such an environment, the ability of newly released granulocytes (*62*) or macrophages (*63*) to scavenge vitamin C from the surrounding fluid may be invaluable for conservation of an essential nutrient as well as reducing the risk of ascorbate becoming a pro-oxidant through its ability to reduce iron (*37*).

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Figure 27

Vitamin C can donate a hydrogen atom and form a relatively stable ascorbyl free radical



β-***Carotene and other carotenoids***

Many hundreds of carotenoids are found in nature but relatively few are found in human tissues, the five main ones being β-carotene, lutein, lycopene, β-cryptoxanthin, and α-carotene (*17, 18, 64*). β-Carotene is the main source of pro-vitamin A in the diet. There are approximately 50 carotenoids with pro-vitamin A activity, but β-carotene is the most important and is one of the most widely distributed carotenoids in plant species (*64*). Approximately 2–6 mg β-carotene is consumed by adults daily in developed countries (*65, 66*) and similar amounts of lutein (*67*) and lycopene (*66*) are probably also consumed. Smaller amounts may be consumed in the developing world (*68, 69*). β-Cryptoxanthin is a pro-vitamin A carotenoid which is found mainly in fruits (*66*). Consumption is small but bio-availability of carotenoids may be greater from fruit than vegetable, so its contribution to dietary intake and vitamin A status may be higher than the amount in the diet would predict.

β-Carotene has the general structure of this group of compounds and has two 6-membered carbon rings (β-ionone rings) separated by 18 carbon atoms in the form of a conjugated chain of double bonds. The latter is responsible for the antioxidant properties of the molecule (*33, 70, 71*). β-Carotene is unique in possessing two β-ionone rings in its molecule which are essential for vitamin A activity. The chemical properties of the carotenoids closely relate to the extended system of conjugated double bonds, which occupies the central part of carotenoid molecules, and various functional groups on the terminal ring structures. The ROS scavenged by carotenoids are singlet oxygen and peroxyl radicals (*33, 72-74*). Carotenoids in general and lycopene specifically are very efficient at quenching singlet oxygen (*72, 73*). In this process the carotene absorbs the excess energy from singlet oxygen and then releases it as heat. Singlet oxygen is generated during photosynthesis; therefore, carotenoids are important for protecting plant tissues, but there is limited evidence for this role in humans. However, ß-carotene has been used in the treatment of erythropoietic protoporphyria (*75*), which is a light-sensitive condition which in some persons respond to treatment with amounts of β-carotene (in excess of 180 mg/day) (*76*). It has been suggested that large amounts of dietary carotenes may provide some protection against solar radiation but results are equivocal. No benefit was reported when large amounts of β-carotene were

used to treat persons with a high risk of non-melanomatous skin cancer (*77*). However, two carotenoids – lutein (3,3'-dihydroxy α-carotene) and zeaxanthin (the 3,3'-dihydroxylated form of β-carotene) – are found specifically associated with the rods and cones in the eye (*78*) and may protect the retinal pigment epithelium against the oxidative effects of blue light (*79, 80*).

Burton and Ingold (*33*) were the first to draw attention to the radical-trapping properties of β-carotene. Using *in vitro* studies, they showed that β-carotene was effective in reducing the rate of lipid peroxidation at the low oxygen concentrations found in tissues. Because all carotenoids have the same basic structure, they should all have similar properties. Indeed, several authors suggest that the hydroxy-carotenoids are better radical-trapping antioxidants than is β-carotene (*81, 82*). It has also been suggested that because the carotenoid molecule is long enough to span the bilayer lipid membrane (*83*), the presence of oxy functional groups on the ring structures may facilitate similar reactivation of the carotenoid radical in a manner similar to that of the phenoxyl radical of vitamin E (*33*).

There is some evidence for an antioxidant role for β-carotene in immune cells. Bendich (84) suggested that β-carotene protects phagocytes from auto-oxidative damage; enhances T and B lymphocyte proliferative responses; stimulates effector T cell function; and enhances macrophage, cytotoxic T cell, and natural killer cell tumoricidal capacity. Some data are in conflict with evidence of protective effects on the immune system (*85, 86*) and other data have found no effect (*87*). An explanation for the discrepancy may reside in the type of subjects chosen. Defences may be boosted in those at risk but it may not be possible to demonstrate any benefit in healthy subjects (*88*).

A requirement for antioxidant nutrients

Free radicals are a product of tissue metabolism, and the potential damage which they can cause is minimised by the antioxidant capacity and repair mechanisms within the cell. Thus in a metabolically active tissue cell in a healthy subject with an adequate dietary intake, damage to tissue will be minimal and most of the damage occurring will be repaired (*36*). An important dietary source of antioxidant nutrients is the intake of fruit and vegetables, and it is now well established that persons consuming generous amounts of these foods have a lower risk of chronic disease than do those whose intake is small (*15, 16, 89*). These observations suggest that the antioxidant nutrient requirements of the general population can be met by a generous consumption of fruit and vegetables and the slogan "5 portions a day" has been promoted to publicize this idea (*90*).

Occasionally, damage may occur which is not repaired and the risk of this happening may increase in the presence of infection or physical trauma. Such effects may exacerbate an established infection or may initiate irreversible changes leading to a state of chronic disease (e.g., a neoplasm or atherosclerotic lesions). Can such effects also be minimised by a generous intake of dietary antioxidants in the form of fruit and vegetables or are supplements needed?

It is generally recognised that certain groups of people have an increased risk of free radical - initiated damage. Premature infants, for example, are at increased risk of oxidative damage because they are born with immature antioxidant status (*91-93*) and this may be inadequate for coping with high levels of oxygen and light radiation. People who smoke are exposed to free radicals inhaled in the tobacco smoke and have an increased risk of many diseases. People abusing alcohol need to develop increased metabolic capacity to handle the extra alcohol load. Similar risks may be faced by people working in environments where there are elevated levels of volatile solvents (e.g., petrol and cleaning fluids, in distilleries, chemical plants, *etc*.). Car drivers and other people working in dense traffic may be exposed to elevated

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levels of exhaust fumes. Human metabolism can adapt to a wide range of xenobiotic substances, but metabolic activity may be raised with the consequent production of more ROS which are potentially toxic to cell metabolism.

Of the above groups, smokers are the most widely accessible people and this has made them a target for several large antioxidant-nutrient intervention studies. In addition, smokers often display low plasma concentrations of carotenoids and vitamin C. However, no obvious benefits to the health of smokers have emerged from these studies and, in fact, β-carotene supplements were associated with an increased risk of lung cancer in two separate studies (*35, 94*) and with more fatal cardiac events in one of them (*95*). Other risk groups identified by their already having had some non-malignant form of cancer, such as non-melanomatous skin cancer (*77*) or a colorectal adenoma (*96*), showed no effect on subsequent recurrences after several years of elevated intakes of antioxidant nutrients. The use of β-carotene (77) or vitamin E alone or in combination with vitamin C (96) showed no benefits. Thus, the results of these clinical trials do not support the use of supplementation with antioxidant micronutrients as a means of reducing cancer or even cardiovascular rates although in the general population, toxicity from such supplements is very unlikely.

Some intervention trials however have been more successful in demonstrating a health benefit. Stitch and colleagues (*97, 98*) gave large quantities of β-carotene and sometimes vitamin A to chewers of betel quids in Kerala, India, and to Canadian Inuits with pre-malignant lesions of the oral tract and showed reductions in leukoplakia and micronuclei from the buccal mucosa. Blot and colleagues (*99*) reported a reduction (13 percent) in gastric cancer mortality in people living in Linxian Province, People's Republic of China, after a cocktail of β-carotene, vitamin E, and selenium. These studies are difficult to interpret because the subjects may have been marginally malnourished at the start and the supplements may have merely restored nutritional adequacy. However, correcting malnutrition is unlikely to be the explanation for the highly successful selenium supplementation study of US patients with a history of basal or squamous cell cancers of the skin (*43*). Interestingly, the intervention with 200 μg/day of selenium for an average of 4.5 years had no effect on the recurrence of the skin neoplasms (relative risk [RR]1.10, confidence interval 0.95–1.28). However, analysis of secondary endpoints showed significant reductions in total cancer mortality (RR 0.5) and incidence (RR 0.63) and in the incidences of lung, colorectal, and prostate cancers. The mean age of this group was 63 years and obviously they were not a normal adult population, but results of further studies are awaited with keen interest. Lastly, results of the Cambridge Heart Antioxidant Study should be mentioned because they provide some support for a beneficial effect of vitamin E in persons who have had a myocardial infarction (*100*). Recruits to the study were randomly assigned to receive vitamin E (800 or 400 mg/day) or placebo. Initial results of the trial suggested a significant reduction in non-fatal myocardial infarctions but a non-significant excess of cardiovascular deaths (*100*).The trial officially ended in 1996, but mortality has continued to be monitored and the authors now report significantly fewer deaths in those who received vitamin E for the full trial (*101*) (*see* ***Chapter 9***).

In conclusion, some studies have shown that health benefits can be obtained by some people with increased risk of disease from supplements of antioxidant nutrients. The amounts of supplements used have, however, been large and the effect possibly has been pharmacologic. Further work is needed to show whether more modest increases in nutrient intakes in healthy adult populations will delay or prevent the onset of chronic disease. The evidence available regarding health benefits to be achieved by increasing intakes of antioxidant nutrients does not assist in setting nutrient requirements.

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APPENDIX 1

Table 1 **Recommended nutrient intakes – minerals***

Age	Calcium (c) *mg/day*	Magnesium *mg/day*	Selenium *µg/day*	Zinc High bioavail-ability *mg/day*	Zinc Moderate bioavail-ability *mg/day*	Zinc Low bioavail-ability *mg/day*	Iron (i) 15% bio-availability *mg/day*	Iron (i) 12% bio-availability *mg/day*	Iron (i) 10% bio-availability *mg/day*	Iron (i) 5% bio-availability *mg/day*	Iodine (o) *µg/day*
Infants											
Premature											30 **(p)** *µg/kg/day*
0 - 6 months	300 **(a)** 400 **(b)**	26 **(a)** 36 **(b)**	6	1.1 **(e)**	2.8 **(f)**	6.6 **(g)**	**(k)**	**(k)**	**(k)**	**(k)**	15 **(p)***µg/kg/day*
7-11 months	400	53	10	0.8 **(e)** 2.5 **(h)**	4.1 **(h)**	8.3 **(h)**	[6] **(l)**	[8] **(l)**	[9] **(l)**	[19] **(l)**	135
Children											
1-3 years	500	60	17	2.4	4.1	8.4	4	5	6	12	75
4-6 years	600	73	21	3.1	5.1	10.3	4	5	6	13	110
7-9 years	700	100	21	3.3	5.6	11.3	6	7	9	18	100
Adolescents											
Males 10 - 18 years	1,300 **(d)**	250	34	5.7	9.7	19.2	10 (10-14 yrs) 12 (15-18 yrs)	12 (10-14 yrs) 16 (15-18 yrs)	15 (10-14 yrs) 19 (15-18 yrs)	29 (10-14 yrs) 38 (15-18 yrs)	135 (10-11 yrs) 110 (12 + yrs)
Females 10 - 18 years	1,300 **(d)**	230	26	4.6	7.8	15.5	9 (10-14 yrs) **(m)** 22 (10-14 yrs) 21 (15-18 yrs)	12 (10-14 yrs) **(m)** 28 (10-14 yrs) 26 (15-18 yrs)	14 (10-14 yrs) **(m)** 33 (10-14 yrs) 31 (15-18 yrs)	28 (10-14 yrs) **(m)** 65 (10-14 yrs) 62 (15-18 yrs)	140 (10-11 yrs) 100 (12 + yrs)
Adults											
Males 19 - 65 years	1,000	260	34	4.2	7.0	14.0	9	11	14	27	130
Females											
19 - 50 years (pre-menopausal)	1,000	220	26	3.0	4.9	9.8	20	24	29	59	110
51 - 65 years (menopausal)	1,300	220	26	3.0	4.9	9.8	8	9	11	23	110
Older adults											
Males 65 + years	1,300	230	34	4.2	7.0	14.0	9	11	14	27	130
Females 65 + years	1,300	190	26	3.0	4.9	9.8	8	9	11	23	110
Pregnancy											
First trimester		220		3.4	5.5	11.0	**(n)**	**(n)**	**(n)**	**(n)**	200
Second trimester		220	28	4.2	7.0	14.0	**(n)**	**(n)**	**(n)**	**(n)**	200
Third trimester	1,200	220	30	6.0	10.0	20.0	**(n)**	**(n)**	**(n)**	**(n)**	200
Lactation											
0-3 months	1,000	270	35	5.8	9.5	19.0	10	12	15	30	200
4-6 months	1,000	270	35	5.3	8.8	17.5	10	12	15	30	200
7-12 months	1,000	270	42	4.3	7.2	14.4	10	12	15	30	200

* For the purposes of the composite tables of RNI values, the body weights used were derived from the 50th percentile of NCHS data until adult weights of 55 kg for females and 65 kg for males were reached. The weights used are the following: 0-6 mo = 6 kg; 7–12 mo = 8.9 kg; 1-3 yo = 12.1 kg; 4-6 yo = 18.2 kg; 7-9 yo = 25.2 kg; 10-11 yo M = 33.4 kg; 10-11 yo F = 34.8 kg; 12-18 yo M = 55.1 kg; 12-18 yo F = 50.6 kg; 10-18 yo M = 55.1 kg; 10-18 yo F = 50.6 kg; 19-65 yo M = 65 kg; 19-65 yo F = 55 kg

Notes - Minerals

(a) Human breast milk.
(b) Infant formula.

Calcium:

(c) The data used in developing calcium RNIs originate from developed countries, and there is controversy as to their appropriateness for developing countries. This notion also holds true for most nutrients, but based on current knowledge, the impact appears to be most marked for calcium.
(d) Particularly during the growth spurt.

Zinc:

(e) Human-milk fed infants only.
(f) Formula-fed infants, moderate zinc bio-availability.
(g) Formula-fed infants, low zinc bio-availability due to infant consumption of phytate rich cereals and vegetable protein based formula.
(h) Not applicable to infants consuming human milk only**.**

Iron:

(i) There is evidence that iron absorption can be significantly enhanced when each meal contains a minimum of 25 mg of Vitamin C, assuming three meals per day. This is especially true if there are iron absorption inhibitors in the diet such as phytate or tannins.
(k) Neonatal iron stores are sufficient to meet the iron requirement for the first six months in full term infants. Premature infants and low birth weight infants require additional iron.
(l) Bio-availability of dietary iron during this period varies greatly.
(m) Non-menstruating adolescents.
(n) It is recommended that iron supplements in tablet form be given to all pregnant women because of the difficulties in correctly evaluating iron status in pregnancy. In the non-anaemic pregnant woman, daily supplements of 100 mg of iron (e.g., as ferrous sulphate) given during the second half of pregnancy are adequate. In anaemic women higher doses are usually required.

Iodine

(o) Data expressed on a per kg body weight basis is sometimes preferred, and this data is as follows:

premature infants = 30 µg/kg/day	infants 0-12 months = 19 µg/kg/day
children 1 - 6 years = 6 µg/kg/day	children 7 - 11 = 4 µg/kg/day
adolescents and adults 12 + years = 2 µg/kg/day	pregnancy and lactation = 3.5 µg/kg/day

(p) In view of the high variability in body weights at these ages the RNIs are expressed as µg/kg body weight/day.

(**NCHS data source:** WHO, Measuring Change in Nutritional Status. Guidelines for Assessing the Nutritional Impact of Supplementary Feeding Programmes for Vulnerable Groups, World Health Organization, 1983)

Table 2 **Recommended nutrient intakes [g] [h] – water and fat soluble vitamins***

	WATER-SOLUBLE VITAMINS									FAT-SOLUBLE VITAMINS			
Age	**Thiamin** *mg/day*	**Riboflavin** *mg/day*	**Niacin (a)** *mg NE/day*	**Vit. B_6** *mg/day*	**Panto-thenate** *mg/day*	**Biotin** *µg/day*	**Folate (c)** *µg DFE/day*	**Vit. B_{12}** *µg/day*	**Vit. C (d)** *mg/day*	**Vit. A (f) (g)** *µg RE/day*	**Vit. D** *µg/day*	**Vit. E (acceptable intakes) (h)** *mg α-TE/ day*	**Vit. K (l)** *µg/day*
Infants													
0 - 6 months	0.2	0.3	2 **(b)**	0.1	1.7	5	80	0.4	25	375	5	2.7 **(i)**	5 **(m)**
7-11 months	0.3	0.4	4	0.3	1.8	6	80	0.5	30	400	5	2.7 **(i)**	10
Children													
1-3 years	0.5	0.5	6	0.5	2	8	160	0.9	30	400	5	5 **(k)**	15
4-6 years	0.6	0.6	8	0.6	3	12	200	1.2	30	450	5	5 **(k)**	20
7-9 years	0.9	0.9	12	1.0	4	20	300	1.8	35	500	5	7 **(k)**	25
Adolescents													
10-18 years													
Males	1.2	1.3	16	1.3	5	25	400	2.4	40	600	5	10	35-65
Females	1.1	1.0	16	1.2	5	25	400	2.4	40	600	5	7.5	35-55
Adults													
Males 19 - 65 years	1.2	1.3	16	1.3 (19-50 yrs) 1.7 (50 + yrs)	5	30	400	2.4	45	600	5 (19-50 yrs) 10 (50 + yrs)	10	65
Females													
19-50 years (pre- menopausal)	1.1	1.1	14	1.3	5	30	400	2.4	45	500	5	7.5	55
51-65 years (menopausal)	1.1	1.1	14	1.5	5	30	400	2.4	45	500	10	7.5	55
Older adults, 65 + years													
Males	1.2	1.3	16	1.7	5		400	2.4	45	600	15	10	65
Females	1.1	1.1	14	1.5	5		400	2.4	45	600	15	7.5	55
Pregnancy	1.4	1.4	18	1.9	6	30	600	2.6	55	800	5	**(i)**	55
Lactation	1.5	1.6	17	2.0	7	35	500	2.8	70 **(e)**	850	5	**(i)**	55

* For the purposes of these composite tables of RNI values, the body weights used were derived from the 50th percentile of NCHS data until adult weights of 55 kg for females and 65 kg for males were reached. The weights used are the following: 0-6 mo = 6 kg; 7–12 mo = 8.9 kg; 1-3 yo = 12.1 kg; 4-6 yo = 18.2 kg; 7-9 yo = 25.2 kg; 10-11 yo M = 33.4 kg; 10-11 yo F = 34.8 kg; 12-18 yo M = 55.1 kg; 12-18 yo F = 50.6 kg; 10-18 yo M = 55.1 kg; 10-18 yo F = 50.6 kg; 19-65 yo M = 65 kg; 19-65 yo F = 55 kg

Notes - Vitamins

Niacin

(a) NE = niacin equivalents, 60-to-1 conversion factor for tryptophan to niacin.

(b) Preformed niacin.

Folate

(c) DFE = dietary folate equivalents; μg of DFE provided = [μg of food folate + (1.7 x μg of synthetic folic acid)].

Vitamin C

(d) An RNI of 45 mg was calculated for adult men and women and 55 mg recommended during pregnancy. It is recognised however that larger amounts would promote greater iron absorption if this can be achieved.

(e) An additional 25 mg is needed for lactation.

Vitamin A:

(f) Vitamin A values are "recommended safe intakes" instead of RNIs. This level of intake is set to prevent clinical signs of deficiency, allow normal growth, but does not allow for prolonged periods of infections or other stresses.

(g) Recommended safe intakes as μg RE/day; 1 μg retinol=1 μg RE; 1 μg β-carotene=0.167 μg RE; 1 μg other provitamin A carotenoids=0.084 μg RE.

Vitamin E:

(h) Data were considered insufficient to formulate recommendations for this vitamin so that "acceptable intakes" are listed instead. This represents the best estimate of requirements, based on the currently acceptable intakes that support the known function of this vitamin.

(i) For pregnancy and lactation there is no evidence of requirements for vitamin E that are any different from those of older adults. Increased energy intake during pregnancy and lactation is expected to compensate for increased need for infant growth and milk synthesis. Breast milk substitutes should not contain less than 0.3 mg α-tocopherol equivalents (TE)/100 ml of reconstituted product, and not less than 0.4 mg TE/g PUFA. Human breast milk vitamin E is fairly constant at 2.7 mg for 850 ml of milk.

(k) Values based on a proportion of the adult acceptable intakes.

Vitamin K:

(l) The RNI for each age group is based on a daily intake of 1 μg/kg/day of phylloquinone, the latter being the major dietary source of Vitamin K.

(m) This intake cannot be met by infants who are exclusively breast-fed. To prevent bleeding due to vitamin K deficiency, all breast fed babies should receive vitamin K supplementation at birth according to nationally approved guidelines.

(**NCHS data source:** WHO, Measuring Change in Nutritional Status. Guidelines for Assessing the Nutritional Impact of Supplementary Feeding Programmes for Vulnerable Groups, World Health Organization, 1983)

Exhibit H

DRI

DIETARY REFERENCE INTAKES

Calcium
Vitamin D

Committee to Review Dietary Reference Intakes for Vitamin D and Calcium
Food and Nutrition Board

A. Catharine Ross, Christine L. Taylor, Ann L. Yaktine, and
Heather B. Del Valle, *Editors*

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"Knowing is not enough; we must apply.
Willing is not enough; we must do."
—Goethe

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by **Irwin H. Rosenberg**, Friedman School of Nutrition Science and Policy, Tufts University, and **Enriqueta C. Bond**, Burroughs Wellcome Fund (retired). Appointed by the National Research Council and Institute of Medicine, they were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

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Preface

It has been an honor to chair this committee tasked with reviewing Dietary Reference Intake (DRI) values for calcium and vitamin D. In this preface, I would like, first and foremost, to thank those persons without whose help this report would not have been possible. I also would like to comment briefly on the nature of the task we had at hand, and how our committee proceeded, from its first meeting in 2009 to the final stage of its report.

The work of our committee was preceded by three important papers and reports. At a time when interest in vitamin D had reached new heights, and many various claims for benefits were reported, health professionals in the governments of the United States and Canada worked together to address the question: Since the 1997 IOM report on DRIs, including vitamin D, is there sufficient new evidence on this micronutrient to warrant a new DRI study? The publication from this group, "Dietary reference intakes for vitamin D: justification for a review of the 1997 values"[1] concluded that there were sufficient new data to warrant a reevaluation. In funding the DRI review for vitamin D, the sponsors also judged that calcium should be reviewed as well, given its interrelationship with vitamin D. I thank the many individuals from the U.S. and Canadian governments who put into motion the processes that led to this report. Moreover, understanding that

[1]Yetley, E. A., D. Brule, M. C. Cheney, C. D. Davis, K. A. Esslinger, P. W. Fischer, K. E. Friedl, L. S. Greene-Finestone, P. M. Guenther, D. M. Klurfeld, M. R. L'Abbe, K. Y. McMurry, P. E. Starke-Reed and P. R. Trumbo. 2009. Dietary reference intakes for vitamin D: justification for a review of the 1997 values. American Journal of Clinical Nutrition 89(3): 719-27.

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a review of the literature would be a tremendous undertaking by itself, this group also commissioned an independent systematic review of the literature on vitamin D and health outcomes for the use of this DRI committee, and intended to update an earlier systematic review on vitamin D and bone health. The systematic review carried out by Dr. Joseph Lau and his colleagues at the Tufts Evidence-based Practice Center, and a preceding systematic review led by Dr. Ann Cranney of the University of Ottawa, both greatly aided the work of the current committee.

In the Statement of Task, the sponsors requested that our report be developed using a risk assessment framework. Such a framework is not one that committee members would naturally have been familiar with at the outset, and some readers of this report may also wonder, "What is that?" The process is discussed and diagrammed in the report in Chapter 1 and referred to throughout. We were greatly helped in adhering to the risk assessment approach by Christine Taylor, Ph.D., Study Director for this DRI study, whose previous background paper, "Framework for DRI Development,"[2] provided us with a much-needed understanding of the uses of risk assessment and the steps in conducting it that we would follow. Chris' insights, as well as her discipline, good humor, and willingness to engage over and over in discussions to obtain a broad understanding and consensus were very much at the heart of the committee's process. I thank her for being the amazing study director she has been. Our committee's work also benefited from the excellent research and support of Ann Yaktine, Ph.D., Heather Del Valle, and Heather Breiner. Linda Meyers, Ph.D., Director, Food and Nutrition Board, kept a watchful eye on our progress and willingly provided guidance as needed. The committee never lacked for exceptionally well-qualified, rigorous, hardworking, professional, and friendly support from the FNB staff, and I sincerely thank each one of them.

It may be of interest to briefly comment on the committee's approach, and how work evolved during its deliberations. The development of IOM reports is a consensus process. Thus, throughout we worked together, dividing specific tasks according to expertise but making sure that discussions proceeded and decisions were always made as a group. During this time, research did not stand still; not a week passed without new publications on these nutrients. We spent a good deal of effort, and staff performed invaluable service for us, in arraying new data, comparing aspects of study design, etc. The committee worked not only at the scheduled committee meetings, but also in a myriad of working groups by conference calls and emails. It was important to keep firmly in mind that DRIs are values meant for im-

[2]Taylor, C. L. 2008. Framework for DRI Development: Components "Known" and Components "To Be Explored." Washington, DC.

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proving public health—the health of the *general population* of the United States and Canada. They provide recommendations for adequate and safe daily intakes of nutrients consumed over *many* years, possibly a lifetime, not just for days, weeks, months, or a year. Thus, the need for sound, causal evidence to make the evidence-based recommendations in this report was always at the forefront of our thinking and deliberations. The terms *causality, dose–response, evidence-based, totality of evidence, uncertainty, caveats* were often on the committee minds and prominent in our discussions. On some points, we consulted with experts, whom we thank for generously providing their input in response to our needs, sometimes on quite short notice. New data on the intakes of vitamin D and calcium in the United States and Canada arrived from the Centers for Disease Control and Prevention and Health Canada just as we needed them, and here I would like to thank the persons in these organizations who worked diligently to make these new intake data available for the committee's use. As DRI values evolved, we thought carefully about the implications of these recommendations for practitioners and decision makers in public health and policy who will use this report in their work, and for special populations in both the United States and Canada. Lastly, we considered research recommendations, linking our recommendations to knowledge gaps identified while using the risk assessment framework. This, of course, was a future-directed activity, and we hope that our recommendations will clarify the types of research and resulting new information that will make determining DRIs for calcium and vitamin D easier and more accurate in the future.

Throughout, the committee members worked together with common purpose and always amicably, even when viewpoints differed, and this made working on this study a remarkable experience for all of us. I sincerely thank all the members of the committee for sharing their expertise and greatly enriching the development of this report.

Finally, it is important to acknowledge the many people who assisted the committee with its work and who provided technical input and invaluable perspectives through a variety of venues ranging from white papers to participation in workshops and public information gathering meetings. Foremost, the committee is grateful to Dr. Hector DeLuca, who served as a tireless consultant and generously offered his wisdom and considerable experience to the committee. Many discussions were enriched by his input. Others who provided scientific evaluations and background information for the committee include: Dr. David Bushinsky, Dr. Thomas Carpenter, Dr. Gary Curhan, Dr. Gordon Guyatt, Dr. Craig Langman, Dr. Dwight Towler, and Dr. Susan Whiting. The committee is deeply appreciative of the heroic efforts of those who worked long hours to provide the committee timely national data on calcium and vitamin D intake as well as measures of serum 25-hydroxyvitamin D concentrations, specifically the

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National Center for Health Statistics (Mr. Clifford Johnson, Dr. Lester R. Curtin, and Dr. Te-Ching Chen), the U.S. Department of Agriculture (Ms. Alanna Moshfegh and Ms. Joanne Holden), the National Cancer Institute (Dr. Kevin Dodd), and Statistics Canada (Mrs. Jeanine Bustros, Mr. Didier Garriguet, Mr. Christopher Oster, and Miss Dawn Warner). Also, invaluable and illuminating analytical assistance was provided by statisticians at Cornell University, Dr. Francoise Vermeylen and Dr. Shamil Sadigov. Finally, the committee wishes to thank the sponsors of this report for their support and without whom there would not have been the opportunity to carry out this important study.

A. Catharine Ross, *Chair*
Committee to Review Dietary Reference
Intakes for Vitamin D and Calcium

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*Appendixes B through K are not printed in this book, but can be found on the CD at the back of the book or online at http://www.nap.edu.

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Summary

Calcium and vitamin D are undoubtedly essential nutrients for the human body. The key questions are: What processes can these nutrients affect in terms of desirable health outcomes, and how much of each nutrient is needed to achieve the effect?

During the past 10 years, there has been increasing interest in the possibility of enhanced roles for vitamin D in human health. A number of researchers in the scientific community have suggested relationships between vitamin D intake and health outcomes ranging from cancer prevention to increased immunity; others have suggested possible roles in preventing diabetes or preeclampsia during pregnancy. The media have also taken an interest, and public expectations have been raised. At the same time, physicians have been ordering blood tests that seem to suggest, based on use of criteria that have yet to be validated, that many in our North American population are vitamin D deficient. For calcium, there is concern that some may not be obtaining sufficient amounts given the foods they eat. Calcium has been increasingly added to foods, and calcium supplement use, particularly among older persons, is widespread. There is controversy concerning levels of nutrient intake, and at times the concept that "more is better" emerges. However, for both calcium and vitamin D, there is another underlying question: How much is too much?

Against this backdrop, the Institute of Medicine (IOM) was requested by the U.S. and Canadian governments to conduct a review of data pertaining to calcium and vitamin D requirements and to identify Dietary Reference Intakes (DRIs) based on current scientific evidence about the roles of calcium and vitamin D in human health. The DRIs, as nutrient

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reference values, are used by various stakeholders, ranging from those who set national nutrition policy to health practitioners in community settings. Such reference values specify, for normal, healthy persons, an average daily requirement for the nutrient, known as the Estimated Average Requirement (EAR). They also identify levels of intake that are likely to meet the needs of about 97.5 percent of the population (the Recommended Dietary Allowance, or RDA). Further, they include a Tolerable Upper Intake Level (UL) above which the potential for harm increases.

THE COMMITTEE AND ITS CHARGE

The two governments requested that the IOM conduct a study to assess current data and to update as appropriate the DRIs for vitamin D and calcium. The study was to include consideration of chronic disease indicators (e.g., reduction in risk of cancer or diabetes) and other (non-chronic disease) indicators/outcomes, and to assess the ability of each to serve as the basis for specifying adequate intake or excess intake. The final DRI indicators were to be selected based on the strength and quality of the evidence.

To carry out the request, the IOM established an ad hoc consensus committee of 14 scientists. The committee met eight times, held a public workshop and open sessions to gather information and receive input on the nature of the available data, maintained a website that accepted comments and data from stakeholders, conducted a review of existing data, and developed a report that included the specification of DRI values. Committee members had expertise in the areas of vitamin D and calcium or a related topic area, with specific expertise related to pregnancy and reproductive nutrition, pediatrics and infant nutrition, minority health and health disparities, cellular metabolism, toxicology and risk assessment, dermatology, immunology, endocrinology, skeletal health, oncology, cardiovascular health, epidemiology; nutrition monitoring, and biostatistics. Three members of the committee had served on other DRI committees.

DRI CONTEXT FOR COMMITTEE'S WORK

This report marks the first DRI review since the completion of the 1997-2004 DRIs, which in contrast with their predecessors were based on a different approach to respond to expanded uses of the values and newer understandings of the role of nutrients. The DRIs now incorporate the statistical concept of a distribution, including the distributions of requirements and intakes. The major components of the DRIs are shown in Box S-1.

The first DRIs, contained in six volumes, are now used in both the United States and Canada. The governments of these two countries have

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BOX S-1
Dietary Reference Intake Components*

Estimated Average Requirement (EAR): Reflects the estimated median requirement and is particularly appropriate for applications related to planning and assessing intakes for groups of persons.

Recommended Dietary Allowance (RDA): Derived from the EAR and meets or exceeds the requirement for 97.5 percent of the population.

Tolerable Upper Intake Level (UL): As intake increases above the UL, the potential risk of adverse effects may increase. The UL is the highest average daily intake that is likely to pose no risk of adverse effects to almost all individuals in the general population.

Adequate Intake (AI): Used when an EAR/RDA cannot be developed; average intake level based on observed or experimental intakes.

*Also, Acceptable Macronutrient Distribution Range (AMDR): An intake range for an energy source associated with reduced risk of chronic disease.

also supported a recent evaluation of the DRI development process, which has informed the approach used to develop this report. The evaluation pointed to the need for enhanced "transparency" about the decisions made, more clarification about uncertainties in the values, and use of a risk assessment framework to organize the scientific assessments. Risk assessment encompasses a series of decision steps and anticipates the need to address uncertainties through documentation and the use of expert judgment.

THE COMMITTEE'S APPROACH AND EXAMINATION OF DATA

To set the stage for its review, the committee gathered background information on the metabolism and physiology of calcium and vitamin D (Chapters 2 and 3). It then identified those relationships that could potentially serve as indicators for establishing nutrient reference values for adequate intakes of the nutrients. To ensure comprehensiveness, the committee included relationships that appeared marginal by standard scientific principles as well as those suggested to be of interest by stakeholders. Box S-2 lists these potential indicators in alphabetical order. The close inter-relationship between calcium and vitamin D often resulted in potential indicators being relevant to both nutrients.

Chapter 4 provides the committee's review of potential indicators,

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BOX S-2
Potential Indicators of Health Outcomes for Nutrient Adequacy for Calcium and Vitamin D

Cancer/neoplasms
- All cancers
- Breast cancer
- Colorectal cancer/colon polyps
- Prostate cancer

Cardiovascular diseases and hypertension
Diabetes (type 2) and metabolic syndrome (obesity)
Falls
Immune responses
- Asthma
- Autoimmune disease
  - Diabetes (type 1)
  - Inflammatory bowel and Crohn's disease
  - Multiple sclerosis
  - Rheumatoid arthritis
  - Systemic lupus erythematosus
- Infectious diseases
  - Tuberculosis
  - Influenza/upper respiratory infections

Neuropsychological functioning
- Autism
- Cognitive function
- Depression

Physical performance*
Preeclampsia of pregnancy and other non-skeletal reproductive outcomes
Skeletal health (commonly bone health)
- Serum 25-hydroxyvitamin D, as intermediate
- Parathyroid hormone, as intermediate
- Calcium absorption
- Calcium balance
- Bone mineral content/bone mineral density
- Fracture risk
- Rickets/osteomalacia

*In the discussions related to review of potential indicators, physical performance is considered together with falls.

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based on literature identified by the committee and incorporating the systematic evidence-based reviews from the Agency for Healthcare Research and Quality (AHRQ). In sum, with the exception of measures related to bone health, the potential indicators examined are currently not supported by evidence that could be judged either convincing or adequate in terms of cause and effect, or informative regarding dose–response relationships for determining nutrient requirements. Outcomes related to cancer/neoplasms, cardiovascular disease and hypertension, diabetes and metabolic syndrome, falls and physical performance, immune functioning and autoimmune disorders, infections, neuropsychological functioning, and preeclampsia could not be linked reliably with calcium or vitamin D intake and were often conflicting. Although data related to cancer risk and vitamin D are potentially of interest, a relationship between cancer incidence and vitamin D (or calcium) nutriture is not adequately and causally demonstrated at present; indeed, for some cancers, there appears to be an increase in incidence associated with higher serum 25-hydroxyvitamin D (25OHD) concentrations or higher vitamin D intake. The role of vitamin D related to falls and physical performance, cardiovascular disease, autoimmune disorders, and immune functioning has also received considerable attention, and remains unresolved. These potential roles of vitamin D are currently best described as hypotheses of emerging interest, and the conflicting nature of available evidence cannot be used to establish health benefits with any level of confidence. In contrast, the evidence surrounding bone health provides a reasonable and supportable basis to allow this indicator to be used for DRI development.

In making its conclusions about potential indicators other than bone health, the committee noted the observation previously highlighted by others tasked with examining the evolution of evidence for nutrient and disease relationships: that evidence about relationships between specific nutrients and a disease or health outcome remains typically elusive, for a number of reasons. These include the difficulty of isolating the effects of a single nutrient under investigation from the confounding effects of other nutrients and non-nutrient factors; the multi-factorial etiology of the chronic diseases the committee considered; the paucity of data from randomized controlled clinical trials, which typically provide the highest level of scientific evidence relevant for DRI development; and the mixed and inconclusive results from observational studies.

For indicators associated with excess intakes of calcium and vitamin D, a process similar to that for reference values for adequacy was undertaken and potential indicators of excess intake were identified (see Box S-3). The ULs serve as a measure for chronic intake of a free-living, unmonitored population. They are not specified for clinical research; it may be appro-

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BOX S-3
Potential Indicators of Adverse Outcomes for Excess Intake of Calcium and Vitamin D

Calcium
- Hypercalcemia
- Hypercalciuria
- Vascular and soft tissue calcification
- Nephrolithiasis (kidney stones)
- Prostate cancer
- Interactions with iron and zinc
- Constipation

Vitamin D
- Intoxication and related hypercalcemia and hypercalciuria
- Serum calcium
- Measures in infants: retarded growth, hypercalcemia
- Emerging evidence for all-cause mortality, cancer, cardiovascular risk, falls and fractures

priate to conduct clinical research with doses exceeding the UL, as long as there is monitoring and the protocol is carefully considered.

KEY CHALLENGES

Beyond the challenge of limited data and the resulting uncertainties, the study faced two additional challenges. The first is that vitamin D, an essential nutrient, is also synthesized in the skin following exposure to sunlight. Thus, the examination of data is complicated by the confounding factors this introduces. Further, vitamin D requirements could not address the level of sun exposure because public health concerns about skin cancer preclude this possibility. There have not been studies to determine whether ultraviolet B (UVB)–induced vitamin D synthesis can occur without increased risk of skin cancer. The best approach was to estimate vitamin D requirements under conditions of minimal sun exposure.

Second, vitamin D when activated functions as a hormone and is regulated by metabolic feedback loops. The intertwining of the effects of vitamin D and calcium represents an extreme case of nutrient–nutrient inter-relationships. Indeed, many studies administered these nutrients together rather than separately. For this reason, distinguishing the health outcomes for one nutrient versus the other was challenging.

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THE COMMITTEE'S OUTCOMES

An assumption in developing the DRIs for calcium is that they are predicated on intakes that meet requirements for vitamin D; similarly, DRIs for vitamin D rest on the assumption of intakes that meet requirements for calcium.

Dietary Reference Intakes for Calcium

DRIs for calcium were established as EARs and RDAs except for infants up to 12 months of age for whom AIs were specified. The DRIs for calcium are shown in Table S-1.

TABLE S-1 Calcium Dietary Reference Intakes by Life Stage (amount/day)

Life Stage Group	AI	EAR	RDA	UL
Infants				
0 to 6 mo	200 mg	—	—	1,000 mg
6 to 12 mo	260 mg	—	—	1,500 mg
Children				
1–3 y	—	500 mg	700 mg	2,500 mg
4–8 y	—	800 mg	1,000 mg	2,500 mg
Males				
9–13 y	—	1,100 mg	1,300 mg	3,000 mg
14–18 y	—	1,100 mg	1,300 mg	3,000 mg
19–30 y	—	800 mg	1,000 mg	2,500 mg
31–50 y	—	800 mg	1,000 mg	2,500 mg
51–70 y	—	800 mg	1,000 mg	2,000 mg
> 70 y	—	1,000 mg	1,200 mg	2,000 mg
Females				
9–13 y	—	1,100 mg	1,300 mg	3,000 mg
14–18 y	—	1,100 mg	1,300 mg	3,000 mg
19–30 y	—	800 mg	1,000 mg	2,500 mg
31–50 y	—	800 mg	1,000 mg	2,500 mg
51–70 y	—	1,000 mg	1,200 mg	2,000 mg
> 70 y	—	1,000 mg	1,200 mg	2,000 mg
Pregnancy				
14–18 y	—	1,100 mg	1,300 mg	3,000 mg
19–30 y	—	800 mg	1,000 mg	2,500 mg
31–50 y	—	800 mg	1,000 mg	2,500 mg
Lactation				
14–18 y	—	1,100 mg	1,300 mg	3,000 mg
19–30 y	—	800 mg	1,000 mg	2,500 mg
31–50 y	—	800 mg	1,000 mg	2,500 mg

NOTE: AI = Adequate Intake; EAR = Estimated Average Requirement; RDA = Recommended Dietary Allowance; UL = Tolerable Upper Intake Level.

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The EARs and RDAs relied primarily upon calcium balance studies for persons 1 to 50 years of age. The effect of menopause on bone resulted in specifying different EARs and RDAs for women and men 51 to 70 years of age. After the age of 70 years, the effects of aging on bone loss resulted in EARs and RDAs that are the same for men and women. The AIs for infants are based on the calcium content of human milk. There is no evidence that calcium requirements are different for pregnant and lactating females compared with their non-pregnant or non-lactating counterparts.

The ULs for calcium for adults are based on data related to the incidence of kidney stones, largely from work conducted with postmenopausal women who use calcium supplements. Newer data from a feeding study provided evidence of intake levels among infants not associated with elevated calcium excretion, and allowed derivation of a UL for infants. The UL for children and adolescents 9 to 18 years of age gives consideration to the pubertal growth spurt and increases the UL as compared with that for children 1 to 8 years of age.

Dietary Reference Intakes for Vitamin D

DRI values for vitamin D (Table S-2) were established as EARs and RDAs for all life stage groups except infants up to 12 months of age for which an AI was specified. These reference values assume minimal sun exposure.

Measures of serum 25OHD level serve as a reflection of total vitamin D exposure—from food, supplements, and synthesis. Although serum 25OHD level cannot be considered a validated health outcome surrogate, it allowed comparison of intake or exposure with health outcomes. Newer data also allowed the simulation of a requirement distribution based on serum 25OHD concentrations. A level of 40 nmol/L (16 ng/mL) was consistent with the intended nature of an average requirement, in that it reflects the desired level for a population median—it meets the needs of approximately half the population. Moreover, benefit for most in the population is associated with serum 25OHD levels of approximately 50 nmol/L (20 ng/mL), making this level a reasonable estimate for a value akin to "coverage" for nearly all the population. Available data were used to link specified serum levels of 25OHD with total intakes of vitamin D under conditions of minimal sun exposure in order to estimate DRIs.

For children and adolescents 1 to 18 years of age, EARs and RDAs are specified on the basis of serum 25OHD concentrations of 40 and 50 nmol/L (16 and 20 ng/mL), respectively. Likewise this approach was used for young adults and adults from 19 through 50 years of age and was supported by data on osteomalacia. The EAR for persons older than 50 years of age is the same as that for younger adults, as the simulated requirement

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TABLE S-2 Vitamin D Dietary Reference Intakes by Life Stage (amount/day)

Life Stage Group	AI	EAR	RDA	UL
Infants				
0 to 6 mo	400 IU (10 µg)	—	—	1,000 IU (25 µg)
6 to 12 mo	400 IU (10 µg)	—	—	1,500 IU (38 µg)
Children				
1–3 y	—	400 IU (10 µg)	600 IU (15 µg)	2,500 IU (63 µg)
4–8 y	—	400 IU (10 µg)	600 IU (15 µg)	3,000 IU (75 µg)
Males				
9–13 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
14–18 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
19–30 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
31–50 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
51–70 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
> 70 y	—	400 IU (10 µg)	800 IU (20 µg)	4,000 IU (100 µg)
Females				
9–13 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
14–18 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
19–30 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
31–50 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
51–70 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
> 70 y	—	400 IU (10 µg)	800 IU (20 µg)	4,000 IU (100 µg)
Pregnancy				
14–18 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
19–30 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
31–50 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
Lactation				
14–18 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
19–30 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
31–50 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)

NOTE: AI = Adequate Intake; EAR = Estimated Average Requirement; IU = International Units; RDA = Recommended Dietary Allowance; UL = Tolerable Upper Intake Level.

distribution suggested no effect due to age. However, there is notable variability around these estimates in the case of bone health for older persons. This suggests that the assumption about the variance associated with coverage for 97.5 percent of the population should be greater for this older group than for the younger group. Therefore, the RDA value for persons older than 70 years of age was increased to a level greater than the two standard deviations used for other groups. In fact, available data provide more information about maximal population coverage than they do about average requirements for these life stage groups. The factors taken into account included changes in bone density and fracture risk. For infants, an AI was established based on evidence that maintaining serum 25OHD levels in the range of 40 to 50 nmol/L (16 to 20 ng/mL) was desirable,

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coupled with observational data suggesting that 400 International Units (IU) (10 μg) per day was adequate to maintain this level.

The ULs for vitamin D were especially challenging because available data have focused on very high levels of intake that cause intoxication and little is known about the effects of chronic excess intake at lower levels. The committee examined the existing data and followed an approach that would maximize public health protection. The observation that 10,000 IU (250 μg) of vitamin D per day was not associated with classic toxicity served as the starting point for adults; this value was corrected for uncertainty by taking into consideration emerging data on adverse outcomes (e.g., all-cause mortality), which appeared to present at intakes lower than those associated with classic toxicity and at serum 25OHD concentrations previously considered to be at the high end of physiological values. Possible ethnic/racial differences were taken into account as well. The UL for adults is used for 9 to 18 years olds, but is "scaled down" for children 1 to 8 years of age. Earlier studies remain the best basis for ULs for infants.

DIETARY INTAKE ASSESSMENT

Calcium remains a nutrient of concern given that median calcium intakes from foods in both the United States and Canada are close to the EAR values for most groups. In particular, girls 9 to 18 years of age are falling below desirable intakes when only food sources of calcium are considered, as are women over the age of 50 years. Available data from the United States on the total intake of calcium when dietary supplements are considered suggest that older women have noticeably increased calcium intakes with supplement. For girls, the increase in intake attributable to supplement use is small. No life stage groups exceeded the UL for calcium when foods alone were considered. However, when supplement use was taken into account (United States only), women at the 95th percentile of calcium intake appeared to be at risk for exceeding the UL. The data underscore the possible need to modestly increase calcium intake among older girls; among older women, a high calcium intake from supplements may be concerning.

Although daily median vitamin D intake from foods in both countries for all life stage groups was below the established reference value, these data should be considered in light of the average serum 25OHD concentrations. U.S. serum 25OHD concentrations on average were well above 40 nmol/L (16 ng/mL), the level established as consistent with an intake equivalent to the EAR; in fact, all mean serum 25OHD concentrations were above 50 nmol/L (20 ng/mL). In the case of serum 25OHD concentrations from Canadian surveys, mean serum 25OHD levels for all life stage groups were at or above 60 nmol/L (24 ng/mL). The fact that these values

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are higher for the Canadian than for the U.S. population may be in part due to differences in assay methodologies used.

IMPLICATIONS AND SPECIAL CONCERNS

The final risk assessment step is risk characterization, which highlights implications of the DRI outcomes and special concerns including the population segments shown in Box S-4. The nature and extent of the risk associated with these population segments vary.

Uncertainties

On balance, the uncertainties surrounding the DRI values for calcium are less than those for vitamin D because the evidence base is considerably larger for calcium, and the physiology and metabolism of calcium are better understood. The following key issues were identified as introducing uncertainty into DRI values for calcium and vitamin D, as based on bone health outcomes:

- The tendency for study protocols to administer a combination of calcium and vitamin D, reducing the opportunity to ascertain effects of each nutrient independently;
- The lack of data examining the responses and health outcomes

BOX S-4
Population Segments and Conditions of Interest

Adiposity
Persons living at upper latitudes in North America
Persons who experience reduced vitamin D synthesis from sun exposure
- Dark skin (including immigrant groups and exclusively breast-fed infants)
- Use of sunscreen
- Indoor environments and institutionalized older persons

Alternative diets or changes in dietary patterns
- Dairy and animal product exclusion
- Changes in dietary patterns of indigenous Canadian populations

Use of calcium supplements
Oral contraceptive use
Premature infants
Interaction between vitamin D and prescription drugs

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from graded doses of calcium or vitamin D intake so as to elucidate dose–response relationships;
- The interaction between calcium and vitamin D to the extent that it would appear that adequate calcium intake greatly diminishes the need for vitamin D relative to bone health outcomes;
- The unique situation in which a nutrient (vitamin D) is physiologically managed by the body as a hormone, introducing a myriad of variables and feedback loops related to its health effects;
- The paucity of data and resulting uncertainty concerning sun exposure, which confounds the interpretation of dose–response data for intakes of vitamin D. This, coupled with the apparent contribution of sun exposure to overall vitamin D nutriture in North American populations, leads to an inability to characterize and integrate sun exposure with dietary intake recommendations as much as may be appropriate, given the concern for skin cancer risk reduction. Thus, for individuals who experience sun exposure, the uncertainty of the DRI is greater than for those who do not;
- The lack of clarity concerning the validity of the serum 25OHD measure as a biomarker of effect;
- The variability surrounding measures of serum 25OHD concentrations owing to different methodologies used;
- The evidence of the non-linear nature of the relationship between serum 25OHD concentrations and total intake of vitamin D, suggesting that lower levels of intake have more impact on serum 25OHD concentrations than previously believed and that higher intakes may have less impact;
- The limited number of long-term clinical trials related to calcium and vitamin D intake and health outcomes; and
- The need to set ULs based on limited data in order to ensure public health protection.

For vitamin D, the challenges introduced by issues of sun exposure are notable. This nutrient is unique in that it functions as a hormone and the body has the capacity to synthesize it. However, concerns about skin cancer risk preclude incorporating the effects of sun exposure in the DRI process. At this time, the only solution is to proceed on the basis of the assumption of minimal sun exposure and set reference values assuming that all of the vitamin D comes from the diet. This is a markedly cautious approach given that the vast majority of North Americans obtain at least some vitamin D from inadvertent or intentional sun exposure. Therefore, the estimated intake data for vitamin D cannot stand alone as a basis for broad public health action. Rather, national policy should consider intake data in the context of measures of serum 25OHD, a well-established biomarker of

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total vitamin D exposure (endogenous synthesis and diet including supplements). Although estimates of vitamin D intake appear to be less than needed to meet requirements, the serum 25OHD data available—when coupled with the committee's assessment of serum 25OHD levels consistent with EAR and RDA values—suggest that requirements are being met for most if not all persons in both countries. Moreover, the possibility of risk for subpopulations of concern due to reduced synthesis of vitamin D, such as persons with dark skin or older persons in institutions, is minimized given the assumption of minimal sun exposure as a basis for the DRIs.

CONCLUSIONS ABOUT VITAMIN D DEFICIENCY IN THE UNITED STATES AND CANADA

Serum levels of 25OHD have been used as a measure of adequacy for vitamin D, as they reflect intake from the diet coupled with the amount contributed by cutaneous synthesis. The cut-point levels of serum 25OHD intended to specify deficiency for the purposes of interpreting laboratory analyses and for use in clinical practice are not specifically within the charge to this committee. However, the committee noted with some concern that serum 25OHD cut-points defined as indicative of deficiency for vitamin D have not undergone a systematic, evidence-based development process.

From this committee's perspective, a considerable over-estimation of the levels of vitamin D deficiency in the North American population now exists due to the use by some of cut-points for serum 25OHD levels that greatly exceed the levels identified in this report as consistent with the available data. Early reports specified a serum 25OHD concentration of at least 27.5 nmol/L (11 ng/mL) as an indicator of vitamin D adequacy from birth through 18 years of age, and a concentration of at least 30 nmol/L (12 ng/mL) as an indicator of vitamin D adequacy for adults 19 to 50 years of age. In recent years, others have suggested different cut-points as determinants of deficiency and what has been termed "insufficiency." In the current literature, these include values ranging from less than 50 nmol/L (20 ng/mL) to values above 125 nmol/L (50 ng/mL). Use of higher than appropriate cut-points for serum 25OHD levels would be expected to artificially increase the estimates of the prevalence of vitamin D deficiency.

The specification of cut-points for serum 25OHD levels has serious ramifications not only for the conclusions about vitamin D nutriture and nutrition public policy, but also for clinical practice. At this time, there is no central body that is responsible for establishing such values for clinical use. This committee's review of data suggests that persons are at risk of deficiency relative to bone health at serum 25OHD levels of below 30 nmol/L (12 ng/mL). Some, but not all, persons are potentially at risk for

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inadequacy at serum 25OHD levels between 30 and 50 nmol/L (12 and 20 ng/mL). Practically all persons are sufficient at serum 25OHD levels of at least 50 nmol/L (20 ng/mL). Serum 25OHD concentrations above 75 nmol/L (30 ng/mL) are not consistently associated with increased benefit. There may be reason for concern at serum 25OHD levels above 125 nmol/L (50 ng/mL). Given the concern about high levels of serum 25OHD as well as the desirability of avoiding mis-classification of vitamin D deficiency, there is a critical public health and clinical practice need for consensus cut-points for serum 25OHD measures relative to vitamin D deficiency as well as excess. The current lack of evidence-based consensus guidelines is problematic and of concern because individuals with serum 25OHD levels above 50 nmol/L (20 ng/mL) may at times be classified as deficient and treated with high-dose supplements of vitamin D containing many times the levels of intake recommended by this report.

Closing Remarks

At this time, the scientific data available indicate a key role for calcium and vitamin D in skeletal health and provide a sound basis for DRIs. The data do not, however, provide compelling evidence that either nutrient is causally related to extra-skeletal health outcomes or that intakes greater than those established in the DRI process have benefits for health. The last chapter of this report specifies the research needs and reflects an urgent and worthwhile agenda. If carried out, this research will assist greatly in clarifying DRIs for vitamin D and calcium in the future.

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1

Introduction

For more than half a century, specification of the quantities of nutrients needed to meet human requirements—dietary reference values—has been carried out at the national level in the United States and Canada. Reference values known in the United States as Recommended Dietary Allowances (RDAs) and in Canada as Recommended Nutrient Intakes (RNIs) were used well into the 1990s (IOM, 2008). They were established primarily to set nutrition and health policy (IOM, 2008) and have found broad application in government programs ranging from standards for school meals to the basis for food fortification. They have also been used to counsel individuals about dietary intake. Over the years, both governments have funded on-going updates and reviews of these reference values.

In 1994, in response to important changes in the nutrition field as well as the recognition that for many nutrients the single-value RDA or RNI did not meet the expanding needs for nutrient reference values, the Institute of Medicine (IOM) in Washington, DC, began an initiative to develop a new, broader set of values known as the Dietary Reference Intakes or DRIs (IOM, 2008). The U.S. and Canadian governments have jointly supported this initiative, and the resulting DRIs are now used in both countries. As a result of the initiative, the DRIs as reference values now

- Include an estimate of an average (or median) requirement as well as an estimate of an intake level that meets, and in turn exceeds, the needs of most (97.5 percent) of the population;
- Include upper levels of intake to ensure no harm from nutrient intake;

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- Incorporate chronic disease indicators when the data allow; and
- Highlight concepts of probability and risk for defining reference values.

With this new model as a backdrop, the IOM in 1997 issued the first set of DRIs. The nutrients included in the first of what became a series of DRI reports were: calcium, phosphorus, magnesium, vitamin D, and fluoride (IOM, 1997). Therefore, the 1997 DRIs for calcium and vitamin D—the nutrients that are the topic of this 2010 review—have been in existence for 13 years. In 2008, the U.S. and Canadian governments made the decision that there were now sufficient new data to warrant funding another study of the DRIs for vitamin D (Yetley et al., 2009). They included calcium in this study because of its close inter-relationship with vitamin D. A 14-member ad hoc expert committee was convened by the IOM in 2009 to take on this task; its work was to be completed by 2010. Committee members had general expertise in the areas of vitamin D and calcium or a closely related topic area, with specific expertise related to endocrinology, bone and skeletal health, immunology, oncology, dermatology, cardiovascular health, pregnancy and reproductive nutrition, pediatrics and infant nutrition, epidemiology, cellular metabolism, toxicology and risk assessment, nutrition monitoring, biostatistics, and minority health and health disparities. Three members of the committee had served on other DRI committees.

The current consideration of the DRIs for vitamin D and calcium takes place at a time when the interest in vitamin D is enormous. This vitamin—with its hormone-related activities—has received much media attention and has been the subject of countless publications and lay press reporting of its benefits for an array of health outcomes. Concerns about widespread vitamin D deficiency in North American populations are often expressed. This committee's focus was, first, to review objectively the existing evidence concerning the benefits and health outcomes associated with vitamin D as well as calcium, using the well-established scientific principles for judging the quality and relevance of data from intervention as well as observational studies. The members of the committee next integrated the available data and, within the context of the risk assessment approach for establishing DRIs, carried out activities to specify DRIs for calcium and vitamin D. The reference values established in 1997 were noted by the committee, but they were not binding on the committee's work.

THE TASK

The charge to the committee was to assess current relevant data and update, as appropriate, the DRIs for vitamin D and calcium. The review was to include consideration of chronic disease indicators (e.g., reduction

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in risk of cancer) and other (non-chronic disease) indicators and health outcomes. The definitions of these terms are discussed below. Consistent with the framework for DRI development, the indicators to assess adequacy and excess intake were to be selected based on the strength and quality of the evidence and their demonstrated public health significance, taking into consideration sources of uncertainty in the evidence. Further, the committee deliberations were to incorporate, as appropriate, systematic evidence-based reviews of the literature.

Specifically, in carrying out its work, the committee was to:

- Review evidence on indicators to assess adequacy and indicators to assess excess intake relevant to the general North American population, including groups whose needs for or sensitivity to the nutrient may be affected by particular conditions that are widespread in the population such as obesity or age-related chronic diseases. Special groups under medical care whose needs or sensitivities are affected by rare genetic disorders or diseases and their treatments were to be excluded;
- Consider systematic evidence-based reviews, including those made available by the sponsors as well as others, and carefully document the approach used by the committee to carry out any of its own literature reviews;
- Regarding selection of indicators upon which to base DRI values for adequate intake, give priority to selecting indicators relevant to the various age, gender, and life stage groups that will allow for the determination of an Estimated Average Requirement (EAR);
- Regarding selection of indicators upon which to base DRI values for upper levels of intake, give priority to examining whether a critical adverse effect can be selected that will allow for the determination of a so-called benchmark intake;
- Update DRI values, as appropriate, using a risk assessment approach that includes (1) identification of potential indicators to assess adequacy and excess intake, (2) selection of the indicators of adequacy and excess intake, (3) intake-response assessment, (4) dietary intake assessment, and (5) risk characterization.
- Identify research gaps to address the uncertainties identified in the process of deriving the reference values and evaluating their public health implications.

THE DIETARY REFERENCE INTAKE FRAMEWORK

The framework for DRI development has been described by others (IOM, 2006, 2008; Taylor, 2008) and will be outlined here to set the con-

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text for this report. The original framework for DRIs was put in place in 1994 (IOM, 1994), and the reviews of nutrients were completed in 2004. During the 4-year period between 2004 and 2008, it was the subject of discussions concerning its needed improvements as well as it successes (IOM, 2008). The present DRI effort described in this report for vitamin D and calcium is the first to be issued since the 2004 to 2008 evaluative discussions.

In developing and enhancing the DRI framework, two goals were identified. The first is that the framework should ensure and foster transparency of the decision-making process. The second goal is that the framework should anticipate the need to make decisions in the face of limited data and, in turn, offer options for making scientific judgments. Scientific judgment in the face of limited data is important, given the interest in protecting public health and the reality that "no decision is not an option"—that is, a science-based judgment is more useful than no recommendation at all. In other words, the framework must operate under conditions of uncertainty.

The framework that has evolved for DRI development is increasingly recognized as akin to that developed in other fields and referred to as *risk assessment*. Risk assessment is a component of risk analysis, a process for managing situations where public health interventions and monitoring come into play. It analyzes and controls the "risks" that may be experienced by a population of interest (Taylor, 2008). In the case of DRI development, the "risk" is nutrient intakes that are too low or too high. Although the terminology associated with the discipline of risk analysis may at times be unfamiliar to those in the nutrition field, the discipline's structure and application are a good match for DRI development (Taylor, 2008).

Risk analysis, as considered generically for all fields of study, typically is described as including three components: risk assessment, risk management, and risk communication. These are often illustrated as overlapping circles. The component known as *risk assessment* has received attention as an organizing scheme for the DRI study committee review process, and is described separately in a section below. Overall, however, the basic assumptions underlying all of risk analysis are relevant to DRI development. At its most basic, risk analysis is predicated on the assumption that *scientific deliberations should be organized in a manner that meets user/sponsor needs while maintaining the scientific integrity of the assessment* (NRC, 1983). Further, the following general assumptions of risk analysis relate directly to the overall development of DRIs, particularly concerning scientific judgments when uncertainties and limited data exist (Taylor, 2008):

- *Failure to provide a reference value ("no decision") is often not a viable option from the perspective of protecting public health.* It is better to offer

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those operating in the public health arena an informed decision based on the best available scientific expertise and judgment, even if not perfect or very precise, than to offer no information, which by default provides no guidance for evaluating or dealing with the current situation.

- *Available datasets are often incomplete*, and scientific uncertainties must be dealt with through use of scientific judgment and judicious, transparent documentation.
- Meeting the scientific needs of users/sponsors requires a framework for ensuring *understanding of the needs* and a *useful presentation* of the scientific assessments, as well as the *independence* of the scientific evaluations and protection of the scientific reviewers from undue stakeholder influence.

Finally, the DRI framework recognizes the considerable utility in organizing and rating the available data through the use of systematic reviews (Taylor, 2008; Russell et al., 2009), which are now a well-established process in many fields of medicine. However, unlike a systematic review of a medical intervention, a systematic review for the relationship between nutrient intake and a health outcome is much broader. In contrast with focused clinical interventions, most nutrients have direct and indirect effects on a wide range of health outcomes and could potentially reduce the risk of chronic diseases. In turn, the breadth of outcomes—and thus research that needs to be assessed—is greater than that for a medical intervention; as a result, considerable care is required in formulating and prioritizing the key questions to be addressed (Chung et al., 2010).

Definition of Dietary Reference Intakes

The DRIs are comprised of several reference values that relate to the concept of a distribution of requirements and a distribution of intakes. These different values are tools for assessing and planning diets and are most applicable for use with groups of people because the exact nutritional requirements of an individual cannot be known. The application of DRI nutrient reference values for these general purposes is wide and diverse. They range from use by federal government agencies in making national nutrition policy or developing federal nutrition and food assistance programs, to work at the local level in assessing diets of groups and individuals. Public health protection and promotion is the common interest. Further, DRIs address nutrients in foods overall. Because people structure diets primarily by selecting individual foods as opposed to selecting a set of nutrients, an important role of government and related advisory groups has been the task of translating quantitative nutrient reference values into

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food-based recommendations for the generally healthy U.S. and Canadian populations. That was not the task of this committee for whom the focus has been the quantitative nutrient requirements and upper levels of intake.

Currently, the mainstays of DRI development are the EAR, and the Tolerable Upper Intake Level, or UL (also referred to at times as Upper Levels of Intake). The RDA is to be derived from the EAR and reflects an estimate of an intake that meets the needs of 97.5 percent of the population's requirements. It is not a target intended to be met by all individuals, and intakes below the RDA cannot be assumed to be inadequate because the RDA by definition exceeds the actual requirements of all but 2 to 3 percent of the population. The Adequate Intake (AI) was originally incorporated into the framework to address the inevitable uncertainties associated with specifying requirements for infants, given the challenges in obtaining sufficient information for this group, but has expanded to include use when available data for any life stage group are too limited to establish a requirement. The AI is the subject of some debate, given that it does not appear to readily "fit" into the probability assumptions for DRI use (Taylor, 2008). There are also other reference values, as described in other IOM documents (IOM, 2006), but as these are not relevant to this report, they are not described here.

Estimated Average Requirement

The EAR is the average daily nutrient intake level that is estimated to meet the nutrient needs of half of the healthy individuals in a life stage or gender group. Although the term "average" is used, the EAR is actually an estimated *median* requirement (IOM, 2006). Therefore, by definition, the EAR exceeds the needs of half of the population and is less than the needs of the other half (Taylor, 2008).

The 1994 to 2004 DRI process placed emphasis on the distribution of requirements for a population, rather than focusing on a single value constructed to "cover" the great majority of the population, as had been the case in earlier efforts (Taylor, 2008). This, along with the development of newer methodologies for assessing and planning adequate intakes for groups, made the EAR a central reference value, along with the UL. The 10 years of DRI development moved the process from a black-and-white cutoff in the form of an RDA to consideration of a probability model. Doing so made it clear that there is a distribution of requirements in the population (Taylor, 2008).

The EAR itself presents little controversy as an expressed reference value. Beyond the question of how to handle EAR estimation in the face of limited data, most of the issues that surround EAR development are

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related to the uncertainty surrounding the value and ensuring appropriate discussions about the variation in requirements. A challenge lies in obtaining adequate data to allow a reasonable approximation of the variability in requirements and hence the distribution of the requirement among individuals (Taylor, 2008).

Recommended Dietary Allowance

The RDA is calculated from the EAR. It is dependent upon estimating the variance around the EAR and reflects a point estimate defined generally as two standard deviations above the EAR (Taylor, 2008). Although some refer to this reference value as "the requirement plus a safety factor," this is potentially misleading in that it underplays the importance of the variability around the median. The RDA is intended to reflect the EAR plus two standard deviations.

This RDA calculation starts with the assumption that the distribution of a nutrient requirement is generally normal. However, this is not the case for a number of nutrients. There is also the need to describe the variance around the EAR. Such data are usually limited; when the variance is not known, the coefficient of variation is assumed, commonly as 10 percent. There is concern expressed by some that RDAs cannot be considered to be scientifically derived because too often the variance around the EAR cannot be determined precisely from the available data, and is therefore unknown, and the assumptions made about the variance may be inappropriate (Taylor, 2008).

The estimation of the RDA results in a value that is above the intake required for about 97.5 percent of the population. The RDA thus exceeds the requirements of nearly all members of the life stage group. Current guidance (IOM, 2000a, 2003) stipulates that the RDA is useful for some applications with individuals, but it is not appropriate when working with groups of persons for the purposes of assessing and planning for nutrient intake (Taylor, 2008).

Adequate Intake

The possibility of the AI—except for reference values for infants—was not considered when the DRI framework was first developed in 1994 (IOM, 2008). The AIs emerged as a result of the deliberations of the early study committees during the implementation of the initial DRI process. When the available data were judged lacking for the purposes of estimating an EAR, an AI was set. The value was seen as filling the gap that would have existed had no value been issued (Taylor, 2008).

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The AI is defined as a value based on observed or experimentally determined estimates of nutrient intake by a group of people who are apparently healthy and assumed to be maintaining an adequate nutritional state. Examples of adequate nutritional states include normal growth, maintenance of normal levels of nutrients in plasma, and other aspects of nutritional well-being or general health. The AI is obviously derived differently from the EAR/RDA, and a distribution of requirements cannot be offered.

Tolerable Upper Intake Level

As intake increases above the UL, the potential risk of adverse effects may increase; it is a level above which the risk for harm begins to increase. The UL is the highest average daily nutrient intake level likely to pose no risk of adverse health effects for nearly all people in a particular group. The need to set a UL grew out of two major trends; increased fortification of foods with nutrients and the use of dietary supplements by more people in larger doses (IOM, 2006).

The UL is not a recommended level of intake, but rather the highest intake level that can be tolerated without the possibility of causing adverse effects in most people. The value applies to chronic daily intake among free-living persons in the community (IOM, 2006). It has often been misused as a determination of levels to be allowed in controlled clinical trials. However, ULs are not defined to fit this purpose, and higher levels may be approved for controlled research purposes if there is a rationale for the levels to be used and if monitoring and other safety precautions are put in place. Rather, the UL is meant for public health protection. The biggest challenge in establishing ULs is the paucity of data indicating the effects of chronic intakes of high levels of nutrients. Experimental animal data as well as observational data are useful and relevant under these circumstances.

Applications of DRIs

The application of the DRIs in real world settings has been the subject of detailed IOM reports (IOM, 2000a, 2003). The EAR is the foundation of DRI development and is relevant to the planning and assessing of diets as they relate to population groups. The EAR is a reference value often important to the government sponsors of the report who may use requirement distributions to set national food policy, establish criteria for food programs, and make decisions about the adequacy of the food supply.

An individual's nutrient requirement cannot be readily determined, and the use of DRIs for the purposes of assessing and planning diets of individuals is challenging. If an individual's daily intake is typically below the

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EAR, there is likely a need for improved intake. If daily intake is typically between the EAR and the RDA, there is probably a need for improvement because the probability of adequacy, although more than 50 percent, is less than 97.5 percent. However, intakes below the RDA cannot be assumed to be inadequate because the RDA by definition exceeds the actual requirements of all but 2 to 3 percent of the population; many with intakes below the RDA may be meeting their individual requirements (IOM, 2006).

Life Stage Groups

The DRIs are expressed on the basis of reference values for a number of different life stage groups. These life stages have been stipulated generally on the basis of variations in the requirements of all the nutrients under review. A recent IOM report (IOM, 2006) described these general groupings as follows.

Infancy

Infancy covers the first 12 months of life and is divided into two 6-month intervals. In this report infancy is designated as 0 to 6 months (meaning from birth to 5.9 months or about the first 182 days of life) and as 6 to 12 months (meaning from 6.0 months to 11.9 months or approximately the second 182 days of life). Intake is relatively constant during the first 6 months after birth. That is, as infants grow, they ingest more food; however, on a body-weight basis their intake remains the same. During the second 6 months of life, growth rate slows. As a result, total daily nutrient needs on a body-weight basis may be less than those during the first 6 months of life (IOM, 2005). In general, special consideration was not given to possible variations in physiological need during the first month after birth or to the intake variations that result from differences in milk volume and nutrient concentration during early lactation (IOM, 2005). Specific recommended intakes to meet the needs of formula-fed infants are not set as part of the DRI process.

Children: Ages 1 Through 3 Years

In terms of height, toddlers experience a faster growth rate compared with older children, and this distinction provides the biological basis for establishing separate recommended intakes for 1- to 3-year-olds compared with 4- to 8-year-olds. However, data on which to base DRIs for toddlers are often sparse; in many cases, DRIs must be derived by extrapolating data taken from the studies of infants or adults.

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Children: Ages 4 Through 8 Years

During early childhood, children ages 4 through 8 or 9 years (the latter depending on the onset of puberty in each gender) undergo major changes in growth rate and endocrine status. For many nutrients, a reasonable number of data have been available on nutrient intake, and various criteria for adequacy serve as the basis for nutrient reference values for this group. For nutrients that lack data on the requirements of children in this age group, the nutrient reference values must be based on extrapolations from other life stage groups.

Children/Adolescence: Ages 9 Through 13 Years and 14 Through 18 Years

The adolescent years are divided into two categories. Several conclusions support the biological appropriateness of creating two adolescent age groups within the DRI framework (IOM, 2006):

- The mean age of onset of breast development for white girls in North America is 10 years; this is a physical marker for the beginning of increased estrogen secretion (in African American girls, onset is about a year earlier, for unknown reasons).
- The female growth spurt begins before the onset of breast development, thereby supporting the grouping of 9 through 13 years.
- The mean age of onset of testicular development in boys is 10.5 through 11 years.
- The male growth spurt begins 2 years after the start of testicular development, thereby supporting the grouping of 14 through 18 years.

Young Adulthood and Middle Age: Ages 19 Through 30 Years and 31 Through 50 Years

Adulthood was divided into two age groups, in part due to consumption of higher nutrient intakes during early adulthood compared with later in life. Mean energy expenditure decreases from ages 19 through 50 years, and nutrient needs related to energy metabolism may also decrease (IOM, 2006).

Older Adults: Ages 51 Through 70 Years and Over 70 Years

The age period of 51 through 70 years spans active work years for most adults. After age 70, people of the same age increasingly display different

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levels of physiological functioning and physical activity (IOM, 2000b). Age-related declines in nutrient absorption and kidney function also may occur.

Pregnancy and Lactation

Unique changes in physiology and nutrition needs occur during pregnancy and lactation. For the DRI framework, consideration is often given to the following factors:

- The needs of the fetus during pregnancy and the production of milk during lactation;
- Adaptations to increased nutrient demand, such as increased absorption and greater conservation of many nutrients; and
- Net loss of nutrients due to physiological mechanisms, regardless of intake.

Owing to the last two factors, for some nutrients there may not be a basis for setting reference values for pregnant or lactating women that differ from the values set for other women of comparable age.

Indicators for DRI Development

Indicators for DRIs are defined as the health outcomes that serve as the basis for estimating a nutrient requirement. Within the fields of biology and medicine, the term "indicators" has been defined differently and in some cases the definition may not be the same used for DRI purposes. In the case of indicators for DRIs, they can take various forms and many different indicators have been used in the more than 15 years of DRI experience (Taylor, 2008). The term in other settings encompasses what are variously referred to as *endpoints*, *surrogates*, *biomarkers,* or *risk factors*. Additionally, the term *clinical outcome*, also referred to as *health outcome*, is used to refer to the ultimate measurable effect of interest for nutrients, which is, of course, an indicator. Other measures preceding the occurrence of a clinical outcome can be predictive of the clinical outcome itself, although this is not necessarily the case and they must be validated before this can be assumed.

The term *biomarker*, like the term indicator, is defined differently within different fields of study. In the field of nutrition it is often referred to in the same way in which this report uses the term indicator. In order for them to equate, however, the biomarker must be causally related to the outcome indicator. Important terms in common parlance are *biomarker of exposure* and *biomarkers of effect*. The former is a validated measure that can be relied upon to reflect intake or exposure in the case of nutrients. A biomarker of

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effect is an indicator and can be relied upon to be causally related to and predictive of the health outcome of interest.

The guiding principles for selecting indicators as they are used in DRI development is that they must be feasible, valid, reproducible, sensitive, and specific (WHO, 2006). As pointed out by others (WHO, 2006), they must, however, be used intelligently and appropriately. In addition to causal association, general characteristics of indicators for DRI development include the following:

- Changes in the indicator are plausibly related to changes in the risk of an adverse health outcome.
- Changes in the indicator are usually outside the homeostatic range.
- Changes in the indicator are generally associated with adverse sequelae.
- Measurement of the indicator can be accomplished accurately and is reproducible between laboratories.

DRI Risk Assessment

Beginning in the 1990s, the process of risk assessment formally entered into DRI development as the basis for the model for establishing ULs for nutrients (IOM, 1998). However, the risk assessment organizing scheme is as applicable to the activities focused on requirements for ensuring nutritional benefit (i.e., the EAR) as it is to establishing ULs. Risk assessment reflects a flexible, objective scientific scheme for making transparent and accountable decisions, whatever the indicator of interest. It is applied across a range of disciplines and has been generically described as shown in Figure 1-1.

The word "risk" causes some in the nutrition field difficulty, in that it does not seem appropriate to link the benefits of nutrient intake to the concept of "risk," despite the ultimate purpose of reducing the risk for intakes too low to provide the health benefits (Taylor, 2008). Other risk assessment terminology may also seem inappropriate, such as the decision steps labeled as "hazard identification" and "hazard characterization," as well as the final step of "risk characterization." Nonetheless, the approach that has evolved for estimating EARs rests on a sequence of decisions that are similar to those specified within generic risk assessment (Taylor, 2008).

Given that the DRI development process couples the considerations for nutrient adequacy with those for excess intakes, there are advantages to applying the same organizing scheme for both ULs and EARs. For instance, incorporating the same general decision-making process to derive both adequate and excess intakes allows side-by-side comparisons of the process as it progresses. This could be of value in identifying unintended conse-

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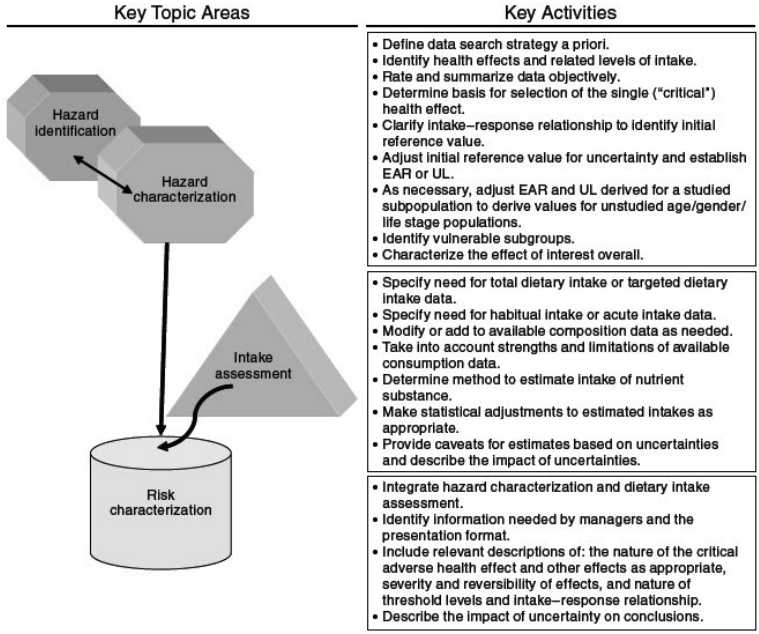


FIGURE 1-1 The four generic steps of risk assessment.
SOURCE: Modified from WHO (2006).

quences or inconsistencies among the various DRI development activities. One example is the procedures used for extrapolation relative to EAR and UL values. Study committees would likely notice potential incompatibilities if the evaluations for both adequate and excess intakes were compared in a side-by-side risk assessment framework. Additionally, as the methodological challenges in the studies used to evaluate risks are likely to be associated with both inadequate and excess intakes, a consistent framework for analyzing both is logical (Taylor, 2008).

The steps associated with risk assessment, as applied in this report on vitamin D and calcium, are briefly described below.

Step 1: "Hazard Identification" or Indicator Review and Selection

An initial starting point for this report—as for all deliberations based on risk assessment—is the identification and review of the potential indicators to be used in developing the DRIs. Based on this review, the indicators

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to be used are selected. As described within the DRI framework, this step of indicator identification (or hazard identification) is outlined as follows.

- **Literature reviews and interpretation** Subject-appropriate and well-done systematic evidence-based reviews as well as other relevant scientific reports and findings serve as a basis for deliberations and development of findings and recommendations for the nutrient under study. De novo literature reviews carried out as part of the study are well documented, including, but not limited to, information on search criteria, inclusion/exclusion criteria, study quality criteria, summary tables, and study relevance to the task at hand consistent with generally accepted methodology used in the systematic review process.
- **Identification of indicators to assess adequacy and excess intake** Based on results from literature reviews and information gathering activities, the evidence is examined for potential indicators related to adequacy for requirements and the effects of excess intakes of the substance of interest. Chronic disease outcomes are taken into account. The approach includes a full consideration of all relevant indicators, identified for each age, gender, and life stage group for the nutrients under study as data allow.
- **Selection of indicators to assess adequacy and excess intake** Consistent with the general approach, indicators are selected based on the strength and quality of the evidence and their demonstrated public health significance, taking into consideration sources of uncertainty in the evidence. They are in consideration of the state of the science and public health ramifications within the context of the current science. The strengths and weaknesses of the evidence for the identified indicators of adequacy and adverse effects are documented.

Step 2: "Hazard Characterization" or Intake-Response Assessment and Specification of Reference Values

The intake–response (more commonly referred to as dose–response) relationships for the selected indicators of adequacy and excess are specified to the extent the available data allow. If the available information is insufficient, then appropriate statistical modeling techniques or other appropriate approaches that allow for the construction of intake-response curves from a variety of data sources are used. In some instances, most notably for the derivation of UL relative to excess intake, it is necessary to make use of specified levels or thresholds in the absence of the ability to describe a dose–response relationship, specifically a no observed effect level

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or a lowest observed effect level. Further, the levels of intake determined for adequacy and excess are adjusted as required, appropriate, and feasible by uncertainty factors, variance in requirements, nutrient interactions, bioavailability and bioequivalence, and scaling or extrapolation.

Step 3: Intake Assessment

Consistent with risk assessment approaches, after the reference value is established, based on the information derived from scientific studies, an assessment of the current intake of (or exposure to) the nutrient of interest is carried out in preparation for the risk characterization step. That is, the known "exposure" to the substance (or the known intake in the case of nutrients) is examined in light of the reference value established. Where information is available, an assessment of biochemical and clinical measures of nutritional status for all age, gender, and life stage groups can be a useful adjunct.

Step 4: "Risk Characterization" or Discussion of Implications and Special Concerns

Risk characterization is a hallmark of the risk assessment approach. For DRI purposes, it includes an integrated discussion of the public health implications of the DRIs and how the reference values may need to be adjusted for special vulnerable groups within the normal population. As appropriate, discussions on the certainty/uncertainty associated with the reference values are included as well as ramifications of the committee's work that the committee has identified as relevant to its risk assessment tasks.

THE APPROACH

The committee began its task in early 2009 and held a total of eight meetings through 2010. Committee members first reviewed the documents concerning the DRI framework (IOM, 2006, 2008; Taylor, 2008) so that members were well versed in the context of their work related to reference values. One of the committee's first activities was to open a website where anyone could submit data or comments to the committee concerning vitamin D and calcium. Any information that was available to the public could be considered by the committee. During its first meeting, the committee made plans for a 1-day public workshop so that information could be presented and explained to the committee, and questions asked of stakeholders.

In order to set the stage for its review, the committee gathered current background information on the metabolism of calcium and vitamin

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D, including life stage differences in metabolism (Chapters 2 and 3). This information may be helpful to those less familiar with the biology and physiology of the two nutrients that are the subject of this report.

Consistent with the risk assessment approach, the committee then initiated the first step of risk assessment in Chapter 4—that is, the work to identify potential indicators. As described in Chapter 4, it reviewed the evidence related to those relationships that could potentially serve as the indicators for establishing DRIs. In order to ensure comprehensiveness, the committee included, as potential indicators, relationships that appeared marginal by standard scientific principles, as well as those suggested to be of interest by stakeholders.

An important set of analyses for the committee's work was the evidence-based reviews on vitamin D (and vitamin D in combination with calcium) carried out by the Agency for Healthcare Research and Quality (AHRQ) (Cranney et al., 2007; Chung et al., 2009). These are referred to throughout the report as AHRQ-Ottawa and AHRQ-Tufts, respectively, at times without a specific reference citation. The methods and results chapters from AHRQ-Ottawa and AHRQ-Tufts are included in their entirety in the appendix section of this report. These large, comprehensive analyses were prepared by AHRQ at the request of the U.S. and Canadian governments and were conducted independently from this committee's work. They provided valuable in-depth information on the quality of the available studies and the overall nature of the database for DRI development for vitamin D and to a lesser extent for calcium.

The AHRQ-Ottawa and AHRQ-Tufts analyses represent the current thinking on approaches to developing dietary reference values in which expansive and at times conflicting bodies of evidence must be arrayed and evaluated in as objective a manner as possible. The key to ensuring the relevance of such analyses to the DRIs as well as their rigor and objectivity is to integrate subject matter experts with methodologists at the planning stages of the systematic reviews. Although the importance of evidence synthesis in medicine was recognized in the 1970s, its widespread use has taken place more recently, especially with the concern that the judgments and opinions of experts could be inadvertently biased (Moher and Tricco, 2008). The questions identified for the analysis must be reflective of the physiological and biological issues, and the inclusion/exclusion criteria must be agreed upon and specified a priori. As described by Moher and Tricco (2008), the four main components of the relevant questions are (1) the population or problem; (2) the intervention, the independent variable, or exposure; (3) the comparators; and (4) the dependent variable or outcomes of interest. The movement to systematic reviews in the nutrition field has been the subject of discussion recently and has been called out as particularly relevant for nutrient reference value development (Russell et al., 2009). Their

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utility is their ability to analyze objectively the available data; their strength derives from including subject matter experts in the planning stages and in the review stages as well. The specific approach used for each of the AHRQ analyses is described in the methodologies section of each (Appendixes C and D) and includes the itemization of the questions asked for the analysis.

It is important to underscore that systematic reviews array much but not all of the data and can assist a DRI committee in identifying relevant indicators. But they do not and cannot establish nutrient reference values, nor do they replace the rigorous integration process and exercise of scientific judgment that characterizes DRI development. That process remains within the purview of the committee.

The committee actively identified other relevant studies not included in the AHRQ analyses or that were published after the close of the AHRQ analyses. These were included in the data consideration. Information from the committee's open sessions as well as the work of committee consultants was also used. In this way, a totality of the body of evidence was established and carefully examined by the committee.

At the close of the literature review process, the committee selected the best indicators to serve as the basis of the DRI values (in Chapter 4). As shown in Chapter 5, the committee then moved to Step 2 in risk assessment, which was to consider the intake-response (or dose–response) relationships based on the available literature. The information identified in Chapter 4 underpins the conclusions reached in Chapter 5. As a result of these discussions, the committee specified first for the purposes of adequacy (EARs, RDAs, and AIs; Chapter 5) and then for preventing excess intakes (ULs; Chapter 6). Step 3 in risk assessment followed, during which the committee performed an intake assessment using current national survey data from the United States and Canada (Chapter 7). For vitamin D, consideration was given to the measures of serum 25OHD concentrations available from national surveys.

In the final step, Step 4, the committee outlined the implications of its work and discussed population segments of interest (Chapter 8). Medical conditions that may relate to special calcium or vitamin D nutriture are specifically outside the scope of the work for this committee and are not addressed in this report. However, a few prevalent clinical groups (e.g., premature infants) are mentioned briefly in Chapter 8. Finally, consistent with its charge, the committee identified research needs for the further development of DRIs for calcium and vitamin D (Chapter 9). Appendix A contains a glossary of terms, acronyms, and abbreviations. With the exception of the Summary and the tables that present the DRIs, this report expresses quantities of calcium as milligrams (mg) and quantities of vitamin D as International Units (IU). In some venues vitamin D is expressed as micrograms (μg) for which 1 μg is equivalent to 40 IU. Serum levels of

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25-hydroxyvitamin D are expressed as nanomoles per liter (nmol/L), but are also often expressed elsewhere as nanograms per milliliter (ng/mL). Values expressed as nmol/L are divided by the conversation factor of 2.5 to obtain the equivalent measure in ng/mL. The Summary and the tables presenting the DRIs express vitamin D using μg as well as IU and express serum 25OHD levels using ng/mL as well as nmol/L.

In sum, Chapters 2 and 3 as developed provide background information about the basic biology of calcium and vitamin D for the readers of this report, but they are not central to the risk assessment process that forms the foundation for this report. The risk assessment approach begins with Chapter 4, which reflects a literature review and evaluation concerning potential indicators for development of DRIs for adequacy; at the close of the chapter, the indicator to be used for the development of DRIs for adequacy is identified. Chapters 5 through 8 contain discussions related to the other steps of risk assessment as specified in the generic model with Chapter 5 providing the reference values related to adequacy of calcium and vitamin D. Chapter 6 overviews the literature related to adverse events and specifies the ULs. Appendix B lists special issues of interest identified by the sponsors of this report and taken into account during committee deliberations.

Finally, it should be noted that this report is not intended to critique or reevaluate the specific conclusions arrived at in the 1997 DRI report related to calcium and vitamin D. This would not be appropriate given the closed nature of those deliberations as well as the specific charge to this committee, which was to review the state of the data currently and come to its own conclusions about DRI values. When necessary to clarify this committee's conclusions, and as relevant to set these new reference values in context, mention is made of the 1997 report.

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2

Overview of Calcium

INTRODUCTION

Calcium as a nutrient is most commonly associated with the formation and metabolism of bone. Over 99 percent of total body calcium is found as calcium hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$) in bones and teeth, where it provides hard tissue with its strength. Calcium in the circulatory system, extracellular fluid, muscle, and other tissues is critical for mediating vascular contraction and vasodilatation, muscle function, nerve transmission, intracellular signaling, and hormonal secretion. Bone tissue serves as a reservoir for and source of calcium for these critical metabolic needs through the process of bone remodeling.

Calcium metabolism is regulated in large part by the parathyroid hormone (PTH)–vitamin D endocrine system, which is characterized by a series of homeostatic feedback loops. The rapid release of mineral from the bone is essential to maintain adequate levels of ionized calcium in serum. During vitamin D deficiency states, bone metabolism is significantly affected as a result of reduced active calcium absorption. This leads to increased PTH secretion as the calcium sensing receptor in the parathyroid gland senses changes in circulating ionic calcium. Increased PTH levels induce enzyme activity (1α-hydroxylase) in the kidney, which converts vitamin D to its active hormonal form, calcitriol. In turn, calcitriol stimulates enhanced calcium absorption from the gut. Not surprisingly, the interplay between the dynamics of calcium and vitamin D often complicates the interpretation of data relative to calcium requirements, deficiency states, and excess intake.

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SOURCES OF CALCIUM

Ingested calcium comes from food sources and dietary supplements. In this report dietary calcium refers to both food sources and supplements combined (although some researchers reserve the term dietary calcium to mean only food sources) and is most often referred to as total calcium intake for clarity. With more than one-half of the U.S. population (Bailey et al., 2010)—and between 24 and 60 percent of Canadians (2004 Canadian Community Health Survey, personal communication, D. Brulé, Health Canada, April 29, 2010)—reporting use of dietary supplements of some type, dietary supplements must be taken into account when considering the sources of calcium in the diet and, in turn, estimating total calcium intake. Current estimates from 2003 to 2006 indicate that the median total intake of calcium from all sources for persons > 1 year of age ranges from 918 to 1,296 mg/day, depending upon life stage (Bailey et al., 2010). Only small amounts of calcium are contributed by water, depending upon geographic location. Chapter 7 of this report contains an assessment of quantitative calcium intake in the U.S. and Canadian populations.

Food

Calcium is classically associated with dairy products; milk, yogurt, and cheese are rich sources of calcium, providing the major share of calcium from foods in the general diet in the United States and Canada. In the United States, an estimated 72 percent of calcium comes from milk, cheese and yogurt and from foods to which dairy products have been added (e.g., pizza, lasagna, dairy desserts). The remaining calcium comes from vegetables (7 percent); grains (5 percent); legumes (4 percent); fruit (3 percent); meat, poultry, and fish (3 percent); eggs (2 percent); and miscellaneous foods (3 percent).[1] Similar data from Canada are not currently available.

Fortification with calcium for a number of foods that do not naturally contribute calcium—such as orange juice, other beverages, and ready-to-eat cereals—is becoming commonplace in the United States (Calvo et al., 2004; Rafferty et al., 2007; Poliquin et al., 2009). These practices challenge the ability of national food composition databases, such as those maintained by U.S. Department of Agriculture (USDA), to keep abreast of these newer products and may result in some underestimation of actual calcium intake from food sources. However, for those persons who choose such foods, total calcium intake is increased.

[1]U.S. Department of Agriculture/Economic Research Service Nutrient Availability Data (2009). Available online at http://www.ers.usda.gov/Data/FoodConsumption/NutrientAvailIndex.htm. Accessed October 19, 2010.

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Dietary Supplements

Among the U.S. population, about 43 percent of all persons—but almost 70 percent of older women—reported calcium intake from supplements, based on a national survey conducted between 2003 and 2006 (Bailey et al., 2010). When calcium from supplement use is taken into account based on these survey data, the average intake increases by about 7 percent for males and 14 percent for females. However, this is not a meaningful snapshot of the effect of supplement use, because non-users of supplements are averaged with users, meaning that the effect is much more skewed than can be reflected by a mean estimate. Similar data are not available for Canada, but the frequency of use data show that 48 to 82 percent of Canadians reported taking a calcium supplement within the previous 30 days (2004 Canadian Community Health Survey, personal communication, D. Brulé, Health Canada, April 29, 2010).

The most common forms of supplemental calcium are calcium carbonate and calcium citrate.[2] The bioavailability of the calcium in these forms is discussed below in the section titled "Other Factors Related to Calcium Nutriture." Generally fewer tablets of calcium carbonate are required to achieve given dose of elemental calcium because calcium carbonate generally provides 40 percent elemental calcium, compared with 21 percent for calcium citrate. Thus, costs tend to be lower with calcium carbonate (Heaney et al., 2001; Keller et al., 2002) than with calcium citrate, and compliance may be higher among patients who do not want to take (or have difficulty swallowing) multiple pills. Chewable calcium carbonate supplements are also available. However, compared with calcium citrate, calcium carbonate is more often associated with gastrointestinal side effects, including constipation, flatulence, and bloating (Straub, 2007). Calcium citrate is less dependent than calcium carbonate on stomach acid for absorption (Hunt and Johnson, 1983; Recker, 1985; Straub, 2007) and thus can be taken without food. It is useful for individuals with achlorhydria, inflammatory bowel disease, or absorption disorders or who are taking histamine-2 receptor blockers or proton pump inhibitors; for residents of long-term care facilities where calcium supplements are not given with meals; and for others whose schedules preclude taking supplements with food (Bo-Linn et al., 1984; Carr and Shangraw, 1987; Straub, 2007). Calcium can compete or interfere with the absorption of iron, zinc, and magnesium. For this reason, persons with known deficiencies of these other minerals who require calcium supplementation usually take calcium supplements between meals (Straub, 2007).

[2]Other forms of calcium dietary supplements include lactate, gluconate, glucoheptonate, and hydroxyapatite; their relevance for life stage groups may vary.

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METABOLISM OF CALCIUM

Absorption

Calcium is absorbed by active transport (transcellularly) and by passive diffusion (paracellularly) across the intestinal mucosa. Active transport of calcium is dependent on the action of calcitriol and the intestinal vitamin D receptor (VDR). This transcellular mechanism is activated by calcitriol and accounts for most of the absorption of calcium at low and moderate intake levels. Transcellular transport occurs primarily in the duodenum where the VDR is expressed in the highest concentration, and is dependent on up-regulation of the responsive genes including the calcium transport protein called transient receptor potential cation channel, vanilloid family member 6 or TRPV6 (Li et al., 1993; Xue and Fleet, 2009). These features—up-regulation of VDR and TRPV6—are most obvious during states in which a high efficiency of calcium absorption is required.

Passive diffusion or paracellular uptake involves the movement of calcium between mucosal cells and is dependent on luminal:serosal electrochemical gradients. Passive diffusion occurs more readily during higher calcium intakes (i.e., when luminal concentrations are high) and can occur throughout the length of the intestine (Ireland and Fordtran, 1973). However, the permeability of each intestinal segment determines passive diffusion rates. The highest diffusion of calcium occurs in the duodenum, jejunum, and ileum (Weaver and Heaney, 2006b).

From a recent series of controlled metabolic studies undertaken by the USDA, mean calcium absorption (also referred to as "fractional calcium absorption," which is the percentage of a given dose of calcium that is absorbed) in men and non-pregnant women—across a wide age range—has been demonstrated to be approximately 25 percent of calcium intake (Hunt and Johnson, 2007). Mean urinary loss averages 22 percent and fecal loss 75 percent of total calcium intake, with minor losses from sweat, skin, hair, etc. In general, mean calcium absorption and calcium intake are directly related (Heaney et al., 1975; Gallagher et al., 1980; Hunt and Johnson, 2007). However, fractional calcium absorption varies inversely with calcium intake when the intake is very low (Malm, 1958; Spencer et al., 1969; Ireland and Fordtran, 1973). For example, when calcium intake was lowered from 2,000 to 300 mg, healthy women increased their fractional whole body retention of ingested calcium, an index of calcium absorption, from 27 percent to about 37 percent (Dawson-Hughes et al., 1993). This type of adaptation occurs within 1 to 2 weeks and is accompanied by a decline in serum calcium concentration and a rise in serum PTH and calcitriol concentrations (see section below titled "Homeostatic Regulation of Calcium"). The fraction of calcium absorbed rises adaptively as intake

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is lowered. However, this rise is not sufficient to offset the loss in absorbed calcium that occurs as a result of the lower intake of calcium—however modest that decrease may be—and thus net calcium absorption is reduced.

Fractional calcium absorption varies during critical periods of life. In infancy, it is high at approximately 60 percent, although the range is large (Fomon and Nelson, 1993; Abrams et al., 1997). Calcium absorption in newborns is largely passive and facilitated by the lactose content of breast milk (Kocian et al., 1973; Kobayashi et al., 1975). As the neonate ages, passive absorption declines and calcitriol-stimulated active intestinal calcium absorption becomes more important (Ghishan et al., 1980; Halloran and DeLuca, 1980; Ghishan et al., 1984).

A recent preliminary report on breast-fed infants in the first 2 months of life (Hicks et al., 2010) reported calcium absorption of approximately 33.7 ± 2.0 mg/100 kcal. In an earlier study using stable isotopes (Abrams et al., 1997), calcium absorption was measured in 14 breast milk–fed infants who were 5 through 7 months of age at the time of the study. Mean absorption was 61 ± 23 percent of intake when approximately 80 percent of the calcium intake was from human milk (IOM, 1997). There was no significant relationship between calcium intake from solid foods and the fractional calcium absorption from human milk. This finding suggests that calcium from solid foods does not negatively affect the bioavailability of calcium from human milk (IOM, 1997). Using measured urinary calcium and estimates of endogenous excretion, net retention of calcium was calculated to be 68 ± 38 mg/day for those infants. Abrams (2010) concluded that in infancy, based on calcium intakes that vary from as low as 200 mg/day in exclusively breast-fed infants in the early months of life to 900 mg/day in older formula-fed infants receiving some solids, calcium absorption depends primarily on the level of intake. The author reported that the absorption fraction can range from somewhat above 60 percent with lower intakes to about 30 percent with higher intakes. As the infant transitions into childhood, fractional calcium absorption declines, only to rise again in early puberty, a time when modeling of the skeleton is maximal. Abrams and Stuff (1994) found fractional absorption in white girls with a mean calcium intake of about 931 mg/day to average 28 percent before puberty, 34 percent during early puberty (the age of the growth spurt), and 25 percent 2 years after early puberty. Fractional absorption remains about 25 percent in young adults. In 155 healthy men and women between 20 and 75 years of age, mean calcium absorption was 24.9 ± 12.4 percent of total intake (Hunt and Johnson, 2007). During pregnancy, calcium absorption doubles (Kovacs and Kronenberg, 1997; Kovacs, 2001). Metabolic status also influences calcium absorption such that severe obesity is associated with higher calcium absorption and dieting reduces the fractional calcium absorption by 5 percent (Cifuentes et al., 2002; Riedt et al., 2006).

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With aging and after menopause, fractional calcium absorption has been reported to decline on average by 0.21 percent per year after 40 years of age (Heaney et al., 1989). Nordin et al. (2004) and Aloia et al. (2010) also reported decreased absorption with age. There are early reports of an inverse correlation between age and calcium absorption in women (Avioli et al., 1965), and several studies have indicated that despite an increase in circulating levels of calcitriol in older women, which would be anticipated to increase calcium uptake, fractional calcium absorption was unaffected (Bullamore et al., 1970; Alevizaki et al., 1973; Gallagher et al., 1979; Tsai et al., 1984; Eastell et al., 1991; Ebeling et al., 1992). Thus, although calcium absorption (active calcium transport) has been reported to decrease with age, it is challenging to take this factor into consideration given that calcium intake must be very high to have a significant effect on calcium uptake via the passive absorption.

Homeostatic Regulation of Calcium

Maintaining the level of circulating ionized calcium within a narrow physiological range is critical for the body to function normally, and control of serum calcium levels is maintained through an endocrine system—a system of glands that secrete hormones and is characterized by controlling factors and feedback mechanisms—that includes a major role for vitamin D metabolites, principally calcitriol, and PTH. Calcium balance within the body is closely linked to the hormonal actions of calcitriol. The vitamin D-related endocrine system that maintains serum calcium levels is discussed in Chapter 3 but is also summarized below and illustrated in Figure 2-1.

The vitamin D metabolic system forms the basis of the calcium homeostatic mechanism in mammals. Total calcium concentration in serum is tightly regulated to remain between 8.5 and 10.5 mg/dL (2.12 and 2.62 mmol/L). If this level deviates slightly, the calcium sensing receptor of the parathyroid gland signals the secretion of PTH, which functions as a calcium sensor. PTH then stimulates the kidney to produce calcitriol, the hormonal form of vitamin D, as well as to activate bone resorption, which will increase extracellular calcium levels. Calcitriol acts in an endocrine manner on the intestine, bone, and kidney to raise serum calcium levels; it also acts on the intestine and, to some extent, the kidneys to raise serum phosphorus levels. As the serum calcium level rises, the feedback mechanism causes the calcium sensing receptor to be turned off and PTH secretion to drop. If there is an overshoot in serum calcium levels, the "C" cells (parafollicular) cells of the thyroid gland secrete calcitonin, which can block bone calcium resorption, helping to keep serum calcium levels in the normal range. Calcitriol, through its receptor, also provides feedback relative to suppressing the production and release of PTH, commonly referred

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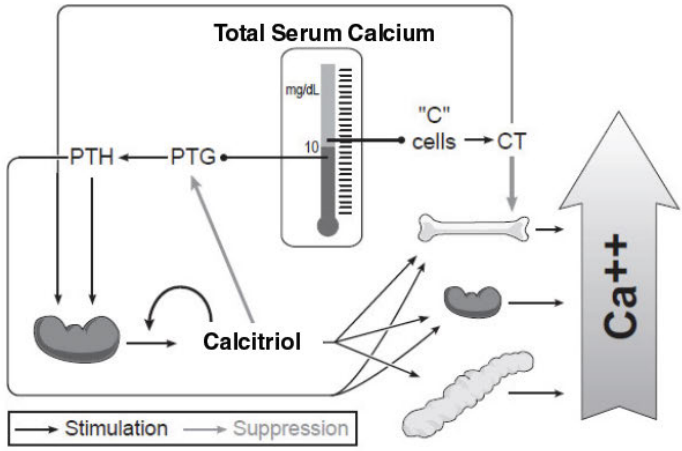


FIGURE 2-1 Endocrine feedback system that maintains serum calcium levels: Involvement of vitamin D and parathyroid hormone (PTH).
NOTE: CT = calcitonin; PTG = parathyroid gland.
SOURCE: Reprinted with permission from Hector DeLuca.

to as PTH suppression. Not shown in the figure is that calcitriol is also directly controlled by the serum phosphorus level; a high serum phosphorus level suppresses the formation of calcitriol, whereas a low level stimulates it.

Excretion

Calcium leaves the body mainly in urine and feces, but also in other body tissues and fluids, such as sweat. Calcium excretion in the urine is a function of the balance between the calcium load filtered by the kidneys and the efficiency of reabsorption from the renal tubules. Nearly 98 percent of filtered calcium (i.e., glomerular filtrate) is reabsorbed by either passive or active processes occurring at four sites in the kidney, each contributing to maintaining neutral calcium balance. Seventy percent of the filtered calcium is reabsorbed passively in the proximal tubule. Active calcium transport is regulated by the calcium sensing receptor located in the ascending loop of Henle, where, in response to high calcium levels in the extracellular fluid, active reabsorption in the loop is blocked through actions of the calcium sensing receptor. In contrast, when the filtered calcium load is low, the calcium sensing receptor is activated, and a greater fraction of the filtered calcium is reabsorbed. In the distal tubule, the ion chan-

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nels known as transient receptor potential cation channel, vanilloid family member 5 or TRPV5 control active calcium transport and this process is regulated by calcitriol and estradiol (Hoenderop et al., 2000). Finally, the collecting duct also can participate in passive calcium transport, although the relative percentage of total calcium reabsorption in the collecting duct is low. Overall, a typical daily calcium loss for a healthy adult man or woman via renal excretion is 5 mmol/day (Weaver and Heaney, 2006a).

Calcium is excreted through the feces as unabsorbed intestinal calcium and is shed in mucosal cells and secretions including saliva, gastric juices, pancreatic juice, and bile. Endogenous fecal calcium losses are approximately 2.1 mg/kg per day in adults and about 1.4 mg/kg per day in children (Abrams et al., 1991). These intestinal losses as well as minor losses in sweat are referred to collectively as endogenous calcium excretion. Endogenous calcium excretion, in contrast to urinary excretion, does not change appreciably with aging (Heaney and Recker, 1994).

PTH can be a major determinant of urinary calcium excretion; during states of low calcium intake, secondary increases in PTH levels result in reduced urinary calcium excretion. Impaired renal function due to aging paradoxically reduces calcium loss due to impaired filtration, but there is also a secondary increase in PTH levels due to reduced phosphate clearance. However, renal 1α-hydroxylase activity declines with impaired renal function, so the net result is calcium loss from the kidney, but also reduced active transport of calcium from the intestine.

Excess Intake

Although excess intake of calcium is almost never due to calcium intake from foods, the use of calcium supplements (including the voluntary fortification of a range of foods that are not naturally sources of calcium) has increased (Ricci et al., 1998; Riedt et al., 2005), and excess calcium intake may occur as a result of high intake from calcium supplements. Excess calcium intake can result in adverse effects. Calcium plays a major role in the metabolism of virtually every cell in the body and interacts with a large number of other nutrients, and as a result, disturbances of calcium metabolism may give rise to a variety of adverse effects (IOM, 1997). A review of the considerations related to adverse effects from excess calcium ingestion can be found in Chapter 6, which focuses on the establishment of Tolerable Upper Intake Levels (ULs).

FUNCTIONS AND PHYSIOLOGICAL ACTIONS OF CALCIUM

Calcium is an integral component of the skeleton, and the skeleton provides a reservoir of calcium for other essential calcium-dependent functions throughout the body. The skeleton serves at least three main func-

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tions. First, calcium, as part of the mineral hydroxyapatite, deposited into the organic matrix of the skeleton, is critical for its structure and is necessary for tissue rigidity, strength, and elasticity. This function allows for normal movement and exercise. Second, the skeleton functions as a source of minerals and alkali and therefore is critical for overall mineral homeostasis. The skeleton is the principal depot for calcium, containing 98 percent of total body calcium. It can be called on repeatedly, through the processes of bone formation and resorption (referred to as remodeling, as discussed below), to maintain circulating levels of calcium at a constant level. While the same qualitative processes apply to skeletal calcium metabolism across the life cycle, there are quantitative differences by age and hormonal status. These life cycle differences for skeletal growth and remodeling are discussed in a section below. Excessive calcium resorption can compromise the integrity and strength of the skeletal tissues. Third, the marrow cavity of bone serves as a major site for the development of hematopoietic cells and as a major compartment of the immune system. Several of the cell types involved in bone remodeling originate in the bone marrow compartment. Stromal or connective tissue cells are found in the bone marrow; at one time, these were thought to be inert, but they are now considered multi-potent stem cells that can become either fat or bone cells under the influence of specific differentiation factors (Muruganandan et al., 2009).

A principal physiological function of calcium apart from its role in maintaining the skeleton, is as an essential intracellular messenger in cells and tissues throughout the body. Although this pool of calcium is quantitatively small, the ionized calcium present in the circulatory system, extracellular fluid, muscle, and other tissues, is critical for mediating vascular contraction and vasodilatation, muscle function, nerve transmission, and hormonal secretion. Ionized calcium is the most common signal transduction element in biology, owing to its ability to reversibly bind to proteins and to complex with anions such as citrate and bicarbonate (Weaver and Heaney, 2006b).

Bone Formation and Remodeling

Bone is composed of a mineral compartment, predominantly calcium hydroxyapatite and an organic matrix, osteoid, composed principally of collagen and non-collagenous proteins and growth factors. The relative contributions of the mineralized and organic compartments depend on the age of the individual; in general, 50 to 70 percent of bone is mineral, 20 to 40 percent is organic matrix, and the rest is water and lipid. The organic matrix is critical for both the structural and functional components of the skeleton, providing elasticity and contributing to regenerative and remodeling properties. Much of the organic matrix is composed of type I collagen fibrils that are organized in such a manner that strength and

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elasticity are combined. Numerous non-collagenous proteins are also present in the organic matrix. Some of them, such as osteocalcin and matrix GLA protein, contain γ-carboxyglutamate, an amino acid with high affinity for calcium that is required for proper mineralization of the matrix (see below). The role of phosphate in bone development should not be overlooked. As described below, first phosphorus is laid down during the mineralization process, and then calcium binds to it. Calcitriol stimulates the uptake of both calcium and phosphorus from the intestine.

Development

The skeleton develops through a process of either intramembranous or endochondral bone formation, depending on location and function. Intramembranous bone formation is the predominant process in the skull, whereas endochondral bone formation occurs in long bone and the axial skeleton. Intramembranous bone is formed by direct differentiation of mesenchymal precursors into osteoblasts, cells of the fibroblast–stromal lineage that produce bone matrix proteins and synthesize a lattice for subsequent mineralization. In contrast, during endochondral bone formation chondrocytic differentiation occurs first, leading to a soft cartilaginous infrastructure. The cartilage then becomes calcified, and the provisional calcified cartilage is subsequently replaced by bone. This occurs by vascular invasion, which allows entry of hematopoietic precursors and osteoclasts, macrophage-like cells that originate from the monocyte–macrophage lineage, which remove apoptotic chondrocytes and cartilage (Provot and Schipani, 2007). New bone is formed by osteoblasts. Osteoblastogenesis follows chondrogenesis after release of growth factors from terminally differentiated chondrocytes. The first bone formed is woven and relatively unorganized. However, through osteoclastic modeling that bone is replaced by lamellar bone, which is highly organized and provides the strength necessary to support soft tissue (Yang and Yang, 2008).

Endochondral bone formation allows for linear development of the growth plate as well as periosteal expansion, which ultimately results in a longer and thicker bone. Mineralization is the final stage in terminal differentiation of the osteoblast and occurs through a complex process whereby ion deposition is followed by crystal formation between the collagen fibrils. This occurs because of undersaturation of calcium hydroxyapatite in the extracellular fluid and the binding of calcium to non-collagenous proteins in the matrix (Favus, 2008). Initially, phosphate drives the mineralization by being laid down in bone as hydroxyapatite; the negative charge of hydroxyapatite then causes calcium to avidly bind to it. In states of phosphorus deficiency, unmineralized osteoid persists despite adequate calcium intake. Bone mechanical properties are then influenced by the distribu-

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BOX 2-1
Bone Remodeling Terms and Definitions

- Cortical bone: One of two types of bone; makes up the outer part of all skeletal structures (nearly 80 percent of the skeleton); is dense and compact with a slow turnover rate and is highly resistant to bending and torsion.
- Trabecular bone: Second of the two bone types; found inside of long bones, vertebrae, pelvis, and other large flat bones; is less dense than cortical bone and has a higher turnover rate.
- Osteoblast: A type of bone cell that is responsible for the production of bone and bone formation.
- Osteoclast: A type of bone cell that resorbs bone using acid and enzymes.
- Bone remodeling: Process that occurs throughout the lifetime that results from the pairing action of osteoclasts (breaking down) and osteoblasts (building up), which replaces damaged bone with new material.
- Bone modeling: A similar process to remodeling, except that new bone is formed at a location different from the site of resorption, such as during times of growth.

SOURCE: Hadjidakis and Androulakis, 2006.

tion, size, and density of the apatite crystals. Too much or too little mineral can lead to impaired bone strength; the former makes the bone too brittle, whereas the latter makes the bone too ductile and weak.

Remodeling

Calcium balance is preserved within the non-bone tissues of the body, because adult bone constantly undergoes remodeling through bone resorption, mainly by osteoclasts and bone formation mainly by osteoblasts.[3] Terminology associated with remodeling is shown in Box 2-1. In adults, virtually all of the human skeleton is remodeled over a 10-year cycle, although trabecular bone turns over more readily. In contrast, bone formation incorporates calcium into the matrix, and this process requires significant time

[3]Not all calcium enters the skeleton through bone formation or leaves the skeleton through bone resorption, as discussed by Parfitt (2003). Moreover, during lactation and in response to other acute demands for calcium, osteocytes have been shown to resorb the matrix surrounding them and then to restore it after the stress is over (Teti and Zallone, 2009).

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and energy. Overall calcium balance is maintained at the skeletal level by opposing actions of bone cells. Skeletal remodeling occurs in microscopic elements of bone referred to as remodeling units or basic multicellular units, which contain the osteoblasts (bone-resorbing cells) and osteoclasts (bone-forming cells). Old osteoblasts then become osteocytes entombed within the bone matrix after mineralization.

The axial and appendicular skeletons are composed of both cortical and trabecular bone. The hard outer shell of bone is cortical, which is remodeled less frequently, but is important for strength and periosteal expansion during puberty and with aging. The trabecular compartment is bathed by bone marrow and is remodeled much more frequently, which is in part due to a much greater surface area and the existence of marrow elements that are in close proximity to the endosteal surface of bone (which contribute progenitor cells for eventual remodeling).

Physical activity—or more specifically mechanical loading—is a critical component of skeletal homeostasis. It is thought that osteocytes in cortical bone sense changes in gravitational forces and elaborate growth factors that initiate remodeling. Unloading of the skeleton in cases such as bed rest or weightlessness (space travel) is associated with a profound uncoupling of remodeling, such that bone resorption is dramatically increased, whereas bone formation is suppressed. These changes cause rapid bone loss and are a major problem for long-term spaceflight. Loading of the skeleton by mechanical means (e.g., weight-bearing exercise such as running, walking, or jumping) can promote bone formation, particularly in early childhood and adolescence, although it has benefits later in life as well.

Concept of Normal, Healthy Bone Accretion

Bone is a dynamic tissue; it is metabolically active, responding to both genetically determined and environmental stimuli that ultimately determine its composition and structural integrity. Bone modeling describes events that occur primarily during growth resulting in increased bone size and modification of its shape in response to genetic determinants and mechanical loading. Bone remodeling occurs in response to stimulation by surface-dependent factors initiated by damage or mechanical loading. It includes bone resorption and deposition but does not alter the size or shape of bone (reviewed in Seeman, 2009).

Although the role of genetic and environmental factors in bone modeling and remodeling has long been debated in the literature, genetics remains the chief determinant of bone mass, which, in turn, is the determinant of bone strength (Krall and Dawson-Hughes, 1993; Jouanny et al., 1995; Jones and Nguyen, 2000; Sigurdsson et al., 2008; Perez-Lopez et al., 2010). Given uncertainties in understanding the cumulative impact of

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genetic and environmental influences on bone mass across life stages, the question of whether attainment of maximal, optimal, or "peak" bone mass can be achieved on a lasting basis through dietary manipulation and/or use of supplements has not been completely resolved.

A 2-year longitudinal multiethnic study (Abrams et al., 2000) of changes in calcium absorption, bone accretion, and markers of bone growth in pre-pubertal girls (7 to 8 years of age) maintained on a calcium intake of 1,200 mg/day found a significant increase in calcium use associated with pubertal development. The increase paralleled markers of bone formation; supporting the hypothesis that calcium intake during the early to late pubertal stage influences peak rates of calcium gain in bone during pubertal development. An earlier study followed pre-pubertal males and females (mean age 8.5 years) for 18 months after calcium supplementation and also found gains in bone mineral content (BMC) and bone area of the lumbar spine; however, the increases in bone accretion disappeared after supplements were withdrawn (Lee et al., 1996). In a longer-term randomized clinical trial, Matkovic et al. (2005) evaluated the effects of calcium supplementation on bone accretion in the transition from childhood into early adulthood. This study found significant increases in bone accretion for total bone density, distal and proximal radius, and metacarpal indexes after 4 years of supplementation; by 7 years, however, only the proximal radius and metacarpal indexes still showed significantly increased bone accretion over non-supplemented controls.

These findings corroborate a role for calcium intake and skeletal size; however, they also suggest that bone accretion diminishes during skeletal consolidation in late adolescence, and attainment of a peak bone mass was transient for some skeletal sites, even though the study subjects continued calcium supplementation through year 7. When considered together, these studies support an increase in skeletal size and mineralization that occurs with calcium supplementation, but fail to show consistently that BMC is retained over the long term, particularly after supplementation is withdrawn.

Effect of Menopause

Studies of bone histomorphometry (Recker et al., 2004) and markers of bone remodeling (Uebelhart et al., 1990) indicate that bone remodeling is accelerated in the perimenopausal and postmenopausal periods. The span of 5 to 10 years surrounding menopause is characterized by a decrease in estrogen production and an increase in resorption of calcium from bone (Stevenson et al., 1981; Riggs, 2002; Masse et al., 2005; Finkelstein et al., 2008), resulting in a marked decrease in bone density. For example, Ebeling et al. (1996) measured changes in markers of bone mineral density (BMD) in a cohort of 281 women who were 45–57 years of age, and found

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the BMD in lumbar spine and femoral neck was decreased by 20 percent in perimenopausal and postmenopausal women compared with premenopausal women. The bone loss is most rapid in the early years of menopause, and then approximately 6 to 7 years postmenopause the loss continues at a slower rate (Pouilles et al., 1995)

The bone loss associated with menopause results from uncoupling in the bone remodeling units, such that resorption of bone is greater than formation of new bone. Over time, such changes lead to skeletal fragility and decreased bone mass. Some cohort studies demonstrate that accelerated bone loss is an independent risk factor for fracture, such that the combination of low bone mass and high rates of bone turnover markedly increase the potential for a future fracture (Garnero et al., 1996). Bone remodeling in postmenopausal osteoporosis includes changes in osteoid thickness, surface area, and volume. Parfitt et al. (1995) determined that defective osteoblast recruitment in women with osteoporosis resulted in decreased osteoid thickness, a characteristic of osteoporosis.

Considerable variability exists among women regarding the effects of menopause on bone loss, and such effects vary according to body mass index and ethnicity (Finkelstein et al., 2008). The effect of estrogen/progesterone treatment on preventing bone loss and reducing fracture risk is well established. However, the use of such therapy has declined as a result of recent reports of adverse non-skeletal effects. Because rapid bone loss occurs after estrogen treatment is discontinued (Gallagher et al., 2002), the potential impact on subsequent fracture rates is of interest but remains unclear.

Skeletal Disorders

Rickets and Osteomalacia

Rickets is the term for the end-stage condition in infants and children that begins with suboptimal bone mineralization at the growth plate and progresses with associated physiological perturbations that include secondary hyperparathyroidism, hypocalcemia, and hypophosphatemia leading to irreversible changes in skeletal structure. The disease is a disorder of the growth apparatus of bone in which growth cartilage fails to mature and mineralize normally. Because the bone is undermineralized it is also soft and ductile, and this leads to bowing of the limbs, widening and compression of the ends of the long bones, etc. The similar condition of osteomalacia (defective mineralization of bone and softening of bone) also occurs, and is seen in adults as well as children. Although these conditions are commonly associated with inadequate vitamin D exposure, each can also result from calcium (or phosphorus) deficiency. Rickets and osteomalacia

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due to a lack of calcium in the diet cannot be corrected by increasing levels of calcitriol (i.e., the active form of vitamin D also referred to as 1,25-dihyroxyvitamin D).

Rickets In rickets, during prolonged deficiency of calcium (and phosphate), the body increases PTH to prevent hypocalcemia by causing osteoclastic absorption of the bone. This, in turn, causes the bone to become progressively weaker, resulting in rapid osteoblastic activity. The osteoblasts produce large amounts of organic bone matrix, osteoid, which does not become calcified (Guyton and Hall, 2001). Consequently, the newly formed, uncalcified osteoid gradually takes the place of other bone that is being reabsorbed. During the later stages of rickets, the serum calcium level falls precipitously, and tetany (neuromuscular spasm) develops. In infants and young children, a long-standing calcium intake deficiency, in association with suboptimal vitamin D exposure, can produce rickets. Indeed, in experimental animals and in humans with extremely low vitamin D levels, genetic absence of calcitriol (vitamin D–dependent rickets [VDDR] type I), or genetic absence of the vitamin D receptor (VDDR type II), the use of increased calcium supplementation or calcium infusions will prevent and treat rickets. These observations indicate that the primary cause of rickets is inadequate delivery of calcium to the bone surface, not a defect in osteoblast function. In other words, the primary role for vitamin D and calcitriol in regulating skeletal homeostasis is indirectly accomplished by stimulating the intestinal absorption of calcium and phosphorus.

The clinical symptoms of rickets include stunted growth and bowing of the extremities. A serum 25-hydroxyvitamin D (25OHD) level of less than 27 to 30 nmol/L is not diagnostic of the disease but is associated with an increased risk for developing rickets (Specker et al., 1992).

Osteomalacia In osteomalacia, as seen in adults, the newly deposited bone matrix fails to mineralize adequately. Poor calcium intake is associated with secondary increases in PTH in an attempt to compensate for low serum calcium levels. The secondary hyperparathyroidism of calcium deficiency states is associated with increased bone resorption and suppression of bone formation. As a result, older adults who have calcium-poor diets and very low vitamin D levels may develop not only osteoporosis, as described below (i.e., a reduction in bone mass), but also osteomalacia (a reduction in mineral within the bone matrix). Osteomalacia is actually the clinical syndrome of undermineralization of bone associated with muscle weakness, bone pain, and fractures. The characteristic histological feature of osteomalacia is unmineralized matrix, which is often represented experimentally as the ratio of osteoid volume to bone volume. Ultimately, reductions in mineralization lead to impaired bone strength and signifi-

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cant softening of the skeleton. The calcium levels in the blood of patients with osteomalacia are often normal despite the undermineralization of bone, underscoring the importance of maintaining the blood calcium level over maintaining the mineralization of the skeleton. However, serum phosphorus levels are frequently low, PTH concentrations are 5 to 10 times the normal levels, and there is an increased level of alkaline phosphatase together with increased markers of bone turnover. Bone scans often indicate dramatically increased skeletal uptake by resident osteoblasts. As recognized by Parfitt et al. (1995) and illustrated by the histological classification scheme used for osteomalacia, what clinicians generally recognize as osteomalacia is the end-stage results from a prolonged severe deficiency of calcium and/or vitamin D. During the earliest stages (preosteomalacia), there exists a calcium-deficient state, even though the osteoid thickness, mineralization lag time, and osteoid volume are still normal. Subsequently, more dramatic changes occur including a greater increase in osteoid thickness, and impaired mineralization.

Osteomalacia is estimated to be present in about 4 to 5 percent of general medical and geriatric patients (Anderson, 1961; Stacey and Daly, 1989; Campbell et al., 1994). However, the clinical syndrome of bone pain, muscle weakness, and impaired bone mineralization is much less frequently recognized. In the face of severe osteoporosis, the diagnosis of osteomalacia can only be made by bone biopsy, usually using the method of double tetracycline labeling, demonstrating impaired mineralization of the skeleton (Villareal et al., 1991; Chapuy et al., 1992; Komar et al., 1993). In fact, osteomalacia is noted histologically in the bones of 20 to 40 percent of first-time hip fracture patients (Jenkins et al., 1973; Aaron et al., 1974; Sokoloff, 1978). These results suggest that these individuals may be presenting with a mixture of osteoporosis and osteomalacia. This clinical scenario can be related to both nutrient insufficiency and the coincidental progression of age-related bone loss.

Osteoporosis and Fractures

Osteoporosis is a skeletal disorder associated with aging and characterized by compromised bone strength due to reduced bone mass and reduced bone quality. Reduced bone mass—as measured by low BMD—increases bone fragility and, in turn, predisposes a person to an increased risk of fracture, notably at the vertebrae, hip, and forearm (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, 2001). As shown in Figure 2-2, the relationship between BMD measures and the incidence of fractures is notable. Overall, osteoporosis-related morbidity and mortality, as well as health care costs, are a significant public health concern (NIH Consensus Development Panel on Osteoporosis Pre-

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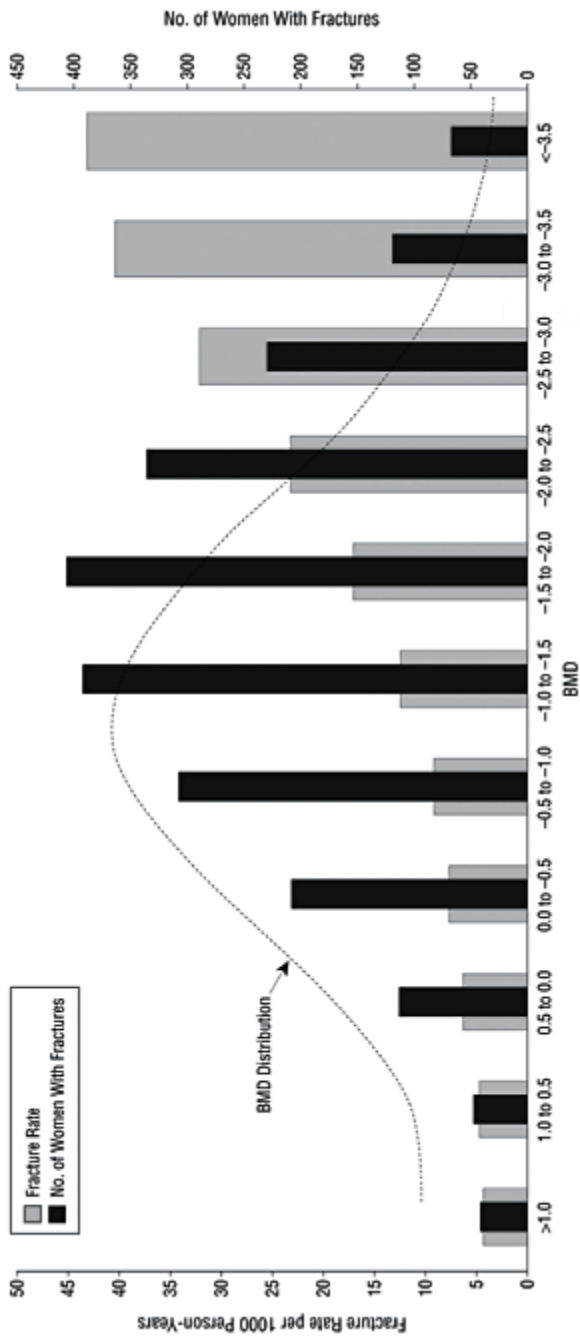


FIGURE 2-2 Bone mineral density (BMD), osteoporotic fracture rate, and number of women with fractures.
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vention, Diagnosis, and Therapy, 2001). Osteoporosis is most commonly associated with women, but the condition also occurs in men.

Menopause can initiate osteoporosis through elevated bone remodeling, which occurs characteristically in postmenopausal women. Remodeling activity, although designed to repair weakened bone, actually makes it temporarily weaker when remodeling is excessive. It can lead to enhanced skeletal fragility (Heaney, 2003). Although it is unclear to what extent calcium intake can mitigate such bone loss, inadequate calcium intake can exacerbate the situation.

Men experience age-related bone loss as well, although not due to menopause. This, in turn, can result in osteoporosis. However, the incidence of fracture risk increases some 5 to 10 years later in men than it does in women (Tuck and Datta, 2007).

CALCIUM ACROSS THE LIFE CYCLE

The body's need for calcium relative to skeletal growth and remodeling varies by life stage. The major physiological activities include bone accretion during skeletal growth and maintenance of bone mass after growth is completed. Later in adult life, net calcium is lost from the body when bone formation no longer keeps up with bone resorption. For all life stages highlighted below, specific studies and conclusions are detailed in Chapter 4.

Infancy

At full-term birth, the human infant has accrued about 26 to 30 g of calcium, most of which is in the skeleton. When calcium transfer from the placenta ceases at birth, the newborn infant is dependent on dietary calcium. Calcium deposition into bone occurs at a proportionately higher rate during the first year of life than during other periods. Breast-fed infants absorb about 55 to 60 percent of the calcium in human milk (Abrams et al., 1997). Formula-fed infants receive more calcium than breast-fed infants because formula contains nearly double the calcium of breast milk. However, fractional calcium absorption is lower in formula-fed infants, averaging about 40 percent among different formula types (Abrams et al., 2002). Studies to establish the level of calcium provided by human milk are long-standing in nature, and little information has emerged to change the conclusions of earlier analyses. Although the composition of milk varies significantly from the start to the end of each feed, the average calcium concentration of milk produced in total for each feeding remains relatively constant over the months of lactation, with an estimated value of 259 ± 59 mg/L at 30 days, followed by a small decrease during the second 6 months. This estimate is based on the average concentrations found in

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several studies from the United States and the United Kingdom, as summarized in Atkinson et al. (1995). Variations in milk calcium content have been found between population groups. For example, in comparison with the above data from the United States, milk calcium concentrations have been found to be lower (by approximately 20 mg/100 mL at 5 months of lactation) in mothers from the Gambia, but this difference appears to be genetic and not due to differences in total intake of calcium (Prentice et al., 1995; IOM, 1997).

Relative to the average amount of milk consumed by infants, there are three key studies based on weighing full-term infants before and after feeding (Butte et al., 1984; Allen et al., 1991; Heinig et al., 1993). While it has been noted that the volume of intake is somewhat lower during the first month of life than in subsequent months (Widdowson, 1965; Southgate et al., 1969; Lonnerdal, 1997) and that a number of factors contribute to variability in intake, an estimate of 780 mL/day is reasonable based on the data from the three test weighing studies. Therefore, given an intake of milk estimated to be 780 mL/day from the infant weighing studies and the average content of 259 ± 59 mg of calcium per liter, the intake of calcium for infants fed exclusively human milk is estimated to be 202 mg/day.

Childhood and Adolescence

Calcium deposition into bone is an ongoing process throughout childhood and into adolescence, reaching maximal accretion during the pubertal growth spurt. Measures of bone density in adolescent girls indicate that about 37 percent of total skeletal bone mass is achieved between pubertal stages 2 (mean age 11 years) and 4 (mean age 15 years), with an average daily calcium accretion rate of 300 to 400 mg/day (Matkovic et al., 1994). For growing children, bone modeling (i.e., formation over resorption) is the predominant skeletal process promoting longitudinal extension of the growth plate and periosteal expansion. Modeling requires mineralization; hence, calcium requirements are increased, particularly during neonatal and pubertal growth spurts. Approximately 40 percent of total skeletal bone mass is acquired within a relatively short window of 3 to 5 years, when gonadal steroids and growth hormone secretion are maximal (Weaver and Heaney, 2006b). During this time, bone formation far outpaces resorption and longitudinal growth, and consolidation of bone occurs. The most recent estimate of average calcium accretion is 92 to 210 mg/day calcium in 9- to 18-year-old boys and girls (Vatanparast et al., 2010), and bone calcium accretion can peak at 300 to 400 mg/day (Bailey et al., 2000).

During this developmental period, calcium absorption is maximal and variation in calcium intake accounts for 12 to 15 percent of the variance in calcium retention for both boys and girls. Increases in total calcium

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transiently enhance bone mass (Lee et al., 1996; Matkovic et al., 2005). These effects disappear during or after cessation of increased calcium intake; final bone mass, measured in randomized trials of calcium supplementation during this period, did not differ between controls and calcium-supplemented individuals (Matkovic et al., 2005). However, this period of bone accretion determines adult bone mass, which, in turn, is a significant predictor of fracture risk late in life.

Young Adults

After puberty and throughout most of adulthood, bone formation and resorption are balanced. During this period, bone mass is consolidated, and calcium requirements are relatively stable. Peak bone mass, the maximum amount of bone that can be accumulated, is reached in early adulthood (Bonjour et al., 1994). The ability to attain peak bone mass is affected by genetic background and by lifestyle factors such as physical activity and total calcium intake. Specific skeletal sites have been found to reach peak bone mass at different ages, and bone mineral accretion has been reported to continue slowly into the third decade of life (Recker et al., 1992). Bone is a dynamic tissue, and a number of clinical studies suggest that increasing bone mass early in life has a transient effect, but does not confer protection against later bone loss and osteoporosis (Gafni and Baron, 2007). The calcium content of bone at maturity is approximately 1,200 g in women and 1,400 g in men (Ilich and Kerstetter, 2000; Anderson, 2001). In men, this level remains relatively constant until the onset of age-related bone loss later in life. In women, the level remains relatively constant until the onset of menopause. Although bone mass generally remains at a plateau during reproductive years, some studies have suggested that mean bone mass gradually reaches a plateau and then declines slowly with age.

Older Adults

Age-related bone loss, in both men and women, results when bone remodeling becomes uncoupled and bone resorption exceeds bone formation. However, the pathogenesis of bone loss is a multi-faceted process. The roles and interactions of various hormonal, genetic, and other factors in bone loss and risk for decreased bone health are not yet clear. Moreover, the ability of increased calcium intake to overcome the effects of bone loss related to menopause or normal aging continues to be debated.

In postmenopausal women, estrogen loss increases the rate of bone remodeling, characterized by an imbalance between osteoclast and osteoblast activity, resulting in irreversible bone loss (Riggs et al., 1998; Seeman, 2003). Estrogen loss can further accelerate bone loss through its effect on

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decreased absorption of calcium and increased urinary loss of calcium (Nordin et al., 2004). Evidence suggests that remodeling in women becomes imbalanced just prior to, during, and immediately after menopause, when the rate of bone loss becomes more rapid. However, the rate of bone loss as a result of menopause varies greatly depending upon a number of factors, including genetics, body composition, other hormonal changes and endogenous production of estradiol.

The effects of lower estrogen levels on calcium balance continue to be debated. However, the principal effect of estrogen deficiency on the skeleton is increased bone resorption. The range of bone loss in the 7 to 10 years around the onset of menopause can range from 3 to 7 percent annually (Kenny and Prestwood, 2000). In women over age 65, the rate of bone loss slows again to 0.5 to 2 percent per year (Greenspan et al., 1994). Later in menopause—and in men over 70 years of age—if reduced calcium intake occurs, it contributes to a secondary form of hyperparathyroidism, which serves as a compensatory mechanism to maintain extracellular calcium balance. This compensation results in accelerated bone resorption, leading to a net loss of bone mass under these conditions.

For men over 65 years of age, the loss of bone is about 1 to 2 percent per year (Orwoll et al., 1990; Hannan et al., 1992). Additionally, reduced glomerular filtration rate is another factor associated with aging that affects renal conservation of calcium in both men and women (Goldschmied et al., 1975) and also leads to secondary hyperparathyroidism, which can cause significant bone loss. This is underscored by patients with renal disease who have renal osteodystrophy, now referred to as chronic kidney disease–mineral disorder (Demer and Tintut, 2010; Peacock, 2010).

Pregnancy and Lactation

Pregnancy

The fetal need for calcium is met by maternal physiological changes, primarily through increased calcium absorption. There is currently debate about whether calcium is also mobilized from maternal skeleton, as discussed in Chapter 4. In any case, calcium is actively transported across the placenta from mother to fetus, an essential activity to mineralizing the fetal skeleton. Calcium accretion in the developing fetus is low until the third trimester of pregnancy when the fetus requires about 200 to 250 mg/day calcium to sustain skeletal growth (Givens and Macy, 1933; Trotter and Hixon, 1974). Intestinal calcium absorption of the mother doubles beginning early in pregnancy—even though there is little calcium transfer to the embryo at this stage (Heaney and Skillman, 1971; Kovacs and Kronenberg, 1997)—and continues through late pregnancy (Kent et al., 1991). Overall,

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relatively few studies have examined the effect of calcium supplementation on either fetal or maternal outcomes.

Maternal serum calcium falls during pregnancy (Pedersen et al., 1984), but this is likely not important from a physiological perspective in that it reflects the fall in serum albumin caused by plasma volume expansion and therefore does not imply calcium deficiency. Reports indicate that the concentration of ionized calcium remains normal during pregnancy (Frolich et al., 1992; Seely et al., 1997).

Pregnant women consuming moderate (800 to 1,000 mg/day [Gertner et al., 1986; Allen et al., 1991]) to high (1,950 mg/day [Cross et al., 1995]) levels of calcium are often hypercalciuric due to increased intestinal calcium absorption (i.e., absorptive hypercalciuria), and as such pregnancy itself can be a risk factor for kidney stones.

Within the developing human fetus, calcium metabolism is regulated differently from that of its mother. Serum calcium, ionized calcium, and phosphorus are raised above the maternal values, while PTH and calcitriol are low. The high calcium and phosphorus as well as the low levels of PTH all contribute to suppression of the renal 1α-hydroxylase and maintenance of low levels of calcitriol.

In adolescents, whose skeleton is still growing, pregnancy could theoretically reduce peak bone mass and increase the long-term risk of osteoporosis. Although most cross-sectional studies comparing BMD in teens early post-partum to never-pregnant teens (reviewed by Kovacs and Kronenberg, 1997) suggest that BMD or bone mass after adolescent pregnancy is not adversely affected, a few smaller associational studies report that adolescent age at first pregnancy is associated with lower BMD in the adult (Sowers et al., 1985, 1992; Fox et al., 1993). Chantry et al. (2004) analyzed data from the Third National Health and Nutrition Examination Survey (NHANES III) on BMD as measured by dual-energy X-ray absorptiometry (DXA) for 819 women ages 20 to 25 years and found that women pregnant as adolescents had the same BMD as nulliparous women and women pregnant as adults.

Lactation

Breast milk calcium content is homeostatically regulated, and maternal calcium intake does not appear to alter the breast milk calcium content (Kalkwarf et al., 1997; Jarjou et al., 2006). Generally, human breast milk will provide two to three times the amount of calcium to the infant during 6 months of lactation as the pregnant woman will have provided to the fetus during the preceding 9 months of pregnancy. To meet the calcium demands of pregnancy, key physiological changes in the female will also occur, but the adaptations differ from those that take place during pregnancy

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(Kovacs and Kronenberg, 1997; Kalkwarf, 1999; Prentice, 2003; Kovacs, 2005, 2008; Kovacs and Kronenberg, 2008). Maternal bone resorption is markedly up-regulated (Specker et al., 1994; Kalkwarf et al., 1997), and it appears that most of the calcium present in milk derives from the maternal skeleton. Maternal BMD can decline 5 to 10 percent during the 2- to 6-month time period of exclusive breastfeeding. However, it normally returns to baseline during the 6 to 12 months post-weaning (Kalkwarf, 1999). Thus, in the long term, a history of lactation does not appear to increase the risk of low BMD or osteoporosis.

The physiological responses appear to be similar for lactating adolescents. In fact, an analysis using NHANES III data compared BMD from DXA measures in 819 women ages 20 to 25 years (Chantry et al., 2004), and found that young women who had breast-fed as adolescents had higher BMD than those who had not breast-fed, even after controlling for obstetrical variables. This suggests that the normal loss of BMD during lactation and the post-lactation recovery occurs in adolescents as well.

BONE MASS MEASURES ASSOCIATED WITH CALCIUM

Several key bone mass measures are commonly used in the context of calcium nutriture and related health outcomes. The accumulation and level of bone mass can be determined using the calcium balance method or, alternatively, the measurement of BMC or BMD based on DXA. The latter method relies on the assumption that about 32 percent of the measured bone mineral is calcium (Ellis et al., 1996; Ma et al., 1999). These methods are described below.

Calcium Balance

Calcium balance (positive, neutral, or negative) is the measure derived by taking the difference between the total intake and the sum of the urinary and endogenous fecal excretion. Balance studies embody a metabolic approach to examining the relationship between calcium intake and calcium retention and are based on the assumption that the body retains the amount of calcium that is needed. As such, measures of calcium balance (or of "calcium retention") can reflect conditions of bone accretion, bone maintenance, or bone loss. Calcium balance analyses involve measuring as precisely as possible the intake and the output of calcium. Output is usually reflected by urine and fecal calcium; sweat calcium is not usually measured, but its inclusion adds to the precision of the estimates. Calcium balance studies are expensive and require considerable subject cooperation owing to the prolonged stays in metabolic wards. Measures of calcium balance have limitations and are generally cross–sectional in nature, and their

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precision is difficult to ascertain. However, if well conducted, they provide valuable information on calcium requirements relative to the typical intake of the population under study. Long-term balance studies for calcium are generally not carried out because of the difficult study protocol. Calcium balance can also be estimated by using stable isotopes to trace the amount of calcium absorbed, usually in infants from a single feeding (Abrams, 2006).

Calcium balance outcomes that are *positive* are indicative of calcium accretion and are sometimes referred to as net calcium retention; *neutral* balance suggests maintenance of bone, and *negative* balance indicates bone loss. The relevance of the calcium balance state varies depending upon developmental stage. Infancy through late adolescence are characterized by positive calcium balance. In female adolescents and adults, even within the normal menstrual cycle, there are measurable fluctuations in calcium balance owing to the effects of fluctuating sex steroid levels and other factors on the basal rates of bone formation and resorption. Later in life, menopause and age-related bone loss lead to a net loss as a result of calcium due to enhanced bone resorption.

In the 1997 IOM report that focused on calcium DRIs (IOM, 1997), metabolic studies of calcium balance were used to obtain data on the relationship between calcium intakes and retention, from which a non-linear regression model was developed; from this was derived an intake of calcium that would be adequate to attain a predetermined *desirable* calcium retention.[4] The approach used in 1997 was a refinement of an earlier approach suggested to determine the point at which additional calcium does not significantly increase calcium retention, called the *plateau intake* (Spencer et al., 1984; Matkovic and Heaney, 1992).

The balance studies included in the 1997 IOM report (IOM, 1997) met

[4]A footnote to the 1997 IOM report (IOM, 1997) explains the decision not to base considerations on maximal calcium retention: The 1997 committee intended to use a recently described statistical model (Jackman et al., 1997) to estimate an intake necessary to support maximal calcium retention and from which to derive an EAR, and did so in the pre-publication of the report. In the original paper by Jackman et al. (1997), an estimate was made of the lowest level of calcium intake that was statistically indistinguishable from 100 percent maximal retention in some individuals. However, the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (DRI Committee) reviewed the approach in the pre-publication of the report and adopted a different interpretation of the data for the purpose of establishing an AI. The 1997 committee was subsequently advised that there were both statistical and biological concerns with the application of the percent maximal retention model (presented in Appendix E of the 1997 IOM report [IOM, 1997]). The final print of the 1997 report retained the statistical model described by Jackman et al. (1997), but applied it to determine, from the same calcium balance data as was used in the pre-publication report, an estimate of the calcium intake that is sufficient to achieve a defined, desirable level of calcium retention specific to the age groups considered.

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criteria that included the following: subjects had a wide range of calcium intakes, as variability in retention increases at higher intakes; the balance studies were initiated at least 7 days after starting the diet in order for subjects to approach a steady state, as observed by Dawson-Hughes et al. (1988); and, where possible, the adult balance studies included were only for subjects who were consuming calcium at their usual intakes, unless otherwise indicated. By selecting studies conducted on such subjects, the 1997 committee concluded that it obviated the concern about whether the *bone remodeling transient* (i.e., the temporary alteration in the balance between bone formation and bone resorption) might introduce bias in the calcium retentions observed (IOM, 1997). Such selection was not possible in studies in children who were randomized to one of two calcium intakes. However, in children, the impact of the bone remodeling transient related to changing intake is overshadowed by their rapid and constantly changing rates of calcium accretion (i.e., their modeling and remodeling rates are not in steady state, even without an intake change).

For the 1997 DRI development (IOM, 1997), the non-linear regression model describing the relationship between calcium intake and retention was solved to obtain a predetermined *desirable* calcium retention that was specific for each age group. According to the report, the major limitation of the data available was that bone mineral accretion during growth had not yet been studied over a wide range of calcium intakes. Overall, the committee expressed concern about the uncertainties in the methods inherent in balance studies.

Specifics about calcium balance studies that relate to DRI development are provided in Chapter 4, but, as background the recent work of Hunt and Johnson (2007) offers some remedy for the uncertainties surrounding the precision of balance studies. Hunt and Johnson (2007) examined data from 155 subjects—men and women between the ages of 20 and 75 years—who took part in 19 feeding studies conducted at one site (Grand Forks Human Nutrition Research Unit) between 1976 and 1995 in a metabolic unit under carefully controlled conditions.

In their overall analysis, the relationship between intake and output was examined by fitting random coefficient models. Rather than model calcium retention compared with calcium intake by using the Jackman et al. (1997) model, as was done in the 1997 DRI report (IOM, 1997), Hunt and Johnson (2007) modeled output rather than retention to avoid confounding in the precision of estimates that would be caused by including intake as a component of the dependent variable. In the Hunt and Johnson (2007) analysis, the data summary did not show non-linearity and therefore did not justify the use of a more complex non-linear model. The authors noted that the coefficients of the 1997 approach appeared to be greatly influenced by data points above the 99th percentile of daily

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calcium intake and pointed out that the data in their model reflected typical calcium intake between the 5th and approximately 95th percentiles for all boys and men 9 or more years of age, and between the approximately 25th and greater than 99th percentiles for all girls and women 9 or more years of age.

Hunt and Johnson (2007) also pointed out that most (but not all) studies with adults that indicate a positive influence of high total calcium in reducing the rate of bone remodeling were confounded by the presence of vitamin D as an experimental co-variable. In their study, the metabolic diets were similar to the estimated median intake of vitamin D by free-living young women. In short, the analysis may provide a reasonable approach for extracting meaningful data from calcium balance studies that are often confounded by multiple dietary factors. At this point, factorial methods should be briefly noted as the determination of calcium requirements has also made use of a factorial approach as noted in the 1997 DRI report (IOM, 1997). The factorial approach allows the estimate of an intake level that achieves the measured levels of calcium accretion/retention. The method combines estimates of losses of calcium via its main routes in apparently healthy individuals and then assumes that these losses represent the degree to which calcium intake, as corrected by estimated absorption, is required to balance these losses. The weakness in this method is that it is unusual for all of the necessary measurements to be obtained within a single study. Therefore, most calculations using the factorial approach are compiled from data in different studies and thus in different subjects; this can introduce considerable variation and confound the outcomes. This approach, as carried out in the 1997 IOM report on DRIs for calcium and vitamin D (IOM, 1997), where the interest was in desirable retention, is illustrated in Table 2-1.

Bone Mineral Content and Bone Mineral Density

BMC is the amount of mineral at a particular skeletal site, such as the femoral neck, lumbar spine, or total body. BMC is correctly a three-dimensional measurement, but when it is commonly measured by DXA, a cross-section of bone is analyzed, and the two-dimensional output is a real BMD (i.e., BMC divided by the area of the scanned region). True measurements of BMC (volumetric BMD) can be determined non-invasively by computed tomography. Throughout this report, the term "BMD" generally means areal BMD unless specified as volumetric BMD. Most importantly, any of these measures are strong predictors of fracture risk (IOM, 1997). Bone density studies can be considered to reflect average intakes of calcium over a long period of time. When available, such data likely provide

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TABLE 2-1 1997 DRI Factorial Approach for Determining Calcium Requirements During Peak Calcium Accretion in White Adolescents

	Number of Observations	Female Calcium Requirements (mg/day)	Number of Observations	Male Calcium Requirements (mg/day)
Peak calcium accretion	507	212[a]	471	282[a]
Urinary losses	28	106[b]	14	127[c]
Endogenous fecal calcium	14	112[d]	3	108[e]
Sweat Losses		55[f]		55[f]
Total		485		572
Total adjusted for absorption[g]		1,276		1,505

[a]Martin et al. (1997) using peak BMC velocity.

[b]Greger et al. (1978); Weaver et al. (1995).

[c]Matkovic (1991).

[d]Wastney et al. (1996) for mean age 13 years on calcium intakes of 1,330 mg/day.

[e]Abrams et al. (1992).

[f]Taken from Peacock (1991) who adjusted the adult data of Charles et al. (1983) for body weight.

[g]Absorption is 38% for mean age 13 years on calcium intakes of 1,330 mg/day (Wastney et al., 1996).

a better snapshot of long-term calcium intake than does the combination of accretion/retention data.

In children, change in BMC is a useful indicator of calcium retention; change in BMD is less suitable, because it overestimates mineral content as a result of changes in skeletal size from growth (IOM, 1997). In adults, with their generally stable skeletal size, changes in either BMD or BMC are useful measures. In the context of longitudinal calcium intervention trials that measure change in BMC, the measures can provide data on the long-term impact of calcium intake not only on the total skeleton, but also on skeletal sites that are subject to osteoporotic fracture (IOM, 1997). However, because DXA does not distinguish between calcium that is within bone and calcium on the surface (e.g., osteophytes, calcifications in other tissues) or within blood vessels (e.g., calcified aorta), an increase in BMC or BMD, particularly in the spine, may result in false positive readings suggesting high bone mass (Banks et al., 1994).

In DXA, fan beam dual-energy X-ray beams are used to measure bone mass, with correction for overlying soft tissue. Data are converted to BMC and the area represented is measured. The BMD measurement is annotated in grams of mineral per square centimeter. BMC represents the amount of mineral in a volume of bone without consideration of total body

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size. It is thus independent of growth. The DXA method is also limited by excessive soft tissue as present in massively obese individuals. Dual-energy computed tomography measurements, which are much more expensive and require larger X-ray doses can provide density as well as volumetric determinants and are useful for estimating the entire mineral component.

Direct estimation of calcium balance in older adults by BMD is highly dependent on other factors besides calcium intake, such as serum levels of estrogen and PTH, intake of other nutrients (e.g., phosphorus and sodium), as well as adequate intestinal absorption and normal kidney function. Indeed, bone remodeling is not directly regulated by calcium, although it can suppress PTH-induced increases in bone resorption under certain conditions. Circumstances that enhance bone resorption, such as estrogen deficiency, or glucocorticoid use, alter the organic matrix and reduce the thickness and density of trabeculae, independent of calcium intake. In short, density measurements do not directly reflect calcium stores.

OTHER FACTORS RELATED TO CALCIUM NUTRITURE

As described above, not all calcium consumed is absorbed once it enters the gut. In general, the efficiency of calcium absorption is in reverse proportion to the amount of calcium consumed at any one time. Other factors also affect the amount of calcium available to the body.

Bioavailability of Calcium

Humans absorb about 30 percent of the calcium present in foods, but this varies with the type of food consumed. Bioavailability is generally increased when calcium is well solubilized and inhibited in the presence of agents that bind calcium or form insoluble calcium salts. The absorption of calcium is about 30 percent from dairy and fortified foods (e.g., orange juice, tofu, soy milk) and nearly twice as high from certain green vegetables (bok choy, broccoli, and kale). If a food contains compounds that bind calcium or otherwise interfere with calcium absorption, such as oxalic acid and phytic acid, then the food source is considered to be a poor source of calcium. Foods with high levels of oxalic acid include spinach, collard greens, sweet potatoes, rhubarb, and beans. Among the foods high in phytic acid are fiber-containing whole-grain products and wheat bran, beans, seeds, nuts, and soy isolates. The extent to which these compounds affect calcium absorption varies, and food combinations affect overall absorption efficiency. Eating spinach with milk at the same time reduces the absorption of the calcium in the milk (Weaver and Heaney, 1991); in contrast, wheat products (with the exception of wheat bran) do not appear

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to have a negative impact on calcium absorption (Weaver et al., 1991).[5] Vegan sources of calcium may be less bioavailable and, in turn, problematic for ensuring adequate calcium intake (Weaver, 2009).

The calcium salts most commonly used as supplements or food fortificants exhibit similar absorbability when tested in pure chemical form (Rafferty et al., 2007), but the absorbability of calcium from pharmaceutical preparations can fall short of predictions from studies of pure salts (Weaver and Heaney, 2006a). Calcium citrate appears to be better absorbed than calcium carbonate (Harvey et al., 1988); when they are taken with food, however, some researchers (Heaney et al., 1999), but not all (Heller et al., 2000), suggest comparable bioavailability of the two forms of calcium.

Factors in the Diet

Protein

Protein intake stimulates acid release in the stomach, and this, in turn, enhances calcium absorption. However, it has long been known that protein also increases urinary calcium excretion. The effect of protein on calcium retention and hence bone health has been controversial (IOM, 1997). Several observational and clinical studies have examined the effect of high-protein diets on bone (Shapses and Sukumar, 2010). Over a 4-year period in the Framingham Osteoporosis Study (Hannan et al., 2000), a higher protein intake (84 to 152 g/day), was positively associated with change in femoral neck and spine BMD (Shapese and Sukumar, 2010). Additionally, NHANES II suggested a positive association between femoral neck BMD and total protein intake (> 75 g/day) (Kerstetter et al., 2000; Shapses and Sukumar, 2010). In contrast, some epidemiological studies suggest that high protein diets reduce bone mass; this has been attributed to a higher acid load, leading to a buffering response by the skeleton and greater urinary calcium excretion. A recent meta-analysis (Darling et al., 2009) concluded that there is a small benefit of protein for bone health, but the benefit may not necessarily translate into reduced fracture risk in the long term. Shapses and Sukumar (2010) suggested that the currently available data would lead to the conclusion that there is a beneficial effect of increasing protein intake on bone in older individuals who normally have a habitually low intake of protein.

[5]Available online at http://ods.od.nih.gov/factsheets/calcium/ (accessed July 23, 2010).

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Foods and Food Components

Sodium and potassium in the diet may also affect calcium nutriture. High intakes of sodium increase urinary calcium excretion. In contrast, adding more potassium to a high-sodium diet might help decrease calcium excretion, particularly in postmenopausal women (Sellmeyer et al., 2002; IOM, 2005).

Alcohol intake can affect calcium nutriture by reducing calcium absorption (Hirsch and Peng, 1996), although the amount of alcohol required to cause an effect and whether moderate alcohol consumption is helpful or harmful to bone are unknown.

Caffeine from coffee and tea modestly increases calcium excretion and reduces absorption (Heaney and Recker, 1982; Bergman et al., 1990). Two studies have indicated that caffeine intake (two to three or more cups of coffee per day) will result in bone loss, but only in individuals with low milk or low total calcium intake (Barrett-Connor et al., 1994; Harris and Dawson-Hughes, 1994).

Phosphate is also of interest. Food phosphate is a mixture of inorganic and organic forms, and there is no evidence that its absorption efficiency varies with dietary intake. A portion of phosphorus absorption is due to saturable, active transport facilitated by calcitriol. However, fractional phosphorus absorption is virtually constant across a broad range of intakes suggesting that absorption occurs primarily by a passive, concentration-dependent process. Several observational studies have suggested that the consumption of carbonated soft drinks with high levels of phosphate is associated with reduced bone mass and increased fracture risk, but it is likely that the effect is due to replacing milk with soda, rather than to phosphorus itself (Calvo, 1993; Heaney and Rafferty, 2001).

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3

Overview of Vitamin D

INTRODUCTION

Vitamin D, first identified as a vitamin early in the 20th century, is now recognized as a prohormone. A unique aspect of vitamin D as a nutrient is that it can be synthesized by the human body through the action of sunlight. These dual sources of vitamin D make it challenging to develop dietary reference intake values.

Vitamin D, also known as calciferol, comprises a group of fat-soluble seco-sterols. The two major forms are vitamin D_2 and vitamin D_3. Vitamin D_2 (ergocalciferol) is largely human-made and added to foods, whereas vitamin D_3 (cholecalciferol) is synthesized in the skin of humans from 7-dehydrocholesterol and is also consumed in the diet via the intake of animal-based foods. Both vitamin D_3 and vitamin D_2 are synthesized commercially and found in dietary supplements or fortified foods. The D_2 and D_3 forms differ only in their side chain structure. The differences do not affect metabolism (i.e., activation), and both forms function as prohormones. When activated, the D_2 and D_3 forms have been reported to exhibit identical responses in the body, and the potency related to the ability to cure vitamin D–deficiency rickets is the same (Fieser and Fieser, 1959; Jones et al., 1998; Jurutka et al., 2001). Experimental animal studies have indicated that vitamin D_2 is less toxic than vitamin D_3, but this has not been demonstrated in humans.

The activation steps involved in converting vitamin D from the diet and cutaneous synthesis are illustrated in Figure 3-1. Vitamin D, in either the D_2 or D_3 form, is considered biologically inactive until it undergoes two

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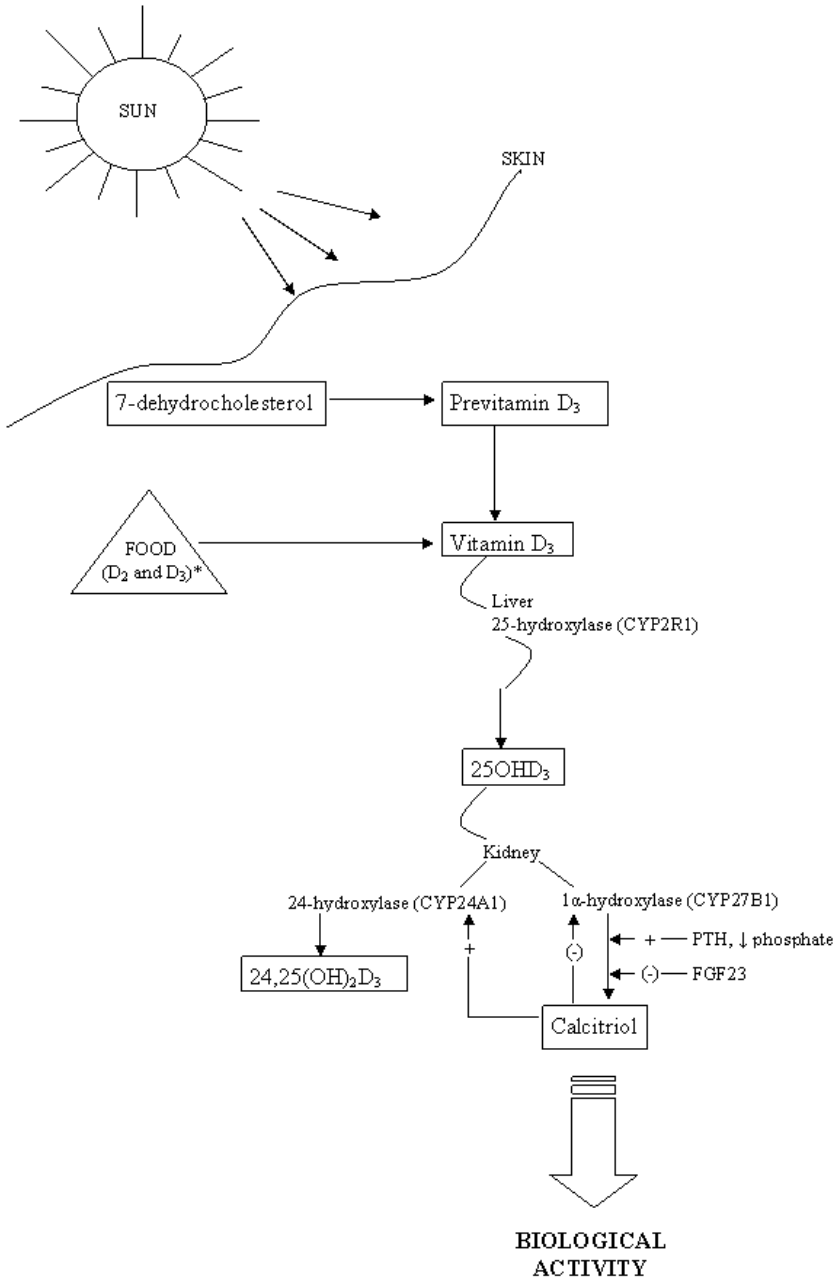


FIGURE 3-1 Overview of vitamin D synthesis, intake, and activation.

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enzymatic hydroxylation reactions. The first takes place in the liver, mediated by the 25-hydroxylase (most likely cytochrome P450 2R1 [CYP2R1]) which forms 25-hydroxyvitamin D (hereafter referred to as 25OHD). The second reaction takes place in the kidney, mediated by 1α-hydroxylase (CYP27B1), which converts 25OHD to the biologically active hormone, calcitriol (1,25-dihydroxyvitamin D). The 1α-hydroxylase gene is also expressed in several extra-renal tissues, but its contribution to calcitriol formation in these tissues is unknown. 25OHD, the precursor of calcitriol, is the major circulating form of vitamin D; it circulates bound to a specific plasma carrier protein, vitamin D binding protein (DBP). DBP also transports vitamin D and calcitriol.

The renal synthesis of calcitriol is tightly regulated by two counteracting hormones, with up-regulation via parathyroid hormone (PTH) and down-regulation via fibroblast-like growth factor-23 (FGF23) (Galitzer et al., 2008; Bergwitz and Juppner, 2010). Low serum phosphorus levels stimulate calcitriol synthesis, whereas high serum phosphorus levels inhibit it. Following its synthesis in the kidney, calcitriol binds to DBP to be transported to target organs. The biological actions of calcitriol, involve regulation of gene expression at the transcriptional level, and are mediated through binding to a vitamin D receptor (VDR), located primarily in the nuclei of target cells (Jones et al., 1998; Jurutka et al., 2001). Additional hydroxylation reactions, such as that mediated by CYP24A1, as shown in Figure 3-1, result in more polar metabolites with greatly reduced or no apparent biological activity.

The classical actions of vitamin D—which by itself is inactive—are due to the functions of the active metabolite, calcitriol. These actions take the form of the regulation of serum calcium and phosphate homeostasis and, in turn, the development and maintenance of bone health (DeLuca, 1988; Reichel et al., 1989; Jones et al., 1998). Non-classical functions are less well elucidated. VDRs are found fairly ubiquitously throughout the body in tissues not involved with calcium and phosphate homeostasis, and the presence of VDRs in these tissues implies that calcitriol may play a more general role or that ligands other than calcitriol can activate the VDR. Furthermore, the specific vitamin D–responsive elements (VDREs), considered the hallmark of vitamin D action, are present in a large number of human genes involved in a wide range of classical and non-classical roles, such as the regulation of cell proliferation, cell differentiation, and apoptosis. It has been suggested that calcitriol exerts immunomodulatory and anti-proliferative effects through autocrine and paracrine pathways (Adams and Hewison, 2008). These wide-ranging actions of calcitriol have further been hypothesized to play a potential role in preventive or therapeutic action in cancer (Masuda and Jones, 2006) and chronic conditions such

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as auto-immune conditions (including type 1 diabetes), cardiovascular disease, and infections (Holick et al., 2007).

Outside of the biological forms of vitamin D, a number of analogues based on the vitamin D structure have been synthesized for use as potential pharmacological agents. These are not, however, dietary or biosynthesized compounds; rather, they are designed for specific applications in research or clinical treatment. Examples of synthetic analogues that have gained importance in clinical medicine are briefly mentioned below.

The term vitamin D is generally used in this report to refer to both the D_2 and D_3 forms as well as their metabolites, although the two forms are distinguished when necessary for clarification (see Box 3-1 for definitions). Vitamin D levels in the diet—from foods and supplements—are expressed in International Units (IU), but may be expressed elsewhere in micrograms (μg). The biological activity of 1 μg of vitamin D is equivalent to 40 IU. Owing to the frequency with which serum 25OHD levels are included in this report text, the levels are expressed only as nanomoles per liter (nmol/L). As shown in Box 3-1, the nanomoles per liter measure can be converted to nanograms per milliliter (ng/mL) by dividing by a factor of 2.5.

BOX 3-1
Terms and Conversions Used in Reference to Vitamin D

Terms:
Vitamin D—also referred to as *calciferol*
Vitamin D_2—also referred to as *ergocalciferol*
Vitamin D_3—also referred to as *cholecalciferol*

25OHD—25-hydroxyvitamin D also referred to as *calcidiol* or *calcifediol*; indicates no distinction between D_2 and D_3 forms. When relevant, forms are distinguished as **25OHD_2** and **25OHD_3**
Calcitriol—1,25-dihydroxyvitamin D_3 (Note: *Ercalcitriol*—refers to 1,25-dihydroxyvitamin D_2, but in this report, the term "calcitriol" will be used for both)
24,25$(OH)_2$D—24,25-dihydroxyvitamin D

IU = International Unit is a measurement based on biological activity or effect; 1 IU of vitamin D is defined as the activity of 0.025 μg of cholecalciferol in bioassays with rats and chicks.

Conversions for Vitamin D_3:
[sources] 40 IU = 1 μg
[serum] 2.5 nmol/L = 1 ng/mL

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SOURCES OF VITAMIN D

Diet

The dietary sources of vitamin D include food and dietary supplements; therefore, "total vitamin D intake" reflects the combined dietary contribution from foods and supplements. There are a few naturally occurring food sources of vitamin D. These include fatty fish, fish liver oil, and egg yolk. Some foods are, however, fortified with vitamin D. After vitamin D was recognized as important for the prevention of rickets in the 1920s (Steenbock and Black, 1924), vitamin D fortification of some foods was initiated on a voluntary basis.

In the United States, fluid milk is voluntarily fortified with 400 IU per quart (or 385 IU/L) of vitamin D (U.S. regulations do not specify the form) (FDA, 2009). In Canada, under the Food and Drug Regulation,[1] fortification of fluid milk and margarine with vitamin D is mandatory. Fluid milk must contain 35–45 IU vitamin D per 100 mL and margarine, 530 IU per 100 g. In addition, fortified plant-based beverages must contain vitamin D in an amount equivalent to fluid milk. In analyses conducted in the 1980s and early 1990s, a significant portion of milk samples in the United States were found to contain less than the specified amount of vitamin D (Tanner et al., 1988). Holick et al. (1992) found that 62 percent of milk sampled from five eastern states contained less than 80 percent and 10 percent contained more than 120 percent of the amount of vitamin D stated on the label. Chen et al. (1993) reported similar findings. A more recent report on vitamin D–fortified milk sampled in New York State over a period of 4 years showed that an average of only 47.7 percent of samples fell within the range of acceptable levels of vitamin D fortification (Murphy et al., 2001). However, recent surveys from the U.S. Department of Agriculture (USDA) indicate that these problems have been corrected. In a presentation to this committee, Byrdwell (2009) reported that a USDA survey of milk samples taken in 2007 from 24 locations across the United States showed that most samples had vitamin D levels within the range of 400 to 600 IU/quart.

In Canada, Faulkner et al. (2000) surveyed milk samples and found that 20 percent of skim milk, 40 percent of 2 percent fat milk, and 20 percent of whole milk, contained the recommended level of vitamin D. Samples collected by the Canadian Food Inspection Agency from 1999 through 2009 and analyzed for vitamin D indicated that during the last 4 years of sample collection, 47 to 69 percent were within the range specified by regulation (personal communication, S. Brooks, Health Canada, April

[1]Available online at http://laws.justice.gc.ca/PDF/Regulation/C/C.R.C.,_c._870.pdf (accessed July 23, 2010).

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30, 2010). In addition, over the past 5 years, the average vitamin D content of analyzed milk samples fell within this range. Over time, manufacturers in the United States have added vitamin D to other foods, and the food industry is increasingly marketing foods fortified with vitamin D (Yetley, 2008). Based on data from a U.S. Food and Drug Administration (FDA) survey that provides information on the labels of processed, packaged food products in the United States, Yetley (2008) reported that almost all fluid milks, approximately 75 percent of ready-to-eat breakfast cereals, slightly more than half of all milk substitutes, approximately one-quarter of yogurts, and approximately 8 to 14 percent of cheeses, juices, and spreads are fortified with vitamin D in the U.S. market. Many product labels included in the survey indicated that the form of added vitamin D was vitamin D_3. However, some milk substitutes are fortified with vitamin D_2. Cereal labels did not specify the form of added vitamin D. Levels of vitamin D ranged from 40 IU per regulatory serving for cereals and cheeses to 60 IU per regulatory serving for spreads and 100 IU per regulatory serving for fluid milk. Several food categories had within-category ranges of 40 to 100 IU of vitamin D per regulatory serving. Serum vitamin D and 25OHD have low penetrance into breast milk, together comprising 40 to 50 IU of antirachitic activity per liter, most of which is contributed by 25OHD (Leerbeck and Sondergaard, 1980; Hollis et al., 1981; Reeve et al., 1982; Specker et al., 1985). Data from the USDA report the vitamin D content of human milk to be 4.3 IU/100 kcal.[2] However, the vitamin D biological activity may be higher than the analyzed values, because human milk contains small amounts of 25OHD in addition to vitamin D_3 (Reeve et al., 1982); further, the biological activity of 25OHD is approximately 50 percent higher than that of vitamin D (Blunt et al., 1968).

The FDA has established that infant formula must contain 40 to 100 IU of vitamin D per 100 kcal.[3] Commercial infant formulas contain approximately 60 IU of vitamin D per 100 kcal, as estimated by the USDA food composition database,[4] and Yetley (2008) reported that commercial milk-based infant formulas collected between 2003 and 2006 contained 87

[2]USDA National Nutrient Database for Standard Reference Release 23. NBD No. 01107. Milk, human, mature, fluid. Available online at http://www.ars.usda.gov/Services/docs.htm?docid=8964 (accessed August 3, 2010).

[3]USDA National Nutrient Database for Standard Reference Release 23. NBD No. 03946. Infant formula, ROSS, SIMILAC LACTOSE FREE ADVANCE, ready-to-feed, with ARA and DHA; and NDB no. 03815. Infant formula, MEAD JOHNSON, ENFAMIL LIPIL, with iron, ready-to-feed, with ARA and DHA. Available online at http://www.ars.usda.gov/main/site_main.htm?modecode=12-35-45-00 (accessed April 28, 2010).

[4]Available online at http://www.nal.usda.gov/fnic/foodcomp/search/ (accessed March 16, 2010).

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to 184 percent of label declarations. In Canada, infant formula is required by regulation to contain between 40 and 80 IU of vitamin D per 100 kcal.

In recent years, dietary supplements containing vitamin D have become more common and have been more frequently consumed. The form of vitamin D used in supplement products can be either vitamin D_2 or vitamin D_3. It would appear from informal observations of the market place that manufacturers are increasingly switching from vitamin D_2 to vitamin D_3, and some are increasing the vitamin D content of their products. Traditionally, many marketed dietary supplements have contained 400 IU per daily dose, but levels in supplements have been increasing. In the United States, vitamin D can now be found in multi-vitamin/multi-mineral formulations as well as a single supplement in a range of dosage levels, including 1,000 to 5,000 IU of vitamin D_3 per dose and even up to 50,000 IU of vitamin D_2 per dose. In Canada, dosage levels of vitamin D above 1,000 IU are obtainable only with a prescription.

Information about current national survey estimates of the intake of vitamin D from foods and supplements can be found in Chapter 7.

Synthesis in the Skin

Vitamin D_3 is synthesized in human skin from 7-dehydrocholesterol following exposure to ultraviolet B (UVB) radiation with wavelength 290 to 320 nm.[5] The process of UVB-mediated conversion of 7-dehydrocholesterol to the previtamin D_3 form and subsequent thermal isomerization to vitamin D_3 occurring in the epidermis is illustrated in Figure 3-2.

The production of vitamin D_3 in skin is a function of the amount of UVB radiation reaching the dermis as well as the availability of 7-dehydrocholesterol (Holick, 1995). As such, the level of synthesis is influenced by a number of factors, as described below in the section entitled "Measures Associated with Vitamin D: Serum 25OHD," including season of the year, skin pigmentation, latitude, use of sunscreen, clothing, and amount of skin exposed. Age is also a factor, in that synthesis of vitamin D declines with increasing age, due in part to a fall in 7-dehydrocholesterol levels and due in part to alterations in skin morphology (MacLaughlin and Holick, 1985).

Toxic levels of vitamin D do not occur from prolonged sun exposure. Thermal activation of previtamin D_3 in the skin gives rise to multiple non–vitamin D forms, such as lumisterol, tachysterol and others (Holick et al., 1981; Webb et al., 1989), as illustrated in Figure 3-2; this limits the

[5]The chemical processes that lead to the formation of vitamin D_3 from its precursor are non-enzymatic and can take place ex vivo and in organic solvents, as well as in vivo. Therefore, vitamin D_3 can also be synthesized commercially.

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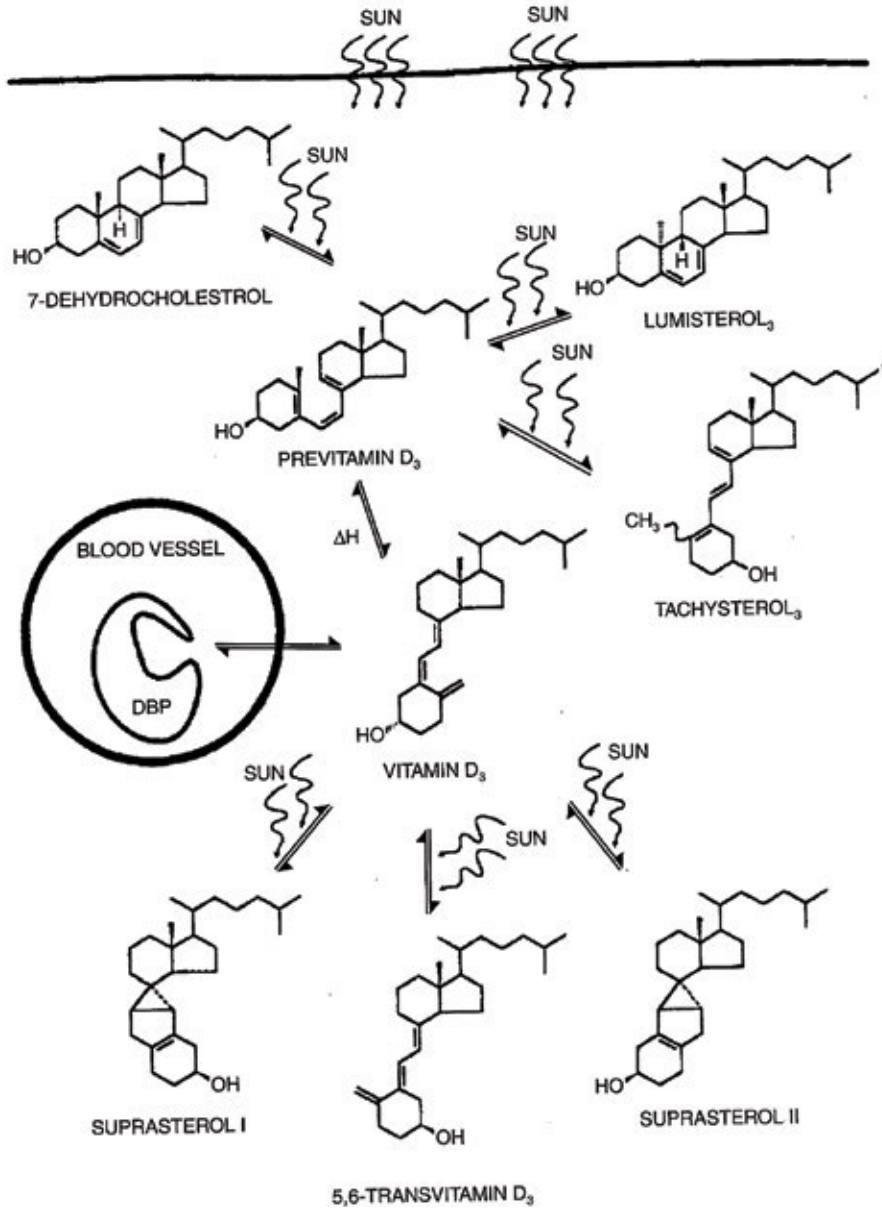


FIGURE 3-2 Photochemical events that lead to the production and regulation of vitamin D_3 (cholecalciferol) in the skin.
NOTE: DBP = vitamin D binding protein.
SOURCE: Holick (1994). Reprinted with permission from the *American Journal of Clinical Nutrition* (1994, volume 60, pages 619-630), American Society for Nutrition.

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formation of vitamin D_3 itself. Vitamin D_3 can also be converted to nonactive forms.

The absolute percentage of circulating 25OHD that arises from cutaneous synthesis versus oral intake of vitamin D in the free-living North American population cannot be clearly specified. Individuals living at Earth's poles during winter months and submariner crew members with very limited or no measurable UVB exposure have detectable levels of 25OHD in blood, arising from dietary sources and likely from previously synthesized and stored vitamin D. This topic is further explored in the section below that focuses on serum 25OHD.

METABOLISM OF VITAMIN D

Absorption

Owing to its fat-soluble nature, dietary vitamin D (either D_2 or D_3) is absorbed with other dietary fats in the small intestine (Haddad et al., 1993; Holick, 1995). The efficient absorption of vitamin D is dependent upon the presence of fat in the lumen, which triggers the release of bile acids and pancreatic lipase (Weber, 1981, 1983). In turn, bile acids initiate the emulsification of lipids, pancreatic lipase hydrolyzes the triglycerides into monoglycerides and free fatty acids, and bile acids support the formation of lipid-containing micelles, which diffuse into enterocytes. Early studies demonstrated that radiolabeled vitamin D_3 appeared almost exclusively in the lymphatics and in the chylomicron fraction of plasma; as well, subjects with impaired bile acid release or pancreatic insufficiency both demonstrated significantly reduced absorption of vitamin D (Thompson et al., 1966; Blomstrand and Forsgren, 1967; Compston et al., 1981). Subsequently, other clinical and experimental animal studies confirmed that vitamin D is most efficiently absorbed when consumed with foods containing fat (Weber, 1981; Johnson et al., 2005; Mulligan and Licata, 2010) and, conversely, that a weight-loss agent that blocks fat absorption also impairs the absorption of vitamin D (James et al., 1997; McDuffie et al., 2002). The optimal amount of fat required for maximal absorption of vitamin D has not been determined.

Within the intestinal wall, vitamin D, cholesterol, triglycerides, lipoproteins, and other lipids are packaged together into chylomicrons. Importantly, while a fraction of newly absorbed intestinal vitamin D is also transported along with amino acids and carbohydrates into the portal system to reach the liver directly, the main pathway of vitamin D uptake is incorporation into chylomicrons that reach the systemic circulation via the lymphatics. Chylomicron lipids are metabolized in peripheral tissues that express lipoprotein lipase, but particularly in adipose tissue and

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skeletal muscle, which are rich in this enzyme. During hydrolysis of the chylomicron triglycerides, a fraction of the vitamin D contained in the chylomicron can be taken up by these tissues. Uptake into adipose tissue and skeletal muscle accounts for the rapid postprandial disappearance of vitamin D from plasma and probably also explains why increased adiposity causes sequestering of vitamin D and is associated with lower 25OHD levels (Jones, 2008). What remains of the original chylomicron after lipolysis is a chylomicron remnant, a cholesterol-enriched, triglyceride-depleted particle that still contains a fraction of its vitamin D content.

Metabolism to the Active Hormonal Form

Vitamin D, regardless of origin, is an inactive prohormone and must first be metabolized to its hormonal form before it can function. Once vitamin D enters the circulation from the skin or from the lymph, it is cleared by the liver or storage tissues within a few hours. The processes that follow are illustrated in Figure 3-3. Vitamin D is converted in the liver to 25OHD, a process carried out by a CYP enzyme that has yet to be fully defined but is likely CYP2R1 (Cheng et al., 2003). The crystal structure of CYP2R1 has been determined with vitamin D in the active site, and the enzyme has been shown to metabolize both vitamin D_2 and vitamin D_3 equally efficiently (Strushkevich et al., 2008). There is little, if any, feedback regulation of this enzyme. A large genome-wide association study of factors that might be determinants of the circulating 25OHD levels identified the human chromosomal 11p15 locus of CYP2R1 as a significant determinant, whereas the loci of the other enzymes purported to have 25-hydroxylase activity (e.g., CYP27A1 and CYP3A4) were not identified (Wang et al., 2010). The other determinants of serum 25OHD besides CYP2R1 have been reported to be DBP (also known as Gc protein), which has six common phenotypes (Laing and Cooke, 2005) as well as 7-dehydrocholesterol reductase and CYP24A1. Increasing intake of vitamin D results in higher blood levels of 25OHD, although perhaps not in a linear manner (Stamp et al., 1977; Clements et al., 1987).

At this point, 25OHD bound to DBP circulates in the blood stream and, when calcitriol is required due to a lack of calcium (or lack of phosphate), 25OHD is 1α-hydroxylated in the kidney to form calcitriol, the active form, by the 1α-hydroxylase enzyme (also known as CYP27B1) (Tanaka and DeLuca, 1983). This metabolic step is very tightly regulated by blood calcium and phosphate levels through PTH and the phosphaturic hormone, FGF23, and constitutes the basis of the vitamin D endocrine system that is central to maintaining calcium and phosphate homeostasis (see discussion below on functions and physiological actions). FGF23 acts by reducing the expression of renal sodium–phosphate transporters and reducing serum calcitriol levels.

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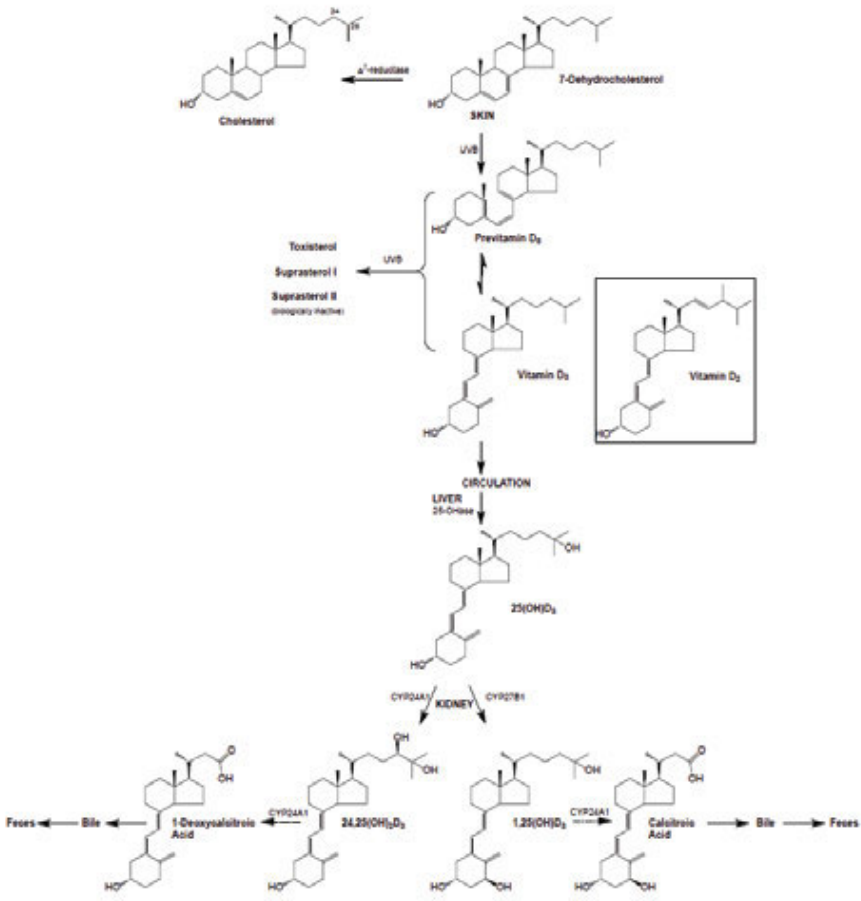


FIGURE 3-3 The metabolism of vitamin D_3 from synthesis/intake to formation of metabolites. The process is the same for vitamin D_2 once it enters the circulation.
NOTE: CYP = cytochrome P450 (a large and diverse group of enzymes).
SOURCE: Reprinted with permission from Hector DeLuca.

Production of the CYP27B1 enzyme is stimulated by PTH, which is secreted in response to a lack of calcium. It is also stimulated by the hypophosphatemic action of FGF23 on renal phosphate excretion, but to a lesser extent. When PTH is suppressed, or FGF23, produced by osteocytes, is stimulated, 1α-hydroxylation is markedly reduced (Liu et al., 2007; Quarles, 2008). Furthermore, calcitriol can act as a suppressor of CYP27B1, although the mechanism is not fully understood.

Calcitriol has its strongest metabolic activity in inducing its own destruction by stimulating the 24-hydroxylase enzyme (now known as CYP24A1;

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Figure 3-1) (Jones et al., 1998). The enzyme CYP24A1 is found in all target tissues and is induced in response to calcitriol interacting with the VDR. CYP24A1 is largely responsible for the metabolic degradation of calcitriol and its precursor, 25OHD, and its deletion in the mouse results in 50 percent lethality at weaning and an inability to efficiently clear the active form of vitamin D (Masuda et al., 2005). CYP24A1 carries out a series of reactions resulting ultimately in production of calcitroic acid from calcitriol and 1-desoxycalcitroic acid from $24,25(OH)_2D$, the major metabolite of 25OHD. These products are excreted through the bile into the feces (Jones et al., 1998); very little is eliminated through the urine (Kumar et al., 1976). The active forms of vitamin D_2 are also catabolized by CYP24A1 into a series of biliary metabolites, somewhat analogous to those of vitamin D_3.

As described above, all naturally occurring vitamin D compounds interact with DBP. Calcitriol and vitamin D have significantly lower affinity for this protein than does 25OHD. Whereas vitamin D has an average lifetime in the body of approximately 2 months, 25OHD has a lifetime of 15 days, and calcitriol has a lifetime measured in hours (Jones et al., 1998). Aside from these key elements in vitamin D metabolism, more than 30 other metabolites have been found, including the 3-epi series of vitamin D compounds (DeLuca and Schnoes, 1983; Siu-Caldera et al., 1999). Their importance seems minimal and need not be discussed here.

Although the route of catabolism between $1\alpha,25(OH)_2D_2$ and $1\alpha,25(OH)_2D_3$ differs beyond the initial 24-hydroxylation step, because 24-hydroxylation is primarily a deactivation step (Brommage and DeLuca, 1985; Horst et al., 1986; Lohnes and Jones, 1992; Jones et al., 1998), the rate of this initial step should be the important indicator of the loss of biological action. Comparisons of initial rate kinetics of the 24-hydroxylase enzyme (CYP24A1) activity toward $1\alpha,25(OH)_2D_2$ and $1\alpha,25(OH)_2D_3$ and their precursors suggest that the rates of inactivation by CYP24A1 in vitro are virtually identical (Jones et al., 2009; Urushino et al., 2009). Although side-chain hydroxylation of $1\alpha,25(OH)_2D_2$ represents the primary route of metabolism in the target cell, clearance of the metabolic products in vivo is complicated by additional non-specific liver CYPs (e.g., CYP3A4) (Gupta et al., 2004, 2005) that are inducible by $1\alpha,25(OH)_2D_3$ in certain extra-hepatic tissues (Thompson et al., 2002) and also Phase II enzymes, including uridine diphosphate–glucuronosyl transferases, which are known to subject vitamin D metabolites to glucuronidation (LeVan et al., 1981; Hashizume et al., 2008). The pharmacokinetic consequence of the sum of these catabolic systems, as shown in studies in rats, is a slightly reduced half-life for $1\alpha,25(OH)_2D_2$ compared with $1\alpha,25(OH)_2D_3$ (Knutson et al., 1997).

There are reports that vitamin D_2 and vitamin D_3 are differentially susceptible to these non-specific inactivating modifications, such as those occurring in the liver in response to a variety of drugs. These enzymes in-

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clude the liver and intestinal CYPs that are known to metabolize vitamin D compounds differently, such as CYP27A1, which 25-hydroxylates vitamin D_3 and 24-hydroxylates vitamin D_2 (Guo et al., 1993), and CYP3A4, which 24- and 25-hydroxylates vitamin D_2 substrates more efficiently than vitamin D_3 substrates (Gupta et al., 2004, 2005) and 23*R*- and 24*S*-hydroxylates $1\alpha,25(OH)_2D_3$ (Xu et al., 2006); the latter enzyme has recently been shown to be selectively induced by $1\alpha,25(OH)_2D$ in the intestine (Thompson et al., 2002; Xu et al., 2006). Both CYP27A1 and CYP3A4 are known to have significantly lower Michaelis-Menten constants (K_m values) for $25OHD_3$ compared with CYP2R1 (Guo et al., 1993; Sawada et al., 2000), in the micromole per liter range; this questions their physiological but not their pharmacological relevance. Recent work (Helvig et al., 2008; Jones et al., 2009) has shown that both human intestinal microsomes and recombinant CYP3A4 protein break down $1\alpha,25(OH)_2D_2$ at a significantly faster rate than $1\alpha,25(OH)_2D_3$, suggesting that this non-specific CYP might limit vitamin D_2 action preferentially in target cells, where it is expressed and when the substrate is in the pharmacological dose range. The same type of mechanism involving differential induction of non-specific CYPs may underlie the occasional reports of co-administered drug classes, such as anticonvulsants (Christiansen et al., 1975; Tjellesen et al., 1985; Hosseinpour et al., 2007), causing accelerated degradation of one vitamin D form over the other.

Storage

Adipose tissue stores of vitamin D probably represent "non-specific" stores sequestered because of the hydrophobic nature of vitamin D, but the extent to which the processes of accumulation or mobilization are regulated by normal physiological mechanisms remains unknown at this time. Rosenstreich et al. (1971) first identified adipose tissue as the primary site of vitamin D accumulation from experiments in which radiolabeled vitamin D was administered to vitamin D–deficient rats. Tissue levels of radioactivity measured during vitamin D repletion and during a subsequent period of deprivation showed that adipose tissue acquired the greatest quantity of radioactive compound and had the slowest rate of release. Work by Liel et al. (1988) suggested that there was enhanced uptake and clearance of vitamin D by adipose tissue in obese subjects compared with those of normal weights. Similarly, Wortsman et al. (2000) concluded that in obese subjects, vitamin D was stored in adipose tissue and not released when needed. Finally, Blum et al. (2008) found that, in elderly subjects supplemented with 700 IU of vitamin D per day, for every additional 15 kg of weight above "normal" at baseline, the mean adjusted change in 25OHD level was approximately 10 nmol/L lower after 1 year of supplementation.

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The authors estimated that in order for subjects with body mass indexes (BMIs) above the normal range to obtain an increase in serum 25OHD level similar to that of subjects with weight in the normal range, an additional 17 percent increase in vitamin D above the administered dose of 700 IU/day would be needed for every 10 kg increase in body weight above baseline in their study population.

The implication of these studies is that vitamin D deposited in fat tissue is not readily available, and obese individuals may require larger than usual doses of vitamin D supplements to achieve a serum 25OHD level comparable to that of their normal weight counterparts. In support of the hypothesis that vitamin D is stored in adipose tissues, weight reduction studies show that serum 25OHD levels rise when obese individuals lose body fat (Riedt et al., 2005; Zitterman et al., 2009; Tzotzas et al., 2010). Conclusive statements regarding changes in serum 25OHD levels after gastric bypass surgery cannot be made, as a result of confounding factors, such as weight change, possible malabsorption, and diet. There is evidence of a rise in serum 25OHD levels after surgery (Mahdy et al., 2008; Aasheim et al., 2009; Goldner et al., 2009; Bruno et al., 2010), as well as evidence that there is no change after surgery (Riedt et al., 2006; Fleischer et al., 2008; Valderas et al., 2009). Gehrer et al. (2010) indicated that serum 25OHD levels decrease after gastric bypass surgery, although the quality of the methods used is questionable.

Excretion

As described previously, the products of vitamin D metabolism are excreted through the bile into the feces, and very little is eliminated through the urine. This is in part due to renal reuptake of vitamin D metabolites bound to DBP, as mediated by the cubilin–megalin receptor system (Willnow and Nykjaer, 2005).

Excess Intake

Excess intake of vitamin D—but not sun exposure, which is associated with a series of thermal and photoisomerization reactions (see Figure 3-2)—can lead to a state of vitamin D "intoxication" or "hypervitaminosis D." Chemically synthesized vitamin D became available late in the third decade of the 20th century; reports of vitamin D intoxication were first found from 1928 to 1932 and continued throughout most of the 20th century (DeLuca, 2009). The condition of hypervitaminosis D leads to hypercalcemia and eventually to soft tissue calcification and resultant renal and cardiovascular damage (DeLuca, 1974). In the case of animal models, at necropsy, vitamin D–intoxicated rats show widespread calcification of organs and tissues.

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The form of the vitamin implicated in the intoxication is 25OHD (Vieth, 1990; Jones, 2008). In fact, it has been shown in dietary supplementation studies using the CYP27B1 knockout mouse, which is incapable of making calcitriol, sufficiently high concentrations of serum levels of 25OHD can cause changes in vitamin D–dependent general expression even in the absence of calcitriol (Rowling et al., 2007; Fleet et al., 2008).

FUNCTIONS AND PHYSIOLOGICAL ACTIONS OF VITAMIN D

Calcium and Phosphate Homeostasis

The dominant function of vitamin D in its hormonal form (calcitriol or 1,25-dihydroxyvitamin D) is the elevation of plasma calcium and phosphate levels, which are required for mineralization of bone (DeLuca, 1979b; Holick, 1996). Furthermore, the elevation of plasma calcium to normal levels is also required for the functioning of the neuromuscular junction as well as vasodilatation, nerve transmission, and hormonal secretion.

Calcitriol—functioning as part of the endocrine system for maintaining serum calcium levels as outlined in Chapter 2—elevates plasma ionized calcium levels to the normal range by three different mechanisms (see Figure 2-1 in Chapter 2). The first mechanism, which does not require PTH, is the well-established role of calcitriol in stimulating intestinal calcium absorption throughout the entire length of the intestine, although its greatest activity is in the duodenum and jejunum. It is clear that calcitriol directly stimulates intestinal calcium and, independently, phosphate absorption.

In the second mechanism, calcitriol plays an essential role in the mobilization of calcium from bone, a process requiring PTH (Garabedian et al., 1972; Lips, 2006). It induces the formation and activation of the osteoclast to function in the mobilization of calcium from bone, as discussed in Chapter 2. In short, calcitriol facilitates the formation of osteoclasts by stimulating the secretion of a protein called receptor activator for nuclear factor κ B (RANK) ligand, which, in turn, is responsible for osteoclastogenesis and bone resorption (Suda et al., 1992; Yasuda et al., 2005).

In the third mechanism, calcitriol together with PTH stimulates the renal distal tubule reabsorption of calcium, ensuring retention of calcium by the kidney when calcium is needed (Sutton et al., 1976; Yamamoto et al., 1984). These well-known functions dominate vitamin D physiology and many of the functional proteins involved in these processes have been identified, although the exact molecular mechanisms of all of these systems have yet to be elucidated.

Thus, overall, calcitriol acts on the intestine, bone, and kidney as described above, and as illustrated in Figure 2-1 in Chapter 2, to elevate

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serum calcium levels, closing the calcium loop. As serum calcium levels rise, PTH secretion drops. If serum calcium levels become too high, the parafollicular cells ("C" cells) of the thyroid secrete calcitonin, which blocks calcium resorption from bone and helps to keep calcium levels in the normal range. Calcitriol, through its receptor, the VDR, suppresses parathyroid gene expression and parathyroid cell proliferation, providing important feedback loops that reinforce the direct action of increased serum calcium levels (Slatopolsky et al., 1984; Silver et al., 1986).

Not shown in Figure 2-1 in Chapter 2 is the mechanism of action of vitamin D in regulating serum phosphorus levels, certain aspects of which remain obscure. What is known is that (1) a deficiency of phosphate stimulates CYP27B1 to produce more calcitriol, which in turn stimulates phosphate absorption in the small intestine; and (2) calcitriol can also induce the secretion of FGF23 by osteocytes in bone, which results in phosphate excretion in the kidney (Liu et al., 2008), as well as feedback on vitamin D metabolism.

Other Actions

It is noteworthy that the VDR is present in the nucleus of many tissues that are not involved in the regulation of calcium and phosphate metabolism. For example, the VDR has been clearly described in epidermal keratinocytes, in activated T cells of the immune system, in antigen-presenting cells, in macrophages and monocytes, and in cytotoxic T cells. Gene array studies in many cells and tissues show that calcitriol regulates several hundred genes throughout the body or as much as 5 percent of the human genome (Pike et al., 2008). However, exactly how calcitriol functions in these tissues and the physiological consequences are not clearly known.

Likewise, the importance of the paracrine or autocrine synthesis of calcitriol under non-disease conditions is unclear. The 1α-hydroxylase (CYP27B1) gene has been reported to be expressed in many extra-renal tissues (Hewison et al., 2007). In some cases, this is based upon in vitro production of calcitriol by cell lines as a consequence of culture conditions, but it also includes detection of the messenger ribonucleic acid (mRNA) transcript or protein for CYP27B1 in tissues in vivo (Hewison et al., 2007). There is no doubt that the kidney is physiologically the overwhelming site of production of calcitriol for the circulation, as chronic kidney disease or nephrectomy results in a significant fall in the serum calcitriol level (Martinez et al., 1995). The contribution of calcitriol to the maternal circulation stemming from production by the placenta is not clearly known; based on a case report for an anephric patient, it appears that the placenta produces calcitriol, but its contribution to the maternal circulation is low (Turner et al., 1988). The pregnancy-related rise in calcitriol is due to up-

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regulation of the enzymes in the maternal kidney (Kovacs and Kronenberg, 1997). However, there may be other extra-renal 1α-hydroxylation sites that can act as intracrine systems primarily involved in regulation of cell or tissue growth: skin, gastrointestinal tract, or glandular tissue, such as prostate and breast (Diesing et al., 2006). In mice missing the *Vdr* gene (*Vdr*-null), calcitriol and the VDR play a role in lactational physiology; there is accelerated mammary development during pregnancy, but delayed involution of the mammary tissue after lactation (Zinser and Welsh, 2004). Extra-renal CYP27B1 may be up-regulated during inflammation (Ma et al., 2004; Liu et al., 2008) or down-regulated in cancerous tissue proliferation (Bises et al., 2004; Wang et al., 2004). Furthermore, extra-renal production of calcitriol is clearly found in certain pathological diseases, including granulomatous conditions such as sarcoidosis, lymphoma, and tuberculosis (Adams et al., 1989), which can be associated with hypercalcemia. If sarcoidosis is left untreated, the extra-renally produced calcitriol can enter the circulation, resulting in hypercalciuria and eventually hypercalcemia.

There is emerging evidence that calcitriol plays a role in the immune system that has not yet been clearly described. Exogenous calcitriol can suppress autoimmune diseases, but with hypercalcemia as an important side effect (DeLuca and Cantorna, 2001). It has been shown that the local conversion of 25OHD into calcitriol in monocytes or macrophages results in an increase in cellular immunity by stimulating the production of cathelicidin, an anti-microbial peptide capable of killing bacteria, particularly *Mycobacterium tuberculosis* (Liu et al., 2006). Recently, Stubbs et al. (2010) showed that renal dialysis patients treated with high-dose vitamin D_3 develop a population of immune cells with increased CYP27B1, VDR, and cathelicidin expression, although the role of these cells in vivo is unknown. Ironically, calcitriol has an opposite effect on the adaptive immune (B and T cell function) response. Calcitriol generally inhibits T helper cell proliferation and B cell immunoglobulin production. In contrast, calcitriol promotes the proliferation of immunosuppressive regulatory T cells and their accumulation at sites of inflammation (Penna et al., 2007).

A role for vitamin D in carcinogenesis evolved initially from in vitro studies as cell culture approaches became more widely available for the evaluation of the mechanisms of action of vitamin D and its metabolites (Masuda and Jones, 2006). The active hormone, calcitriol, was shown to consistently inhibit the growth of cancer cells and promote differentiation in vitro by regulating multiple pathways (Deeb et al., 2007; Kovalenko et al., 2010). Additional studies documented the presence of the VDR in a wide array of cancer cell types. Vitamin D orchestrates cell cycle progression via alterations in key regulators such as cyclin-dependent kinases, retinoblastoma protein phosphorylation, and repression of the proto-oncogene myc as well as by modulating growth factor receptor-mediated signaling

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pathways (Koga et al., 1988; Kawa et al., 1996; Campbell et al., 1997; Xie et al., 1997; Yanagisawa et al., 1999; Sundaram et al., 2000; Gaschott and Stein, 2003; Li et al., 2004). In addition, calcitriol restores or enhances pro-apoptotic effects in cancer cells by several possible pathways, including repression of several pro-survival proteins such as Bc12 and telomerase reverse transcriptase and by activating pro-apoptotic proteins Bax and μ-calpain (James et al., 1996; Diaz et al., 2000; Jiang et al., 2004; Kumagai et al., 2005). Evidence also supports an anti-angiogenic effect of vitamin D. Vascular endothelial growth factor (VEGF) expression by cancer cells is suppressed and endothelial cell responses to VEGF are inhibited by vitamin D, an observation supported by in vivo xenograft studies (Mantell et al., 2000; Bao et al., 2006). The immunoregulatory effects of vitamin D may also have an impact on cancer biology. Inflammation is a critical early step in the carcinogenesis cascade for many cancers, and the ability of vitamin D to exhibit anti-inflammatory effects on cancer cells by down-regulating the pro-inflammatory pathways, such as cyclooxygenase-2, may contribute to cancer inhibition (Moreno et al., 2005). In contrast, the role of vitamin D in cancer immunosurveillance of nascent or established cancers remains to be defined.

The encouraging in vitro findings, tempered with concerns about hypercalcemia, led to the development of many vitamin D analogues in the hope of retaining anti-cancer activity, but without increasing serum calcium, for the pharmacological therapy of cancer, as recently reviewed (Beer and Myrthue, 2004; Masuda and Jones, 2006; Trump et al., 2010).

Vitamin D_2 Versus Vitamin D_3

Vitamins D_2 and D_3, as described previously, differ only in their side chain structure. Physiological responses to both forms of the vitamin include regulation of calcium and phosphate homeostasis and regulation of cell proliferation and cell differentiation of specific cell types, as described above. Qualitatively, vitamins D_2 and D_3 exhibit virtually identical biological responses throughout the body (i.e., through gene expression) that are mediated by the VDR (Jones et al., 1998; Jurutka et al., 2001).

Regarding the potency of the two forms of vitamin D, there are reports that certain animals, such as avian species and New World monkeys (Chen and Bosmann, 1964; Drescher et al., 1969), discriminate against vitamin D_2. However, it has been assumed for several decades that the two forms are essentially equipotent in humans (Christiansen et al., 1975). Recent reports involving human dietary studies have argued for (Trang et al., 1998; Armas et al., 2004) or against (Holick et al., 2008) a metabolic discrimination against vitamin D_2, compared with vitamin D_3. Part of the apparent conflict between these different studies (Trang et al., 1998; Armas et al.,

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2004; Holick et al., 2008) is almost certainly due to differences in size and frequency of dose (which have ranged from 1,000 IU daily doses to 50,000 IU in a single dose); the differences reported suggest a difference in pharmacokinetic parameters between vitamin D_2 and vitamin D_3.

This debate runs parallel to the suggestion that vitamin D_2 is less toxic than its vitamin D_3 counterpart. Experimental animal data from a number of mammalian species ranging from rodents to primates (Roborgh and de Man, 1959, 1960; Hunt et al., 1972; Sjoden et al., 1985; Weber et al., 2001), support the concept that the D_2 form is less toxic than D_3, but there is no evidence available in humans. Nonetheless, the implication of these diverse studies in several mammalian species is that vitamin D_2 compounds may show differences in pharmacokinetics that manifest as lower toxicity from high doses.

There is considerable evidence that most of the steps involved in the metabolism and actions of vitamin D_2 and vitamin D_3 are identical (Jones et al., 1998). The identification of the series of vitamin D_3 metabolites in the late 1960s and early 1970s was followed by the identification of their vitamin D_2 counterparts: $25OHD_2$, $1\alpha,25(OH)_2D_2$, and $24,25(OH)_2D_2$ (Suda et al., 1969; Jones et al., 1975, 1979, 1980a). Noteworthy here is the fact that the structural features unique to the vitamin D_2 side chain did not preclude either the 25- or 1α-hydroxylation steps in activation of the molecule or the first step of inactivation, namely 24-hydroxylation. Studies have also shown that the steps in the specific vitamin D signal transduction cascade do not appear to discriminate discernibly between the two vitamin D homologues at the molecular level (e.g., binding to the transport protein, DBP [Hay and Watson, 1977; Jones et al., 1980a] or binding to the receptor, VDR [Jones et al., 1980b; Reinhardt et al., 1989]). Overall, it can be concluded that specific signal transduction systems designed to respond to vitamin D_3 respond to physiological doses of vitamin D_2 equally well.

At this time, firm conclusions about different effects of the two forms of vitamin D cannot be drawn; however, it would appear that at low doses, D_2 and D_3 are equivalent, but at high doses, D_2 is less effective than D_3. In essence, the potency of the two forms (as judged by the dose required to cure rickets) is assumed to be the same (Park, 1940). Differences in toxicity for humans, as judged by the dose to cause hypervitaminosis D, are unclear, but there is evidence from experimental animal data to suggest that D_2 is less toxic than D_3.

Skeletal Disorders

Vitamin D deficiency results in inadequate mineralization of the skeleton. Commonly referred to as rickets in children and osteomalacia in adults, this disorder has been described in Chapter 2 relative to calcium.

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Vitamin D deficiency is characterized by aberrations in the mineralization of the bone. In children, the deficiency results in rickets (see also Chapter 2), in which the cartilage fails to mature and mineralize normally. Rickets is characterized by widening at the end of the long bones, rachitic rosary, deformations in the skeleton, including craniotabes and deformities of the lower limbs, known as bowed legs and knocked knees. In adults, the deficiency of vitamin D leads to osteomalacia in which the newly deposited bone matrix fails to mineralize adequately, and there are wide unmineralized bone matrix (osteoid) seams.

Vitamin D–dependent rickets type I (VDDR I) is an autosomal recessive trait that results in abnormally low calcitriol levels but normal serum 25OHD levels. The mutation in VDDR I affects the 1α-hydroxylase enzyme and leads to impaired intestinal calcium absorption and the resulting rickets (Fraser et al., 1973). VDDR I manifests in the first year after birth and is treated with calcitriol. Supplemental calcium and phosphate are usually not needed. The second disorder is vitamin D–dependent rickets type II (VDDR II), which results in hypocalcemia, tetany, convulsions, alopecia, and rickets. VDDR II is also an autosomal recessive trait, resulting from a mutation in the *Vdr* gene, which can appear in the second year after birth or go unrecognized until adulthood.

VITAMIN D ACROSS THE LIFE CYCLE

Overall, vitamin D's role at different life stages is less clearly age-related than that of calcium, and also less well understood, with numerous gaps in basic information. Although some aspects of vitamin D nutrition and physiology have been found to differ with life stage, most of the functions of vitamin D are quite consistent across life stages from infancy and childhood, to adolescence, adulthood, and old age. For all life stages highlighted below, specific studies and conclusions are detailed in Chapter 4.

Infancy

Healthy skeletal development in infancy requires adequate intakes of vitamin D as well as calcium. Inadequate vitamin D intake during periods of growth leads to development of vitamin D deficiency rickets, which when it occurs in North American populations typically manifests around 20 months of age (DeLucia et al., 2003). If rickets is diagnosed early, vitamin D therapy can cure it, but not if skeletal deformities are severe and growth plates have started to mature in puberty (DeLuca, 1979a). Infants at risk for developing rickets include those who are exclusively breast-fed, because vitamin D and 25OHD are normally present at low levels in breast milk (Bachrach et al., 1979; Ward et al., 2007). Health Canada currently

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recommends that exclusively breast-fed infants receive a supplement of vitamin D,[6] and the American Academy of Pediatrics guidelines support supplementation of breastfeeding infants with vitamin D (Gartner and Greer, 2003). Commercial infant formula contains vitamin D, as discussed previously in this chapter.

Childhood and Adolescence

This life stage is characterized by bone accretion. During the rapid growth phase of adolescence, almost 50 percent of the adult skeletal mass will be accumulated. The onset of puberty stimulates increased metabolism of 25OHD levels to calcitriol (Aksnes and Aarskog, 1982) and subsequent increased calcium intestinal absorption, decreased urinary calcium excretion, and greater calcium deposition into bone (Wastney et al., 1996). Information on relationships between 25OHD levels and optimal intestinal absorption of calcium or risk for rickets or fracture in children and adolescents is lacking, although Abrams et al. (2005) found evidence for an indirect relationship between low serum 25OHD and increased calcium absorption in young adolescents. Although a recent set of systematic reviews (Cranney et al., 2007; Chung et al., 2009), to be discussed in Chapter 4, did not report specifically on bone mass for this age group in relation to vitamin D nutriture, the reviews suggested the possibility of a relationship between serum levels of 25OHD and bone mineral density (BMD) in adolescents. A recent analysis of vitamin D intake and BMD in male and female adolescents and adults ages 13 to 36 years found positive correlations between vitamin D intake and bone density from adolescence into adulthood among male but not female subjects (van Dijk et al., 2009).

Adults

The life stages associated with younger adults, covering several decades, are characterized by a need for adequate nutrition for bone maintenance. The bone is constantly undergoing remodeling, and the maintenance of normal bone density reduces the risk of skeletal disorders ranging from osteomalacia to the onset of osteoporotic fractures later in life. It is also the time of pregnancy and lactation for some female members of this population.

Older adults, especially those characterized as frail, may have poor dairy and vitamin D intake, decreased sun exposure, reduced dermal conversion of 7-dehydrocholesterol to vitamin D_3 and secondary hyperparathy-

[6]Available online at http://www.hc-sc.gc.ca/fn-an/nutrition/infant-nourisson/vita_d_supp-eng.php (accessed September 1, 2010).

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roidism, all of which contribute to increased risk for poor bone health and osteoporotic fractures. As discussed below, there is inconsistent evidence as to whether intestinal absorption of vitamin D declines with age. In women, bone loss occurs as a result of the decreased estrogen levels that accompany menopause. As aging continues, both men and women experience age-related bone loss. As is the case for calcium intake, it is not well established whether and to what extent intakes of vitamin D may mitigate the bone loss.

Pregnancy and Lactation

The role of vitamin D in pregnancy and fetal development is the focus of current attention. However, at present the role of vitamin D is not clear, and there are very few data by which to examine the questions surrounding the effect of the nutrient on pregnancy and lactation. Animal studies and inferential human data do not readily elucidate a specific function in fetal development, especially with respect to formation and mineralization of the fetal skeleton. Calcitriol levels increase during pregnancy, but factors other than vitamin D appear to stimulate the increased calcium absorption. Although a number of avenues are still being explored, the bulk of the evidence suggests that calcium is moved from the mother to the fetus without requiring calcitriol.

Breast milk is not normally a significant source of vitamin D for the infant and remains unchanged with supplementation at least up to 2,000 IU/day. Existing evidence suggests that vitamin D nutriture does not appear to affect the maternal processes of bone resorption that occur during lactation, nor its restoration post-lactation.

MEASURES ASSOCIATED WITH VITAMIN D: SERUM 25OHD

Serum 25OHD level is widely considered as a marker of vitamin D nutriture, and consideration of serum 25OHD measures for the purposes of nutrient reference value development has generated notable interest. There is agreement that circulating serum 25OHD levels are currently the best available indicator of the net incoming contributions from cutaneous synthesis *and* total intake (foods and supplements) (Davis et al., 2007; Brannon et al., 2008; Davis, 2008). Thus, the serum 25OHD level may function as a *biomarker of exposure*; it is a reflection of the supply of vitamin D to the body and can be a useful adjunct to examining the intake level of vitamin D if the confounders and the measure's variability depending upon a range of variables are kept in mind. However, what is not clearly established is the extent to which 25OHD levels serve as a *biomarker of effect*. That is, there is some question as to whether levels of 25OHD relate to

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health outcomes via a causal pathway and can serve as predictors of such outcomes.

Research recommendations in the previous Dietary Reference Intake (DRI) review of vitamin D (IOM, 1997), as well as an Institute of Medicine (IOM) workshop on DRI research needs (IOM, 2007), called for studies to evaluate the intake requirements for vitamin D as related to optimal circulating 25OHD concentrations across life stage and race/ethnicity groups of U.S. and Canadian populations, taking into account variability in UVB radiation exposures. The issue of the role of serum 25OHD concentrations was also identified by the sponsors of this current study on vitamin D and calcium DRIs as central to the development of DRIs for vitamin D (Yetley et al., 2009). Much in the way of this information gap for serum 25OHD concentrations has not yet been addressed. Nonetheless, measures of serum 25OHD are important considerations in developing DRI values for vitamin D intake. The sections below highlight factors affecting serum 25OHD level and methodologies for its measurement. It is important to note that these discussions refer to 25OHD, not to calcitriol (i.e., 1,25-dihydroxyvitamin D). Calcitriol, the active hormonal form of the nutrient, has not been used typically as a measure associated with vitamin D nutriture or as an intermediate related to health outcomes. Calcitriol is not useful as such a measure, for several reasons. Its half-life is short (hours), its formation is not directly regulated by vitamin D intake, its levels are regulated by other factors (such as serum PTH), and, even in the presence of severe vitamin D deficiency the calcitriol level may be normal or even elevated as a result of up-regulation of the 1α-hydroxylase enzyme.

Factors Affecting Serum 25OHD Levels

Dietary Intake (Foods and Supplements)

Available literature demonstrates that serum 25OHD levels increase in response to increased vitamin D intake, although overall it can be concluded that the relationship is non-linear rather than linear. Factors that may affect the relationship between vitamin D intake and serum 25OHD levels are not entirely clear, and the reliability of such measures may be less than desirable. Moreover, there remains debate over the equivalence of vitamins D_2 and D_3 in the diet (Armas et al., 2004; Rapuri et al., 2004; Vieth, 2004), although it has been assumed that they are 25-hydroxylated at similar rates (see previous discussion of functions and physiological actions of vitamin D).

As part of the 2007 Agency for Healthcare Research and Quality (AHRQ) systematic review (Cranney et al., 2007, 2008), referred to hereafter as AHRQ-Ottawa, exploratory meta-regression analysis was conducted

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of 16 trials in adults, which suggested an association between vitamin D dose and serum 25OHD concentrations. The analysis found that for each additional 100 IU of vitamin D_3, serum 25OHD concentrations rose by 1 to 2 nmol/L. Trials varied in their use of vitamin D_2 or vitamin D_3. A few of the studies reported different effects of vitamin D_2 and vitamin D_3 on serum 25OHD levels. Although the later AHRQ-Tufts analysis (Chung et al., 2009) did not identify newer (compared with AHRQ-Ottawa) randomized controlled trials related to intake of vitamin D and serum 25OHD levels, it did graphically evaluate the net changes in serum 25OHD concentrations against the doses of vitamin D supplementation using data from the trials in adults. The analysis confirmed the relationship between increasing doses of vitamin D and increasing net change in serum 25OHD concentrations in both adults and children, but it also concluded that the dose–response relationships differ depending upon study participants' baseline serum 25OHD levels (≤ 40 vs. > 40 nmol/L) and duration of the supplementation (≤ 3 vs. > 3 months).

In a recent study conducted by Smith et al. (2009), personnel stationed in the Antarctic in winter months (and thus presumed to obtain vitamin D from food and supplements only) were given graded doses of 400, 1,000, or 2,000 IU of vitamin D_3 per day for 5 months. Baseline levels of serum 25OHD rose from approximately 44 nmol/L to 57, 63, and 71 nmol/L, respectively, representing a change in 25OHD levels of 13, 19, or 27 nmol/L. Evident in this study is the continuing fall in 25OHD level in the "no pill" group (to 34 nmol/L) of men who were deprived of sunlight and received approximately 250 to 350 IU of vitamin D (which included foods and any non-study supplements) per day. A possible complicating factor in interpreting these data is that the subjects were consuming diets with a vitamin D content that ranged from 241 to 356 IU/day, in addition to the graded doses of vitamin D from administered supplements, although the amounts of vitamin D obtained from foods (or non-study supplements) were not significantly different between treatment groups. Therefore, any effect of these sources of vitamin D on serum levels would have been consistent between treatment groups.

Another study of serum 25OHD response to total intake under conditions of minimal sun exposure used two populations based in Cork, Ireland (51°N), and Coleraine, Northern Ireland (55°N). The study estimated the dose of vitamin D required to maintain 25OHD levels above certain chosen cutoff values (i.e., 25.0, 37.5, 50.0, and 80.0 nmol/L) during the winter months (Cashman et al., 2008). The researchers found that serum 25OHD levels that ranged between 65.7 and 75.9 nmol/L in late fall fell in all groups receiving 200, 400, and 600 IU of vitamin D_3 per day, as well as in the placebo group, in winter. The decrease in the 600 IU/day group was minimal, from 75.9 to 69.0 nmol/L. Cashman et al. (2008) went on to

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plot the serum 25OHD levels attained after 5 months versus the estimated total vitamin D intake (approximately 0 to 1,400 IU/day) in 215 individuals, as shown in Figure 3-4. They concluded that, in these two populations at 51°N and 55°N, the wintertime intake required to achieve 25OHD cutoff levels of 37.5, 50.0, and 80.0 nmol/L were 796, 1,120, and 1,644 IU/day, respectively.

Importantly, the relationship between vitamin D intake and serum 25OHD response appears not to be linear, given evidence that increasing serum 25OHD level above 50 nmol/L requires more vitamin D intake than does increasing serum 25OHD levels when the starting point is less than 50 nmol/L (Aloia et al., 2008). Factors such as baseline serum 25OHD level in the population may be relevant. Further, there have been reports that the rise in serum 25OHD levels for a given dose tends to stabilize by week 6 (Harris and Dawson-Hughes, 2002; Holick et al., 2008) and that it does

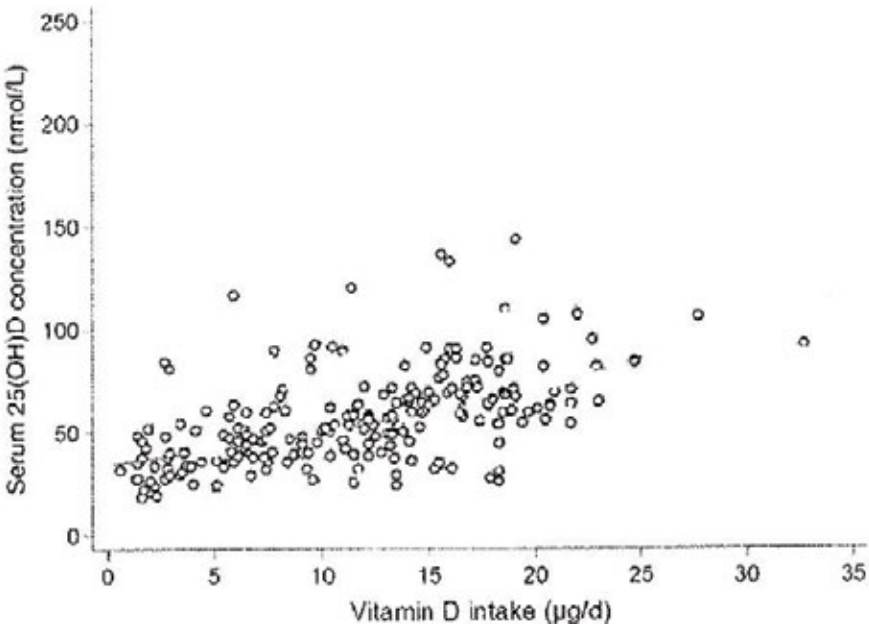


FIGURE 3-4 The relationship between serum 25-hydroxyvitamin D concentrations (in late winter 2007) and total vitamin D intake (dietary and supplemental) in 20- to 40-year-old healthy persons (n = 215) living at northern latitudes (51°N and 55°N).
SOURCE: Cashman et al. (2008). Reprinted with permission from the *American Journal of Clinical Nutrition* (2008, 88, 1535-42), American Society for Nutrition.

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not vary with age at least up to 80 years of age (Harris and Dawson-Hughes, 2002; Cashman et al., 2008, 2009).

Sun Exposure

The cutaneous synthesis of vitamin D, and in turn its contribution to the concentration of serum 25OHD, is initially dependent upon the presence of 7-dehydrocholesterol in the skin. However, many variables can affect the cutaneous synthesis of vitamin D, making it difficult to estimate an average amount of vitamin D and, in turn, serum 25OHD levels that are produced by sun exposure in North America. There is, however, agreement that sun exposure is a significant source of the circulating serum 25OHD in summer for many North Americans, and is notably reduced as a contributor in the winter months. Early work from Webb et al. (1988, 1989) as well as a letter from Holick et al. (1989) have outlined the role of sunlight in regulating cutaneous synthesis of the vitamin and have implicated factors in vitamin D_3 synthesis in the skin to include aging, melanin pigmentation, season of the year, latitude, and use of sunscreen. Matsuoka et al. (1992) has discussed the role of clothing in preventing synthesis.

The 2007 AHRQ-Ottawa systematic review (Cranney et al., 2007) noted that the few available randomized clinical trials conducted between 1982 and the time of the analysis, which focused on the effect of UVB radiation on serum 25OHD level revealed little information about the impact of age, ethnicity, skin pigmentation, BMI, or latitude on serum 25OHD levels. However, UVB exposure increased serum 25OHD levels in vitamin D–deficient and –sufficient subjects with mean increases ranging from 15 to 42 nmol/L.

The difference in seasonal contributions to serum 25OHD level from sun exposure is discussed first. Next, factors affecting the synthesis of vitamin D—and, in turn, the levels of 25OHD in serum—are outlined.

Effect of season on circulating serum 25OHD level Sunlight exposure as a source of cutaneous synthesis of vitamin D is subject to a number of limitations. For example, excess exposure can lead to photo-degradation as a regulatory mechanism to avoid toxicity (Chen et al., 2007). In addition, latitude, time of exposure, and season all affect cutaneous synthesis, depending on the ability of UV rays to stimulate vitamin D production. Differences in seasonal exposure can vary by as much as 6 months at extreme northern and southern latitudes (Lucas et al., 2005; Kull et al., 2009).

A number of studies have examined serum 25OHD levels in different seasons. Van der Mei et al. (2007) compared cross–sectional data from men and women less than 60 years of age living in Australia and concluded that season was a strong determinant of serum 25OHD concentrations. Berry

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et al. (2009) examined white adults ages 20 to 60 years living in the United Kingdom (UK) at latitude 53°N. In this study, women were found to have higher average serum 25OHD levels than men in both summer and winter (9 and 20 percent higher, respectively). In a study of young adults of diverse ethnic backgrounds living in Toronto, Gozdik et al. (2009) found that in winter, serum 25OHD levels of individuals with East and South Asian backgrounds were significantly lower than those of individuals with European ancestry. Kull et al. (2009) measured seasonal variance of 25OHD levels in adults ages 25 to 70 years living in Estonia in northern Europe, where dairy products are not fortified with vitamin D. During the winter, 73 percent of the study population had 25OHD levels that were below 50 nmol/L, and 8 percent had levels that were below 25 nmol/L, compared with 29 percent and 1 percent, respectively, during the summer. Rapuri et al. (2002) examined white, black, and Hispanic women 65 to 77 years of age in Omaha and reported mean serum 25OHD levels of 68 nmol/L in February and 86 nmol/L in August. These studies, despite variations in age, gender, and ethnicity, all suggest that seasonal change can affect cutaneous vitamin D synthesis. The winter low serum 25OHD concentrations and the summer high serum 25OHD concentrations from these studies are summarized in Table 3-1.

Although there are variations in the available data as well as a number of unknowns that may influence such values, they suggest a seasonal change in serum 25OHD concentrations between the winter nadir and the summer zenith of approximately 25 nmol/L. Free-living individuals in the latitudes studied appear to experience an approximately one-third seasonal increase in their circulating serum 25OHD levels as they moved from the winter months to the summer months.

The 25 nmol/L change in serum 25OHD level would appear to be similar in magnitude to change experienced by subjects given 2,000 IU/day in the Antarctic study (Smith et al., 2009). Although these data suggest that average cutaneous synthesis during the summer in northern latitudes equates to 2,000 IU/day, this may be a questionable conclusion given the many variables that come into play, ranging from feedback mechanisms to skin pigmentation to baseline levels of 25OHD. For example, recent work from Olds et al. (2008) suggested a curvilinear relationship between sun exposure and serum 25OHD levels, as well as variation depending upon initial concentrations of 7-dehydrocholesterol levels. At lower doses, vitamin D_3 production rises immediately in response to UV exposure, whereas at higher doses, the rate of production is lower and reaches an earlier plateau.

Effect of skin pigmentation on synthesis The presence of melanin in the epidermal layer is responsible for skin pigmentation. A number of

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TABLE 3-1 Winter Low 25OHD Levels and Summer High 25OHD Levels Around the World

Location (Latitude)	Winter Baseline	Summer High
Toronto, Canada[a] 43°N	35 nmol/L	50 nmol/L[b]
Tasmania, Australia[c] 43°S	40 nmol/L	62 nmol/L
Estonia, northern Europe[d] 59°N	44 nmol/L	59 nmol/L
Salford/Manchester, UK[e] 53°N	46 nmol/L	71 nmol/L
Geelong region, Australia[c] 38°S	57 nmol/L	93 nmol/L
Omaha, USA[f] 41°N	68 nmol/L	86 nmol/L

[a]Gozdik et al., 2009.
[b]high value recorded in autumn.
[c]van der Mei et al., 2007.
[d]Kull et al., 2009.
[e]Berry et al., 2009.
[f]Rapuri et al., 2002.

recent studies have reinforced the relationship of skin pigmentation to the capacity to produce vitamin D_3 after UV exposure, but the results are not all consistent. Armas et al. (2007) studied the incremental change in serum 25OHD levels in individuals with average 25OHD baseline levels of 52 nmol/L and with different skin pigmentation who were exposed to different daily doses of 20 to 80 mJ/cm^2 of UVB light three times per week for 4 weeks on 90 percent of their skin surface area. The work suggested that for individuals at the lighter pigmentation scale,[7] exposed to similar UVB doses (20 to 80 mJ/cm^2) resulted in twice the increase in serum 25OHD concentration compared with individuals at the opposite extreme (i.e., 62 vs. 32 nmol/L change) (Armas et al., 2007) (see modeled representation in Figure 3-5). However, other studies have shown that the response to UV dose is non-linear and dependent on genetic factors (Snellman et al., 2009), duration and dose rate of UV exposure, and baseline serum 25OHD levels (Bogh et al., 2010).

Another population study of 237 subjects at a single geographical location (Toronto, 43°N) also explored the effect of skin pigmentation on

[7]L*=70; the lightest skin tone of northern Europeans.

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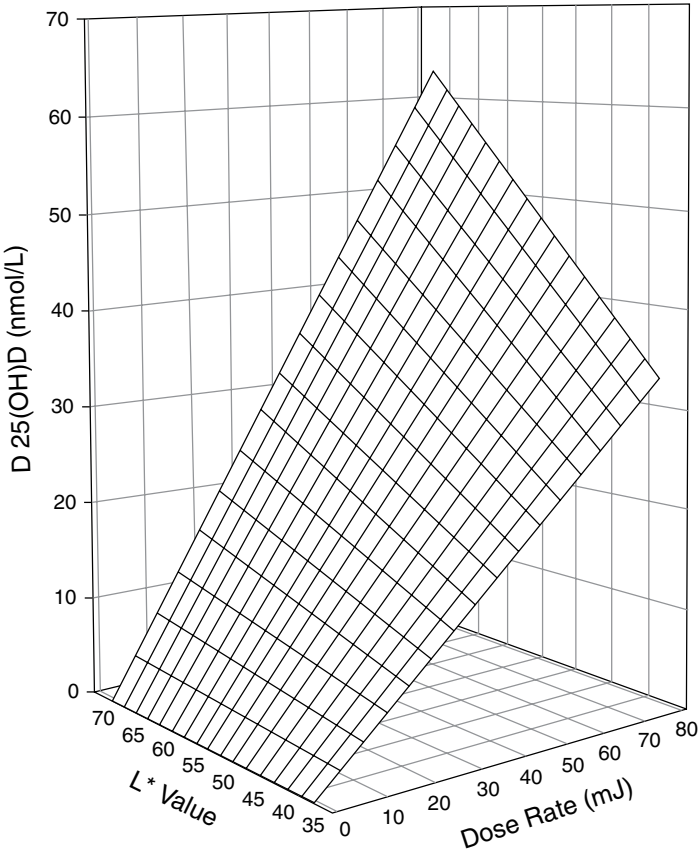


FIGURE 3-5 Three-dimensional scatter-plot of 4-week change in serum 25OHD concentration above baseline expressed as a function of both basic skin lightness (L*) and UVB dose rate. Surface is hyperboloid, plotting equation 1, and was fitted to data using least squares regression methods.
NOTE: Equation 1 is z = b * x * y, where z is the induced increase in serum 25OHD level from baseline (in nmol/L); x is UVB dose (in mJ/cm^2 per session); y is skin lightness (expressed as the L* score value from the reflective meter); and b is the sole parameter to be fitted.
SOURCE: Reprinted with permission from Robert Heaney.

25OHD synthesis (Gozdik et al., 2009). The mean serum 25OHD levels in Canadians of European, East Asian, and South Asian ancestry were 71.7, 44.6, and 33.9 nmol/L, respectively, in late fall; and 51.6, 28.1, and 26.5 nmol/L, respectively, in the winter. The authors found that differences between the three subgroups were strongly associated with skin pigmenta-

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tion as well as amount of time spent outdoors and total vitamin D intake. A recent study of 182 individuals in Denmark, screened in January and February and selected to reflect wide ranges of baseline 25OHD levels and skin pigmentation, found that the increase in 25OHD levels after UVB exposure was inversely correlated with skin pigmentation as well as with baseline 25OHD (Bogh et al., 2010).

A number of small studies have reported serum 25OHD levels to be consistently lower in persons with darker skin pigmentation, and data from NHANES suggest that serum 25OHD levels are highest in whites, lowest in non-Hispanic blacks, and intermediate in Hispanic groups (Looker et al., 2008). Overall, there is considerable evidence that darker skin pigmentation is associated with a smaller increase in serum 25OHD concentration for a given amount of UVB exposure.

Effect of latitude on synthesis Early on, in vitro methods, such as exposure of sealed vials of 7-dehydrocholesterol to UVB radiation under "idealized conditions" at various geographical locations, were used to assess the effect of latitude, time of day, and season on the rate of vitamin D production (Webb et al., 1988). However, this approach cannot completely simulate the in vivo conditions in the body, where many factors serve to regulate this process. Furthermore, although the measurement of vitamin D concentrations in a mixture of irradiation products is analytically simple, vitamin D_3 levels in human serum are rarely used to estimate cutaneous synthesis of the vitamin, in part because of the transient nature of this blood parameter and the difficulty of measuring the low levels in serum (Holick, 1988; Hollis, 2008). Nevertheless, Holick and colleagues used a serum vitamin D_3 assay to augment in vitro methodology and suggested that, at latitudes above 43°N, cutaneous synthesis contributes little serum 25OHD to the system in the winter months between October and March in North America (Webb et al., 1988; Matsuoka et al., 1989).

More recent data may call into question current assumptions about the effect of latitude. In fact, Kimlin et al. (2007), using computer modeling, concluded that it may no longer be correct to assume that vitamin D levels in populations follow latitude gradients. Indeed, the relationship between UVB penetration and latitude is complex, as a result of differences in, for example, the height of the atmosphere (50 percent less at the poles), cloud cover (more intense at the equator than at the poles), and ozone cover. The duration of sunlight in summer versus winter is another factor contributing to the complexity of the relationship. Geophysical surveys have shown that UVB penetration over 24 hours, during the summer months at Canadian north latitudes when there are many hours of sunlight, equals or exceeds UVB penetration at the equator (Lubin et al., 1998). Consequently, there is ample opportunity during the spring, summer, and fall months in the far north for humans (as well as animals that serve as food

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sources) to form vitamin D_3 and store it in liver and fat. These factors may explain why latitude alone does not consistently predict the average serum 25OHD level of a population.

Effect of sunscreen on synthesis Sunscreens are used to protect the skin from ultraviolet A (UVA) and UVB waveband exposure that is associated with deoxyribonucleic acid (DNA) damage—the same UVB exposure that is needed for vitamin D synthesis. Experimental studies suggest that sunscreens can decrease cutaneous vitamin D synthesis (Misra et al., 2008). However, emerging evidence suggests that although sunscreens are effective, many may not actually be blocking UVB because they are improperly or inadequately applied. Thus, sunscreen use may not actually diminish vitamin D synthesis in real world use, although further study is needed to verify its actual impact (Diehl and Chiu, 2010; Springbett et al., 2010).

Other variables affecting synthesis A number of other variables can impede sun exposure and thus inhibit cutaneous vitamin D synthesis. Clothing is an effective barrier to sun exposure and the UVB waveband, but the effectiveness of sun blocking depends on the thickness or weave of the fabric (Diehl and Chiu, 2010). Likewise, ethnic practices, such as extensive skin coverage with clothing, urban environments that reduce or block sunlight, air pollution, and cloud cover that reduces solar penetration can variously reduce sun exposure. In contrast, high altitude reduces the atmospheric protection against UVB waveband and can increase risk for sun damage as well as increase vitamin D synthesis (Misra et al., 2008). There may be a role for measures of physical activity in affecting vitamin D synthesis, although many covariates may be relevant, and some have suggested that genetics can account for some of the differences in synthesis of serum 25OHD.

Confounders Affecting Serum 25OHD Concentrations

Adiposity Interpreting data on serum 25OHD concentrations in obese and overweight persons is particularly challenging. Data from NHANES showed lower circulating levels of 25OHD among young adult obese non-Hispanic white women compared with their leaner counterparts; the relationship appeared to be weaker among non-Hispanic blacks. Differences in physical activity levels partially explain these differences (Looker, 2005, 2007). However, overweight and obese persons in NHANES also reported lower use of dietary supplements than did leaner persons of the same age or gender group (Radimer et al., 2004; Picciano et al., 2007), suggesting that lower dietary exposures could also contribute to the lower serum 25OHD levels in obese and overweight people.

Sequestration of vitamin D into fat likely also plays a significant role in

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reducing the amount that can be presented to the liver for 25-hydroxylation. As noted previously, vitamin D is absorbed with fat as part of chylomicrons and is taken up first by peripheral tissues that express lipoprotein lipase, especially adipose tissue and skeletal muscle. This pathway predicts that increased adiposity should lead to lower serum 25OHD levels and, conversely, that weight loss should reduce peripheral sequestration and enable higher 25OHD levels. Consistent with this, not only does increasing adiposity correlate with lower 25OHD levels, but also a few studies of modest weight loss have found the circulating 25OHD levels to increase despite no increased intake of vitamin D from diet or sunlight exposure (Riedt et al., 2005; Reinehr et al., 2007; Zittermann et al., 2009; Tzotzas et al., 2010). The measured increase in serum 25OHD levels in overweight and obese individuals was about 1.5 nmol/L for a 100 IU/day vitamin D intake over 12 months (Sneve et al., 2008; Zittermann et al., 2009). Others found that obese subjects show a lower rise in serum 25OHD levels in response to both oral vitamin D intake and UVB exposure (Wortsman et al., 2000) or in a retrospective analysis in response to 700 IU/day vitamin D_3 supplementation (Blum et al., 2008). It is interesting that in severely obese individuals after malabsorptive gastric bypass surgery, vitamin D supplementation resulted in a marked rise in serum 25OHD level of approximately 3 nmol/100 IU intake when the dose was 800 to 2,000 IU/day, but only a 1 nmol/L rise when intake was increased to 5,000 IU/day (Goldner et al., 2009).

African American ancestry Serum 25OHD levels are lower in African Americans compared with light-skinned population groups (Looker et al., 2008), yet the risk for fracture is lower for African Americans than for other ethnic groups (Aloia, 2008). It should be noted, however, that there is a wide range of variability among individuals of any race or ethnicity (Aloia, 2008). Serum 25OHD levels in African Americans and whites have been shown to be similarly responsive to vitamin D supplementation at 40°N latitude (equivalent to Philadelphia or Indianapolis), increasing by 1 to 2 nmol/L per 100 IU/day at a dose of 3,440 IU/day (Aloia et al., 2008), although at doses below 2,000 IU/day, serum 25OHD levels do not increase above 50 nmol/L in African American girls (Talwar et al., 2007). The significance of maintaining a higher serum 25OHD level in African Americans is not understood at this time because of a lack of evidence on extra-skeletal effects of vitamin D.

Size and frequency of dose Dosing of vitamin D daily, weekly, or monthly has been tested, and there are reports of annual dosing as well. The results of a study by Chel et al. (2008) suggested that daily (600 IU/day) and weekly doses of vitamin D will increase serum 25OHD levels more than monthly doses, but Ish-Shalom et al. (2008) using a dose of 1,500 IU/day

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found no difference due to timing of the doses. Ish-Shalom et al. (2008) suggested that the attenuated response to monthly dose in the Chel et al. (2008) study may have been due to poor compliance with a powdered monthly supplement compared with pills used for their daily and weekly doses. An alternative explanation is that only a lower (Chel et al., 2008) and not a higher (Ish-Shalom et al., 2008) dose is influenced by timing of the vitamin D supplement. Thus far, studies suggest that weekly and daily dosing give similar serum 25OHD responses.

Assays for Serum 25OHD

Serum 25OHD comprises the sum of $25OHD_2$ and $25OHD_3$. Because of the widespread use of both vitamin D_2 and vitamin D_3 in the United States and Canada, analysts must measure both $25OHD_2$ and $25OHD_3$ in order to provide the total 25OHD level in serum. This is in contrast to the situation in Europe where there has been a tradition of using only vitamin D_3 and where commercial methods that purport to measure only $25OHD_3$ are available.

In North America, several assay types are currently in use, each with strengths and weaknesses (Makin et al., 2010). The two most common types of assays are

- Antibody-based methods, which use a kit or an automated clinical chemistry platform; and
- Liquid chromatography (LC)-based methods, which use automated equipment featuring either UV or mass spectrometric (MS)-detection.

As discussed below, both these methods are equivalent in terms of measuring the physiologically relevant parameter (total 25OHD level in serum), but there remains controversy over the performance of these assays in clinical and research laboratories. Moreover, reports in the literature for serum 25OHD measures should be interpreted with care, taking into account the type of assay employed, use of automation, year of analysis, and context of the analysis.

Overview of Assay Methodology

Assays for total 25OHD level in serum have existed for four decades since the metabolite was first discovered (Blunt et al., 1968). The earliest assays were competitive protein-binding assays (CPBAs), based upon the ability of either $25OHD_2$ or $25OHD_3$ to displace $[^3H]25OHD_3$ from the plasma binding protein, DBP (Belsey et al., 1971; Haddad and Hahn,

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1973). These assays incorporated extraction, chromatographic purification, and detection steps, but the assays were laborious and not easily simplified, owing to the presence of co-extracted interfering substances. They therefore fell out of favor. Non-chromatographic CPBA assays tended to read erroneously high, and some data from this period (1970s) are viewed as extremely questionable for this reason.

In the late 1970s LC-based assays emerged that used refined extraction and chromatographic steps with some form of fixed- or variable-wavelength UV detector to measure the distinctive native absorbance of the vitamin D metabolites with λmax at 265 nm (Jones, 1978; Horst et al., 1979). These allowed for the separate estimation of $25OHD_2$ and $25OHD_3$ from serum samples. This type of assay has undergone much refinement over the years, with improvements in LC, improved automation, or the introduction of diode-array UV detectors that minimize the chance of picking up interfering substances (Lensmeyer et al., 2006). Although these assays require expensive equipment as well as sequential sample analysis, which make them somewhat time-consuming, they are still popular with a minority of analysts.

In the early to late 1980s, antibody-based assays were introduced, which use a proprietary antibody to a vitamin D molecular antigen, usually with a truncated vitamin D side chain. They are therefore devoid of the features that allow the resultant antibody to distinguish between $25OHD_2$ and $25OHD_3$ (Hollis and Napoli, 1985); this is of value, but at the expense of detecting other minor metabolites. Consequently, antibody-based methods measure only total 25OHD in serum. Various commercial assays differ because of the nature of the antibody used, some claiming an advantage that they do not discriminate between $25OHD_2$ and $25OHD_3$ (Hollis and Napoli, 1985), whereas others in fact do underestimate the $25OHD_2$ pool and therefore provide correction factors to compensate for high $25OHD_2$ content. Over the past decade, antibody-based 25OHD assays have become automated into a multiwell plate-format and the increased throughput has made them extremely popular. It is important to note that the majority of the data collected over the past 20 to 30 years have been analyzed using antibody-based assays.

Recently, LC-based assays have also undergone a radical transformation with the replacement of the UV detection step by a "universal detector" in the form of a tandem mass spectrometer (LC-MS/MS) (Jones and Makin, 2000). LC-MS/MS assays allow the analyst to discriminate between $25OHD_2$ and $25OHD_3$ and other serum lipids by their unique molecular masses and mass fragments. Accordingly, these methods measure $25OHD_2$ and $25OHD_3$, and therefore total 25OHD (Makin et al., 2010). Because these methods use short LC retention times, automated robotic extraction and LC separation steps, and computerized MS systems, they can be made relatively operator-free and provide high throughput. Further, their potential

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advantages also include high specificity, high sensitivity, and better reproducibility (< 10 percent). The consensus among analysts is that LC-MS/MS assays will become the "gold standard" for assay performance in the future.

Thus, a variety of methods are available to determine serum 25OHD levels, each with its advantages and disadvantages that must be considered in evaluating the data arising from them.

Assay Performance Concerns

The methodologies used over four decades of assaying 25OHD levels in serum have each presented technical problems. These include, first, CPBA assays without chromatography that read high because of poorly defined "interferences" (Morris and Peacock, 1976; Stamp et al., 1976). Second, some LC-MS/MS assays with short LC run times read high because they cannot resolve 3-epi-$25OHD_3$, an isomer found in abundance in neonatal samples (Singh et al., 2006). Third, some antibody-based assays, particularly some of the automated versions, fail to recover or detect, and therefore underestimate $25OHD_2$ (Carter et al., 2004). Fourth, some antibody-based assays overestimate values by detecting further metabolites of 25OHD (e.g., 24,25$[OH]_2$D and 26,23-lactone) particularly those found in hypervitaminotic D situations (i.e., 25OHD > 250 nmol/L) (Jones et al., 1987); and some antibody-based methods are sensitive to exogenous interferences such as the presence of dilutants (ethanol, non-human serum) used in quality control materials (Phinney, 2009). Thus, no assay is free from problems, and performance must be monitored vigilantly.

Performance has been a concern of analysts and clinicians in the vitamin D field for some time. Inter-laboratory comparisons have been conducted and published for two decades and suggest an unacceptable degree of variability in the different results (Jongen et al., 1984, 1989). This has led to the creation of external quality assurance schemes, such as the Vitamin D External Quality Assurance Scheme or DEQAS[8] (Charing Cross Hospital, London, UK), similar to those used in other areas of clinical chemistry. Since its inception in the early 1990s, DEQAS has grown steadily, such that it now serves as a quarterly monitor of performance of analysts and 25OHD analytical methods for approximately 700 laboratories worldwide (Carter et al., 2010). Although initially DEQAS used gas chromatography-mass spectrometry (GC-MS) as its "gold standard," more recently it has adopted an all-laboratory trimmed mean (ALTM) as the value it uses to judge performance. Using ALTM, DEQAS has served as an early-warning system for method and operator biases that have alerted the commercial kit manufacturers to modify their products or steps in their procedures or withdraw their kits. DEQAS has published performance characteristics (precision,

[8]Available online at http://www.deqas.org/ (accessed March 9, 2010).

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accuracy, and variability) regularly over the past decade (Carter et al., 2004; Carter, 2009; Jones et al., 2009). These publications have been augmented by other independent method comparisons (Glendenning et al., 2006; Lensmeyer et al., 2006; Roth et al., 2008).

In brief, these performance reports suggest some method biases in terms of accuracy and precision as well as variability as high as 15 to 20 percent. However, some skilled analysts can perform better than this with a coefficient of variation less than 10 percent. The recent introduction of the National Institute of Standards and Technology (NIST) reference standards[9] calibrated using a "validated" LC-MS/MS method (Phinney, 2009) offers some hope that the variability of all methods can or will be improved in the future. Indeed, recent data suggest that an improvement is already occurring (Carter and Jones, 2009). At this time, however, serum 25OHD data in the literature must be viewed with care based upon the knowledge that they have been acquired using a variety of methods, each with its own shortcomings and subject to high variability.

Assay Shift and Drift: U.S. National Health and Nutrition Examination Survey

Recently, the National Center for Health Statistics (NCHS) posted an analytical note on its NHANES web page informing users about two issues that should be addressed when analyzing and using serum 25OHD data from NHANES.[10] The first involved making direct comparisons between serum 25OHD levels from NHANES III (1988 to 1994) and those from the 2000 to 2006 NHANES because of a reformulation of the DiaSorin radioimmunoassay (RIA) kit[11] that resulted in shifts in assay results between the two time periods. Second, the note also cautioned that the data from the 2000 to 2006 NHANES were likely affected by drifts in the assay performance (method bias and imprecision) over time. A standard reference material (SRM 972, Vitamin D in Human Serum) and calibration solution (SRM 2972, 25-Hydroxyvitamin D_2 and D_3 Calibration Solutions) are currently available from the NIST. The NCHS plans to incorporate use of this SRM into future measures of 25OHD in NHANES. The web page also indicates that guidance concerning a correction factor and data interpretation regarding this shift and drift will be forthcoming from the agency. In the case of the Canadian survey of 25OHD levels in the Canadian population, the analytical methods used to determine 25OHD concentrations were the

[9]Available online at http://ts.nist.gov/measurementservices/referencematerials/index.cfm (accessed March 15, 2010).

[10]Available online at http://www.cdc.gov/nchs/data/nhanes/nhanes3/VitaminD_analytic note.pdf (accessed March 17, 2010).

[11]DiaSorin Radio-immunoassay (RIA) (Stillwater, MN).

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Diasorin total 25OHD (Liaison) kit[12] (personal communication, S. Brooks, Health Canada, December 18, 2009).

MEASURES ASSOCIATED WITH VITAMIN D: PARATHYROID HORMONE

PTH, which, as described above, plays a role in vitamin D metabolism, is also a known marker for bone resorption, based upon the bone manifestations of secondary hyperparathyroidism—increased bone turnover, increased rates of bone loss, osteoporosis, and increased risk of fractures. Serum PTH level has thus been explored and suggested as a measure indicative of adequate vitamin D nutriture, notably on the basis of the level of serum 25OHD at which serum PTH level rises or, alternatively, the level of serum 25OHD at which serum PTH level no longer declines. This measure is discussed more fully in Chapter 4.

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4

Review of Potential Indicators of Adequacy and Selection of Indicators: Calcium and Vitamin D

APPROACH

The first step in the decision-making process associated with the development of Dietary Reference Intakes (DRIs) is the identification of potentially useful measures—indicators—that reflect a health outcome associated with the intake of the nutrient. As described in Chapter 1, this is classically referred to as hazard identification, the first step of risk assessment. The available data are examined to determine their relevance and validity as well as strengths and limitations for elucidating a relationship between the health outcome of interest (including chronic disease risk) and the intake of the nutrient.

In considering reference values for calcium and vitamin D, there are challenges in organizing a data review to examine these nutrients independently, because they act in concert and are often administered together in experimental studies. To the extent possible, the independent effects of these nutrients were explored and taken into account; when this was not possible or not appropriate, the combined effect was considered. This chapter reviews evidence for calcium and vitamin D jointly to avoid redundancy. Evidence related to potential indicators for adverse effects of excess intake of calcium and vitamin D is reviewed separately in Chapter 6.

Identification of Potential Indicators for Calcium and Vitamin D

The array of potential health outcomes to be considered for these two nutrients was identified using five sources:

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1. Agency for Healthcare Research and Quality (AHRQ) evidence report issued in 2007 (Cranney et al., 2007), hereafter referred to in this chapter as AHRQ-Ottawa without a reference citation; and
2. AHRQ evidence report issued in 2009 (Chung et al., 2009), hereafter referred to in this chapter as AHRQ-Tufts without a reference citation;
3. The Institute of Medicine (IOM) report *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride* (IOM, 1997);
4. Literature searches conducted by the committee;
5. Publicly available input from stakeholders either through written submissions to the committee or as presented during the information gathering workshop.

As outlined in Chapter 1, the ARHQ analyses are highly relevant to DRI development. Evidence-based systematic reviews have been identified as a useful tool for the purposes of dietary reference value development (Russell et al., 2009), and the work of this committee was enhanced by the availability of these two high-quality evidence reports from AHRQ. The approach used, questions asked, data search criteria, and the detailed results from the AHRQ-Ottawa and AHRQ-Tufts can be found in Appendixes C and D.

In sum, the focus of AHRQ-Ottawa was on the:

- Association of specific circulating 25-hydroxyvitamin D (25OHD) concentrations with bone health outcomes in children, women of reproductive age, postmenopausal women, and elderly men;
- Effect of vitamin D dietary intake (fortified foods and/or supplements) and sun exposure on serum 25OHD levels;
- Effect of vitamin D on bone mineral density (BMD) and fracture or fall risk; and
- Identification of potential harms associated with vitamin D exposures above current reference intakes.

The AHRQ-Tufts evidence report analyzed data related to calcium and vitamin D with respect to a broader spectrum of health outcomes. AHRQ-Tufts also served to update and expand AHRQ-Ottawa. Specifically, AHRQ-Tufts focused on the:

- Relationship between vitamin D and growth, cardiovascular disease (CVD), body weight, cancer, immunological outcomes, bone health, all-cause mortality, hypertension/blood pressure, and BMD and bone mineral content (BMC); and

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- Relationship between calcium and growth, CVD, body weight, and cancer.

Neither AHRQ report reviewed calcium alone as a factor in bone health.

A key component of systematic reviews of scientific literature is a specification of the quality of the available data. The AHRQ grading system is summarized in Box 4-1. In the case of the systematic analysis carried out by AHRQ-Ottawa, the Jadad scale (Jadad et al., 1996) was used for quality assessments of randomized controlled trials (RCTs). The Jadad scale is a validated scale designed to assess the methods used to generate random assignments and double blinding. The scale also scores whether there is a description of dropouts and withdrawals by intervention group. Jadad scores range from 1 to 5, and a total score of 3 and above indicates studies of higher quality. Further, to assess the quality of the observational studies, a grading system adapted from R. P. Harris et al. (2001) was used. In the case of the AHRQ-Tufts analysis, a three-category grading system ("A," "B," or "C") was adapted from the AHRQ Methods Reference Guide for Effectiveness and Comparative Effectiveness Reviews (AHRQ, 2007). This system defines a generic grading system that is applicable to each type of study design including interventional and observational studies; it is summarized in Box 4-1.

The committee's literature search identified relevant evidence outside the scope of, or not included in, the two AHRQ reports as well as newer data available after the cutoff date of the AHRQ-Tufts analysis in 2009. The nature of the literature search is outlined in Appendix E. The literature base that was included in the 1997 report of the IOM committee tasked with DRI development for calcium and vitamin D (IOM, 1997) was also considered. Additionally, information gathered as part of a public workshop and several open committee sessions (see Appendix J) and a white paper requested by the committee (Towler, 2009) were taken into account.

Through use of the five data sources listed above, health outcomes of potential interest were identified. They are listed alphabetically in Table 4-1 and are grouped by general outcome. In addition, there is the possibility of intermediate variables that are not validated biomarkers of effect for health outcomes, but which may have the potential to be useful in the development of DRIs. Two such variables were considered: serum 25OHD concentrations and levels of parathyroid hormone (PTH).

Review of Data

General Principles

Within the scientific and clinical literature, there is a general hierarchy of study design. The lowest form of evidence is the idea or opinion,

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BOX 4-1
AHRQ Critical Appraisal and Grading of Evidence

Grading system used by AHRQ-Ottawa:

Basic Jadad score is assessed based on the answer to five questions listed below. Questions that are answered with a "yes" gain 1 point; questions answered with a "no" receive 0 points; the maximum score is 5. A score of 0 to 2 points is considered "low" quality, and a score of 3 to 5 points is considered "high" quality.

1. Was the study described as random?
2. Was the randomization scheme described and appropriate?
3. Was the study described as double-blind?
4. Was the method of double-blinding appropriate? (Were both the patient and the assessor appropriately blinded?)
5. Was there a description of dropouts and withdrawals?

Grading system used by AHRQ-Tufts (based on criteria below):

A = highest quality
Studies have the least bias and results are considered valid. These studies adhere mostly to the commonly held concepts of high quality, including the following: a formal study design; clear description of the population, setting, interventions, and comparison groups; appropriate measurement of outcomes; appropriate statistical and analytical methods and reporting; no reporting errors; less than 20 percent dropout; clear reporting of dropouts; and no obvious bias. Studies must provide valid estimation of nutrient exposure from dietary assessments and/or biomarkers with reasonable ranges of measurement errors and justifications for approaches to control for confounding in their design and analyses.

B = medium quality
Studies are susceptible to some bias, but not sufficient to invalidate the results. They do not meet all the criteria in category "A"; they have some deficiencies, but none likely to cause major bias. The study may be missing information, making it difficult to assess limitations and potential problems.

C = low quality
Studies have significant bias that may invalidate the results. These studies have serious errors in design, analysis, or reporting; there are large amounts of missing information or discrepancies in reporting.

SOURCES: Jadad et al., 1996; Cranney et al., 2007; Chung et al., 2009.

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TABLE 4-1 Alphabetical Listing of Potential Indicators of Health Outcomes for Nutrient Adequacy

Indicator	AHRQ (Ottawa and Tufts)
Cancer/neoplasms	
• All cancers	✓
• Breast cancer	✓
• Colorectal cancer/colon polyps	✓
• Prostate cancer	✓
Cardiovascular diseases and hypertension	✓
Diabetes (type 2) and metabolic syndrome (obesity)	✓
Falls	✓
Immune response	✓
• Asthma	—[a]
• Autoimmune disease	✓
◦ Diabetes (type 1)	✓
◦ Inflammatory bowel and Crohn's disease	✓
◦ Multiple sclerosis	✓
◦ Rheumatoid arthritis	✓
◦ Systemic lupus erythematosus	—[a]
• Infectious diseases	✓
◦ Tuberculosis	—[a]
◦ Influenza/upper respiratory infections	—[a]
Neuropsychological functioning	—[b]
• Autism	—[b]
• Cognitive function	—[b]
• Depression	—[b]
Physical performance[c]	✓
Preeclampsia, pregnancy-induced hypertension, and other non-skeletal reproductive outcomes	✓
Skeletal health (commonly bone health)	
• Serum 25OHD, as intermediate	✓
• Parathyroid hormone, as intermediate	✓
• Calcium absorption	✓
• Calcium balance	✓
• Bone mineral content/bone mineral density	✓
• Fracture risk	✓
• Rickets/osteomalacia	✓

[a]Specific condition not reviewed as a health outcome in AHRQ.

[b]Outcome category not considered in AHRQ.

[c]In the discussions within this chapter, physical performance is considered together with falls to avoid redundancy.

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followed, in ascending order, by case reports, case series, case–control studies, cohort studies, and, finally, the highest form of evidence, the randomized, controlled, double-blind trial (Croswell and Kramer, 2009). Only the RCT can show a causal relationship between an intervention and an outcome. Observational evidence can show only associative links, not causality. The highest level of observational evidence is the cohort study—a large, population-based, prospective investigation to compare an exposed group with an unexposed group. However, the cohort study does not reach the level of evidence of an RCT, because the intervention is not a random or chance event; rather it is the choice of the investigator (Croswell and Kramer, 2009). Nested case–control studies are a type of cohort study and were considered at that level of evidence; in some literature, populations from RCTs were evaluated as a cohort (adjusting for treatment assignment or limiting the analysis to the control group) and thus are at the same level of evidence as other observational research.

A summary of the strengths and weaknesses of the various types of observational studies and RCT studies is shown in Table 4-2. Flaws, biases, and confounding effects are an inevitable aspect of any study design, and the strength of a study therefore depends on the ability of the investigator to control such methodological obstacles. In addition, even well-designed studies can be weakened by complications such as loss to follow-up, missing outcomes, subject non-compliance, and a biased selection process (Baker and Kramer, 2008).

The Process

In addition to its consideration of the AHRQ analyses, the committee conducted searches of several online bibliographical databases, including Medline, Science Direct, and WorldCat/First Search. Evidence searches were carried out to identify relevant RCTs in support of a causal relationship between vitamin D and/or calcium and the health outcome under consideration, and these were weighted as the strongest type of evidence for development of a DRI. The second tier of evidence considered was observational to support associative relationships between vitamin D and/or calcium and a health outcome. Further examination was carried out to determine the quality of the observational evidence and whether the results were in agreement with RCT outcomes for a specific indicator. Potential confounders were also taken into account. Figure 4-1 shows the committee's ranking of evidence by the strength of the study design. In the figure, RCTs prevail over observational and ecological studies as the strongest evidential support and were therefore necessary for a health outcome indicator to be further considered for DRI development. When the totality of evidence, including causal evidence, was supported by concordance

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TABLE 4-2 Comparison of the Strengths and Weaknesses of Observational Study Designs and Randomized Controlled Trials for Use in DRI Development

Study Type/ Definition	Strengths	Weaknesses	Quality Ranking For DRI Development
Ecological *An observational study in which the units of analysis are populations or groups of people, rather than individuals*	• Provides an exploratory overview or indication for a potential association with outcome of interest	• Outcome measures are not predictable at the individual level	Low
Cross–sectional *An observational study in which a statistically significant sample of a population is used to estimate the relationship between an outcome of interest and population variables as they exist at one particular time*	• Allows for study of either a whole population or a representative sample • Provides estimates of prevalence of all factors measured • Facilitates greater generalizability	• Possible selection bias • Susceptible to mis-classification • Poor design for uncommon diseases or conditions • Simultaneous data collection obscures the order of effects	Low moderate
Case–control *An observational epidemiological study of persons with the outcome variable of interest and a suitable control group of persons without the variable of interest*	• Good design for uncommon diseases or conditions • Time and resource efficient	• Does not provide an estimate of incidence or prevalence of the disease, unless data about the population size are available • Possible selection bias • Susceptible to mis-classification • Simultaneous data collection obscures the order of effects	Moderate

continued

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TABLE 4-2 Continued

Study Type/ Definition	Strengths	Weaknesses	Quality Ranking For DRI Development
Cohort *A method of epidemiological study in which subsets of a defined population can be identified as exposed to a factor hypothesized to influence the probability of occurrence of an outcome*	• Good design for common diseases or conditions • Relative timing of exposure and disease is less confusing than with other observational study designs	• Can be expensive and time-consuming • Possible selection bias from loss to follow-up • Statistically inefficient	High moderate
Randomized controlled trial *An experimental study design in which exposure is randomly assigned and in which the frequency of the outcome of interest is compared between one or more groups receiving an experimental treatment and a group receiving a placebo or the current standard of care*	• More similar to experimental study design than to observational design • Provides strongest evidence for causality • Fulfills the basic assumption of statistical hypothesis tests	• Expensive and time-consuming • Subjects may not be representative of all who might receive treatment	High

SOURCE: Gordis (2009).

between RCTs and high-quality observational evidence and had strong biological plausibility, the committee gave further consideration to a potential indicator for development of a DRI. When observational evidence failed to support the findings of RCTs, the indicator's validity for consideration was reevaluated, and a decision to give further consideration was made on the balance of the totality of evidence.

For each potential indicator discussed in this chapter, the review of evidence included consideration of the analytical approach, study population, and research protocol design and the overall quality of the evidence for each study reviewed. The introductory statement for each indicator includes ecological studies. Observations made from such studies require caution in their interpretation because the outcome measures are not known at the individual level, and inferring individual characteristics or

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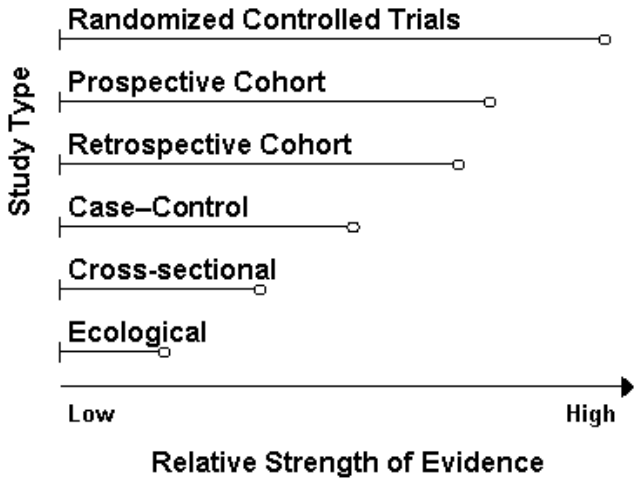


FIGURE 4-1 Ranking study designs: Ranking is shown in descending order of quality from top to bottom; the length of bars is arbitrary and indicates the relative strength of a study design.

relationships from group-level measures would be fallacious. Ecological studies, however, can contribute important information in more than an exploratory manner. Where it was relevant or needed in the absence of human studies, evidence for biological plausibility was included in the review as gleaned from experimental animal and mechanistic studies. The observational evidence reviewed included cross–sectional, case–control, and cohort (prospective and retrospective) studies. As pointed out previously, the strongest evidence among observational studies is from the cohort study. This study design offers an advantage over the case–control design in that it allows for observation of the incidence of a health outcome or the rate at which the health outcome develops in association with vitamin D or calcium intake or status in the population under study. In case–control studies, cases are included without identifying the entire "exposed" and "unexposed" populations from which they were derived, thus inferences drawn about a health outcome related to vitamin D or calcium intake or status are less reliable using this type of design.

As a tool to aid in the review process, the committee developed evidence "maps" for each indicator to provide an overarching view of the balance of relevant evidence from ecological and biological plausibility studies, observational studies, systematic reviews, and RCTs (including trials where the indicator was a primary outcome as well as other evidence from trials where the indicator was a secondary or non-pre-specified outcome). These served largely as an organizing tool and are included in Appendix

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F. The organizational construct of the maps did not allow distinctions between studies relative to the quality of the study design; however, this was considered by the committee in the overall evaluation of data.

The nature of the data surrounding each potential indicator is described below, beginning with a brief statement about the condition under consideration, followed by a summary of the evidence for ecological and biological plausibility studies, observational studies, systematic reviews from the two AHRQ reports, and additional evidence not covered in the AHRQ reviews. Each indicator is then evaluated in a summary discussion of the utility of the evidence for DRI development.

REVIEW OF POTENTIAL INDICATORS

Owing to the importance of a variety of acute and chronic diseases as public health concerns and the accumulating data focused on the hypothesis that vitamin D and/or calcium may have an impact on disease risk, it was crucial that this committee consider a wide spectrum of indicators for DRI development. After reviewing the available data, including recent systematic reviews from AHRQ and other literature, the committee chose to focus on areas where the research database is most compelling and the indicator is of public health concern within the context of DRI development. The following discussions review the roles of vitamin D and calcium in the reduction of risk for the health indicators identified in Table 4-1.

The entirety of evidence for each indicator that was reviewed by the committee cannot be presented in detail here, and the following discussions are a summary of relevant evidence. In drawing its conclusions about an indicator, the committee evaluated the strengths and weaknesses of the studies considered for each indicator, including an examination of the methods used for measuring an indicator, its relevance to total intake and functional or physiological outcomes, and the strength of the study design. This approach is summarized in Box 4-2.

Cancer/Neoplasms

As the second leading cause of death in the United States, cancer is a major public health concern. Cancer encompasses a wide range of malignancies with many variations in etiology and pathogenesis. Thus, the committee considered not only total cancer, but also specific malignancies in which vitamin D and/or calcium have been examined for an interaction thought to play a role.

Cancer is a disease in which genetically damaged cells within a tissue experience uncontrolled growth and invasion with subsequent spread to other host organs. The metastatic spread leads to dysfunction of vital organs causing significant morbidity and culminating in death. An expanding

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BOX 4-2
Evaluation of Evidence for DRI Development

In its review of evidence, the committee used a qualitative approach to determine its confidence in interpreting positive or negative relationships between vitamin D and/or calcium and indicators of disease outcomes for DRI development. In analyzing and weighing the data, the committee considered the following factors:

- Preliminary evidence in support of a relationship between vitamin D and/or calcium and a disease outcome is not always complete or well substantiated.
- Evidence for the effect of vitamin D and/or calcium on disease outcomes is heterogeneous and may not provide strong support for a consistent and predictable outcome.
- Clinical trials have the greatest influence in moderating confidence in a relationship between vitamin D and/or calcium and a disease outcome.

The committee's findings and conclusions were derived from its weighing of the totality of evidence and its ranking of evidence based on examination of study methods, relevance to dietary intake, effect of vitamin D and/or calcium on disease outcome, and overall strength of the study design.

array of experimental studies examining cells in culture and rodent models of cancer are providing evidence that vitamin D may have an impact on carcinogenesis at several organ sites (Deeb et al., 2007; Welsh, 2007; Davis, 2008). In parallel, epidemiological investigations of diverse approaches are examining the role of vitamin D in human cancer (WCRF/AICR, 2007; Yetley et al., 2009). In contrast, very few randomized and controlled prospective intervention trials with vitamin D targeting cancer as the primary outcome have been undertaken, leaving major gaps in understanding of causal relationships. Although more challenging to study in vitro, studies of dietary calcium in rodent models have also suggested a potential role in cancer risk; there are, as discussed below, experimental and clinical studies providing evidence in support of calcium as a modulator of carcinogenesis, particularly in the colon and rectal mucosa.

All Cancers

Cancer represents hundreds of different histopathologically distinct types of malignancy derived from virtually all organs and tissues. Investi-

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gations into the cellular defects contributing to the carcinogenic process indicate that cancers, regardless of tissue origin, share in a specific set of defective biological processes (Hanahan and Weinberg, 2000) that enhance cell proliferation, survival, invasion, and metastasis. Although cancer studies initially suggested the possibility of a tissue-specific gene expression signature unique to a cancer type, it is now appreciated that multiple different mutational patterns contribute to the heterogeneity in biology and response to intervention among humans with cancer.

Biological plausibility Serum 25OHD levels are determined by both dietary intake and endogenous synthesis in the skin upon exposure to ultraviolet B (UVB) light. UVB exposure is often used as an indirect estimate of endogenous production of vitamin D in ecological studies of cancer incidence patterns. Several investigators associated lower UVB exposure with higher cancer mortality beginning decades ago (Apperley, 1941) and continuing with improved methods of estimating exposure (Boscoe and Schymura, 2006), as reviewed by IARC (2008). However, a large literature suggests that increasing latitude cannot be equated with decreasing vitamin D status, and cancer risk factors (exposure to UVB or other forms of ionizing radiation) vary with latitude. Importantly, an opposite gradient is well established for skin cancers, with a greater risk among populations residing in areas of high sun exposure (IARC, 1992). In general, ecological studies based upon estimated UVB exposure, vitamin D status, and cancer risk have many potential biases due to methodological considerations making causal biological inferences, particularly at the level of the individual, impossible.

Systematic reviews and meta-analyses Assessment of total cancer risk has been the subject of systematic reviews, including IARC (2008), WCRF/AICR (2007), and AHRQ-Tufts. Several studies, including those reviewed in AHRQ-Tufts, were examined by the committee in detail. Three intervention trials that examined total cancer as an outcome were identified from these reviews; these trials were originally designed to assess fracture risk, and none included total cancer as a pre-specified primary outcome (see Table 4-3). In both the Trivedi et al. (2003) and Lappe et al. (2007) osteoporosis trials cancer risk was determined from a secondary analysis of safety data that relied upon subjects notifying the investigators of the new diagnosis. Neither trial indicated a significant reduction in cancer incidence with vitamin D supplementation, whether given alone (Trivedi et al., 2003) or in combination with calcium and compared with calcium supplementation alone (Lappe et al., 2007). In the Lappe et al. (2007) trial, however, logistic regression analysis showed a significant reduction in risk for all cancers in the vitamin D plus calcium treatment group when

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TABLE 4-3 Vitamin D, Calcium,[a] and Total Cancer: Results of RCTs Reviewed in AHRQ-Tufts[b]

Reference; Location (Latitude)	Population Description	Background Calcium and Serum 25OHD	Outcome	Intervention, Daily Dose	*n* Event/ *N* Total	Outcomes: Metric (Comparison); Result; 95% CI
Lappe et al., 2007	Postmenopausal women	25OHD: 71.8 nmol/L	Incident cancer (all causes)	Vit D_3 1,000 IU + Ca (citrate 1,400 mg or carbonate 1,500 mg)	13/446	RR (Vit D + Ca vs. Ca) 0.76 0.38–1.55
Nebraska, United States (41°N)	Mentally and physically fit Mean age 67 years			Ca (citrate 1,400 mg or carbonate 1,500 mg)	17/445	
			Incident cancer (restricted to patients who were free of cancer at 1-year intervention)	Vit D_3 1,000 IU + Ca (citrate 1,400 mg or carbonate 1,500 mg)	8/403	RR (Vit D + Ca vs. Ca) 0.55 0.24–1.28
				Ca (citrate 1,400 mg or carbonate 1,500 mg)	15/416	
Trivedi et al., 2003	General population	25OHD: 53.4 nmol/L	Incident cancer (all causes)	Vit D_3 ~ 833 IU (100,000 IU every 4 months)	188/1,345	HR (Vit D vs. placebo) 1.09 0.86–1.36
Oxford, UK (52°N)	Mean age 75 (65–85) years	Calcium intake: 742 mg/day (at 4 years; no difference by treatment allocation)		Placebo	173/1,341	
			Total cancer mortality	Vit D_3 ~ 833 IU (100,000 IU every 4 months)	63/1,345	HR (Vit D vs. placebo) 0.86 0.61–1.2
				Placebo	72/1,341	

NOTE: CI = confidence interval; HR = hazard ratio; IU = International Units; RR = relative risk; UK = United Kingdom; Vit = vitamin.

[a]Calcium is included in Lappe et al. (2007) only.

[b]This table has been truncated for the purposes of this chapter, but it can be found in its entirety in Appendix D.

SOURCE: Modified from Chung et al. (2009).

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compared with the placebo group. Notably, the investigators could not exclude that cancers had been present at baseline or that cancers remained unnoticed at the end of the study. Moreover, the analysis of the multitude of outcomes in safety data raises the possibility of chance results that seem to be statistically significant but are the result of multiple comparisons being made within one data set.

Observational evidence in AHRQ-Tufts included a large 12-year prospective study of a cohort from the Third National Health and Nutrition Examination Survey (NHANES III) that examined associations between serum 25OHD levels and total cancer mortality as well as specific cancer mortalities. Serum 25OHD levels were found to be associated with gender, educational level, and race/ethnicity, but not with season/latitude. No interaction was detected, however, between serum 25OHD level and total cancer mortality (Freedman et al., 2007). In one frequently cited study included in the AHRQ-Tufts review, Giovannucci et al. (2006) prospectively examined a large cohort from the Health Professionals Follow-up Study (HPFS) for 14 years for multiple determinants of vitamin D, including diet, supplements, skin pigmentation, adiposity, and geography, and their associations with cancer mortality. This study found that each incremental increase in serum 25OHD level of 25 nmol/L was associated with a 17 percent reduction in total cancer incidence and a 29 percent reduction in total cancer mortality. Each of the determinants considered was found to influence plasma 25OHD levels among older men. These results should be viewed with caution, however, because of heterogeneity in serum 25OHD levels that is not accounted for by the variables used in the study, which included intakes based on self-administered semiquantitative food frequency questionnaires and self-reported weight and physical activity levels.

Taken together, the studies reviewed by AHRQ-Tufts, IARC (2008), and WCRF/AICR (2007) as a whole are not supportive of a role for vitamin D, with or without calcium in reducing risk for cancer.

Additional evidence from randomized controlled trials In addition to the trials identified in AHRQ-Tufts, a secondary analysis of data from the Women's Health Initiative (WHI) trial examined the effect of combined supplementation of vitamin D and calcium (400 International Units [IU] of vitamin D and 1,000 mg of elemental calcium) on various health outcomes including cancer mortality (Lacroix et al., 2009). The results, with an average of 7 years of follow-up, indicated a non-significant trend toward reduction in risk for cancer mortality among postmenopausal women.

Observational studies One additional large cohort study, not included in the AHRQ reviews, was identified that examined serum 25OHD levels and risk for cancer mortality. This study examined cancer mortality among patients referred for coronary disease after a median of 7.75 years and

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found a significant correlation between low serum 25OHD level defined as less than 25.5 nmol/L, and increased cancer mortality. No associations were detected, however, between calcitriol level and cancer mortality (Pilz et al., 2008). In total, the observational studies reviewed suggest that the association between 25OHD level and risk of death from all cancers is generally weak when considered over a broad range of serum 25OHD levels because of variability in outcomes between the studies reviewed. However, there may be a stronger association between low serum 25OHD levels and cancer risk. The evidence reviewed was not strong enough to conclude that associations between cancer mortality were dependent on latitude, or race/ethnicity.

The role of calcium in cancer risk was examined in one large prospective cohort study over 7 years of follow-up (Park et al., 2009). Calcium intake was found not to be related to total cancer risk in men, but a non-linear reduction in total cancer incidence in women was reported. A decreased cancer risk was found for calcium intakes up to approximately 1,300 mg/day, although no additional risk reduction was observed for higher intakes. Taken together, the heterogeneity among outcomes exhibited in these studies and the discrepancy in outcomes between observational and randomized trial evidence do not support a relationship between vitamin D or calcium and total cancer risk.

Concluding statement The totality of the available evidence from RCTs and observational association studies for a relationship between vitamin D and/or calcium and the risk for either incidence of or mortality from all cancers does not support the use of cancer mortality as an indicator for DRI development. The interpretation of the evidence reviewed is limited by the small number of studies identified and lack of consistency in associations between vitamin D intake or serum 25OHD levels and all cancer mortality. Interpretation is further complicated by the absence of large-scale RCTs examining total cancer risk as a pre-specified primary outcome. Given the lack of consistent evidence on associations between vitamin D intake or serum 25OHD level and total cancer, and the paucity of evidence on cancer as a primary outcome of vitamin D or calcium intervention in randomized trials, as well as inconsistency between findings in the available research for an effect of vitamin D or calcium supplementation or status on reducing risk for cancer, the committee could not draw a conclusion about the utility of the evidence for this indicator to support DRI development.

Breast Cancer

Risk for breast cancer is largely defined by reproductive endocrinology, with increased risk for those with early age of menarche, late menopause, no pregnancy, later age of first pregnancy, shorter duration of lactation, the

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use of postmenopausal hormonal supplementation (Fentiman, 2002; Velie et al., 2005; Narod, 2006; Parsa and Parsa, 2009; Dietel, 2010). Dietary-related factors have been extensively reviewed with alcoholic drinks, adult attained height, and adult weight gain likely contributing to risk and with physical activity showing some benefit (WCRF/AICR, 2007). These characteristics must be considered when evaluating other putative breast cancer risk factors.

Biological plausibility The influence of the active form of vitamin D (calcitriol) on breast cancer cells in vitro is well characterized and includes anti-cancer effects such as cell cycle inhibition, reduced proliferation, enhanced sensitivity to apoptosis, and induction of differentiation markers (Welsh, 2004), which are likely mediated by the vitamin D receptor (VDR) (Matthews et al., 2010). A shortcoming in applying results from cell culture studies to risk for disease, however, is that the dose of calcitriol necessary to achieve tumor inhibition in vivo is frequently associated with hypercalcemic toxicity (Welsh, 2004; Matthews et al., 2010). Novel genomic approaches have begun to elucidate the gene expression signature of vitamin D in breast cancer cells and the mammary glands of mice (Matthews et al., 2010). Many of the genes identified show a consensus vitamin D response element (VDRE) in their promoter elements, indicating that they are specific targets of the vitamin D receptor (VDR[1]) complex (Swami et al., 2003; Matthews et al., 2010). Since the discovery of polymorphisms in the *Vdr* gene, a search for associations of mutations with breast cancer has been undertaken, but with indeterminate results (Bertone-Johnson, 2009; McKay et al., 2009). An inverse association has been postulated between mammographic density, a putative breast cancer risk factor, and serum 25OHD levels in premenopausal women (Berube et al., 2004; Brisson et al., 2007). The role of dietary calcium intake and in breast cancer risk, however, is less well studied, and the potential biological mechanisms of action are not understood.

Systematic reviews and meta-analyses AHRQ-Tufts did not find any qualified systematic reviews that evaluated associations between vitamin D and calcium intake or serum 25OHD levels and risk for breast cancer. Three observational studies of sufficient methodological quality were identified that examined the relationship between 25OHD levels and breast cancer risk. A prospective cohort study described above for total cancer mortality reported that women whose 25OHD levels were in a higher stratification, were at significantly lower risk for breast cancer. There were, however,

[1]In this report, the term VDR is used to refer to the protein. The term *Vdr* is used to refer to the gene, whether in animals or humans.

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only eight women in the higher stratification and a linear trend analysis was not significant (Freedman et al., 2007). A nested case–control study using data from the Nurses' Health Study (NHS) (Bertone-Johnson et al., 2005) found no significant relationship between higher plasma 25OHD concentrations and decreased risk for breast cancer overall, except when the population was restricted to women over 60 years of age. Another nested case–control cohort study of postmenopausal women participating in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial also found no evidence supporting the hypothesis that higher plasma 25OHD concentrations were associated with reduced risk of breast cancer in this cohort (Freedman et al., 2008).

Analysis of the results of RCTs reviewed in AHRQ-Tufts found no significant effect of supplementation with both vitamin D and calcium on breast cancer incidence and no association between the intervention and risk for death from breast cancer. Subjects with lower baseline 25OHD levels were found to be at increased risk for breast cancer; however, the association was not significant after adjusting for body mass index and physical activity (Chlebowski et al., 2008).

A meta-analysis of evidence from observational studies carried out by IARC (2008) evaluated associations between serum vitamin D levels and cancer. The analyses for breast cancer risk indicated no significant or consistent associations. A literature review and all-inclusive meta-analysis of published studies of heterogeneous quality individually examined the impact of estimated vitamin D intake, circulating 25OHD levels, and calcium intake on breast cancer risk (Chen et al., 2010). Their analysis suggests an inverse relationship between risk and level of vitamin D intake, serum $25OHD_3$ level, and calcium intake.

Additional evidence from randomized controlled trials WHI was used as a data source in an 8-year follow-up study for risk of benign proliferative breast disease, a putative premalignant condition associated with increased risk of subsequent cancer (Rohan et al., 2009). This study identified an association between risk for breast cancer and baseline age but found no effect of supplemental calcium and vitamin D intervention on reducing risk for breast cancer.

Observational studies Several case–control and cohort studies conducted subsequent to the systematic reviews were identified that examined associations between dietary and supplemental intake of vitamin D and calcium and risk for breast cancer, and these have shown mixed results. Rossi et al. (2009), a large case–control study in Italy, found an inverse association between vitamin D intake and risk for breast cancer at intakes of 188 IU/day or greater, suggesting a threshold effect; however, when risk was cal-

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culated in the upper three deciles compared with the lower seven deciles the significant difference was attenuated. A population-based case–control study of women ages 25 to 74 years in Canada compared vitamin D and calcium intake from food alone or from food and supplements. When intake above 400 IU of vitamin D per day was compared with no intake, a reduced risk was found. Calcium supplement intake alone, however, did not correlate with reduced risk, although a significant inverse trend was identified (Anderson et al., 2010). Two studies were identified that examined associations between dairy intake and risk of breast cancer. Shin et al. (2002) analyzed data from the NHS 1980 cohort for dairy intake and incident breast cancer. Over 16 years of follow-up, a significant inverse association was found for premenopausal women consuming low-fat dairy products and breast cancer risk. No association was found for calcium and vitamin D intake and postmenopausal breast cancer risk. Supplemental calcium intake had no linear association and supplemental vitamin D intake a weak but non-significant association with breast cancer risk in both premenopausal and postmenopausal women. Using a similar study design, McCullough et al. (2005), in an analysis of participants from the Cancer Prevention Study II Nutrition Cohort, found that two or more daily servings of dairy products were inversely associated with breast cancer risk; however, no association was found for either calcium or vitamin D supplementation. Women with dietary calcium intakes above 1,250 mg/day had lower breast cancer risk than women with intakes at or below 500 mg/day. Altogether, these observational studies were of lower quality and thus not considered as strong support for an association between vitamin D and risk for breast cancer, and they were not well supported by randomized trial evidence.

Concluding statement In summary, although experimental studies are suggestive of a role for vitamin D in breast biology, a review of the available evidence from both RCTs and observational studies of associations between vitamin D and calcium and risk of breast cancer shows a lack of consistency between study outcomes and insufficiently strong evidence to support DRI development. Both retrospective and prospective studies do not show consistent associations between estimated vitamin D intake or 25OHD status and breast cancer risk. A paucity of RCTs of vitamin D, calcium, or both with breast cancer as a primary outcome further limited the strength of the evidence.

Colorectal Cancer/Colon Polyps

Foods, nutrients, and physical activity all interact in a complex array of mechanisms to influence colorectal cancer risk. There is convincing evidence that physical activity protects against colorectal cancer, whereas

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red and processed meat, body fatness, and alcohol may increase the risk (WCRF/AICR, 2007). The committee's review of studies on vitamin D and calcium and risk for colorectal cancers and possible protective benefits identified for calcium and vitamin D was inconclusive.

Biological plausibility A major role of the active form of vitamin D is to enhance calcium absorption by the intestine, and the molecular and cell biology has been well defined (Song and Fleet, 2007; Xue and Fleet, 2009). The VDR and the vitamin D converting enzyme, 1α-hydroxylase, are both expressed in the colon and rectum (Cross et al., 1997; Holt et al., 2002). Vitamin D has been reported to act on colonic epithelial and cancer cells to regulate growth factor and inhibitor expression and signaling pathways, including modulation of the cell cycle, sensitivity to apoptosis, and enhancement of cellular differentiation (Harris and Go, 2004; Yang et al., 2007). Many rodent models of colon carcinogenesis suggest that there is an increased risk for colon cancer associated with vitamin D deficiency; and a decreased risk associated with supplementation (Harris and Go, 2004; Yang et al., 2008; Newmark et al., 2009). However, few studies were identified that examined vitamin D over a range of dose levels. A recent review of findings from the *Vdr*-null mouse model indicates an increase in hyperplasia of the distal colonic epithelium and greater deoxyribonucleic acid (DNA) damage in vitamin D–deficient compared with wild-type mice (Bouillon et al., 2008). The independent role of calcium in modulating colon cancer risk is also under investigation. Although intracellular calcium plays a key role in cell biology and influences growth control processes that may be related to carcinogenesis, serum calcium is tightly regulated over a wide range of intakes. Thus, the potential mechanisms by which serum calcium levels could mediate risk for colon cancer may be through indirect effectors in metabolic pathways involved in tumorigenesis.

Systematic reviews and meta-analyses

Colorectal cancer The AHRQ-Tufts systematic review considered evidence for associations between 25OHD levels and risk for colorectal cancer mortality or incidence. One RCT found no significant difference between colorectal mortality or incidence and supplementation with vitamin D in an elderly population. One cohort study was identified that found an inverse association between high serum 25OHD levels and risk for colorectal cancer mortality, and two nested case–control studies in women found an inverse trend between serum 25OHD level and colorectal cancer incidence. Two nested case–control studies in men and three in both men and women found no significant associations between serum 25OHD level and risk of colorectal cancer.

The IARC (2008) meta-analysis found a significant protective effect for

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serum 25OHD level against risk for colorectal cancer that correlated with each 2.5 nmol/L increase, although there was significant between-study heterogeneity. The results did not significantly differ by gender, mean population age, or cancer subsite (colon or rectum). The review noted that, based on multiple studies of circulating 25OHD and colorectal cancer risk, individuals in the high quartile or quintile of 25OHD level had about half the risk of colorectal cancer as did those in the lowest group. In another systematic review of studies examining associations between serum 25OHD levels and colorectal cancer, Bischoff-Ferrari et al. (2006a) concluded that the protective effect of 25OHD for decreased risk of colorectal cancers began at 75 nmol/L, and optimal levels were between 90 and 100 nmol/L. In contrast to these findings, the AHRQ-Ottawa systematic review reported that the studies reviewed were too inconsistent to permit conclusions to be drawn about specific serum 25OHD levels that conferred a decrease in risk.

Colorectal adenomas/polyps The AHRQ-Tufts systematic review considered evidence for associations between 25OHD levels and risk for colorectal adenomas. Colorectal adenomas or polyps are precursor lesions for colon cancer, and a number of investigations focused on the influence of vitamin D or calcium on the incidence of these surrogate markers for human colon carcinogenesis. A meta-analysis by Wei et al. (2008) of seven studies suggested that at the upper quintiles of circulating 25OHD levels there was a significant decrease in risk for colorectal adenoma. In parallel, these authors conducted a meta-analysis of vitamin D intake and colorectal adenoma risk in seven cohort and five case–control studies and found a marginally significant (11 percent) decreased risk among persons with high compared with low vitamin D intakes. The cut-points for the highest category of vitamin D intake varied between studies, with about one-third of the studies reporting cut-points of approximately 600 IU/day, one-third reporting cut-points between 250 and 600 IU/day, and one-third reporting cut-points of below 250 IU/day.

Stronger evidence has accumulated for a role of dietary calcium. The AHRQ-Tufts analysis identified four good quality cohort studies that evaluated the association between calcium intake and risk for colorectal adenoma. Two of these studies recruited men and women with a history of previous colorectal adenoma. One study found a significant inverse association between total calcium intake and colorectal adenoma recurrence after an average of 3.1 years of follow-up (highest [> 1,279 mg/day] vs. lowest [< 778 mg/day]) intake, whereas another found no significant association. Among two studies of healthy women without a history of colorectal adenoma one found a significant inverse association between total calcium intake and colorectal adenoma (highest vs. lowest intake, whereas the other found a borderline significant trend (highest [median, 1,451 mg/day] vs. lowest [median, 584 mg/day] intake. A Cochrane systematic review identified two randomized trials that found that calcium supplementation

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reduced the incidence of recurrent colorectal adenoma (Weingarten et al., 2008). Overall, the evidence is suggestive that vitamin D and probably calcium may reduce the risk of this intermediate endpoint for colorectal cancer, but the available data are not sufficient to allow a definitive assessment of the effects of vitamin D, calcium, and their interactions on risk for new or recurrent colorectal adenomas.

Additional evidence from randomized controlled trials The committee did not identify any additional relevant RCTs assessing vitamin D or calcium intake and risk for colorectal cancer or adenomas.

Observational studies The European Prospective Investigation into Cancer and Nutrition (EPIC) study has recently reported data on more than 1,200 colorectal cancer cases and an equal number of controls (Jenab et al., 2010). In this report, serum concentrations lower than the pre-defined mid-level concentrations of 25OHD (50 to 75 nmol/L) were associated with higher colorectal cancer risk. Jenab et al. (2010) also reported that higher 25OHD concentrations of 75 to less than 100 nmol/L and 100 nmol/L and higher were associated with a decreased risk. No other relevant observational studies were identified outside the AHRQ reviews. Although this evidence was largely in agreement with the IARC (2008) findings and Bischoff-Ferrari et al. (2006a), the committee did not consider it convincing enough to outweigh the conclusions from both AHRQ reviews.

Concluding statement Taken in aggregate, epidemiological studies examining associations between vitamin D status and colorectal cancer incidence generally support an inverse association, although the shape of the dose–response relationship curve over a wide range of vitamin D intake remains very speculative. The biological plausibility is supported by data from cell culture and rodents, with additional support from surrogate biomarker studies in humans. There remains a paucity of prospective randomized intervention studies, and those available have not shown a significant relationship at this time. Thus, the data are insufficient for the committee to utilize colon cancer as an outcome for establishment of vitamin D DRIs. The data for an effect of dietary calcium on colorectal cancer risk are also highly suggestive of a protective effect, but there are not sufficient data available on dose–response relationships to utilize colorectal cancer as a health outcome for DRI development.

Prostate Cancer

Prostate cancer risk is strongly associated with aging and is clearly dependent upon prolonged exposure to testosterone. Unlike breast cancer

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in women, however, where specific reproductive events define risk, further characterization of the relationship has been challenging. Specific dietary and nutritional hypotheses, including a role for vitamin D and calcium, have been proposed but evidence supporting these relationships is not conclusive.

Biological plausibility Studies in vitro document that prostate cancer and prostate epithelial cells in culture respond to calcitriol with anti-proliferative effects and that calcitriol stimulates cell differentiation (Washington and Weigel, 2010). Evidence indicates that these effects, as for epithelial cells of other tissue origins, are mediated by the VDR expressed on prostate cells (Kivineva et al., 1998; Thorne and Campbell, 2008). Gene expression array studies provide evidence that calcitriol induces a pattern of gene expression that inhibits growth factor signaling and cell cycle progression, promotes differentiation, and is anti-inflammatory and anti-angiogenic (Krishnan et al., 2004; Peehl et al., 2004; Kovalenko et al., 2010). The role of dietary calcium intake in prostate cancer risk is less well studied, with inconsistent results, and the potential biological mechanisms of action are highly speculative.

Systematic reviews and meta-analyses The AHRQ-Tufts systematic review found no qualified systematic reviews assessing associations between serum 25OHD levels and incidence of prostate cancer. Among observational studies reviewed, 8 of 12 nested case–control studies found no association between baseline serum 25OHD levels and risk for prostate cancer, and only 1 (C-rated) (Ahonen et al., 2000) reported a significant association between baseline serum 25OHD levels below 30 nmol/L and higher risk of prostate cancer, compared to those with levels greater than 55 nmol/L. Further, the effect appeared to be stronger for men younger than age 52 at entry into the study. A meta-analysis by Huncharek et al. (2008) of 45 observational studies on dairy and milk intake and risk of prostate cancer showed no significant association between dietary intake of vitamin D and prostate cancer risk.

Additional evidence from randomized controlled trials No relevant RCTs that were not reviewed by AHRQ were identified for vitamin D or calcium intervention and risk for prostate cancer.

Observational studies Three observational studies not included in either AHRQ-Ottawa or AHRQ-Tufts were identified as potentially relevant to prostate cancer as a health indicator for vitamin D and calcium. Schwartz and Hulka (1990) suggested that vitamin D deficiency was a causative

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factor in prostate cancer based upon the observation that the prevalence of vitamin D deficiency increases with age and is greater in those with dark-pigmented skin types and northern European populations, coupled with the observation that mortality rates for prostate cancer appear to be inversely related to sun exposure. However, a more recent case–control analysis of data from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, which examined male smokers, found no association; including any age-related associations to support a relationship between serum 25OHD levels and incidence of prostate cancer (Faupel-Badger et al., 2007). A potential procarcinogenic effect of higher dietary calcium was suggested by the HPFS (Giovannucci et al., 1998), which reported that calcium intake from the diet or from diet and supplements was independently associated with risk of locally advanced or metastatic prostate cancer especially when intakes exceeded 2,000 mg per day. The potential role for calcium as a risk factor for prostate cancer is discussed in detail in Chapter 6.

Because of the complexity of assessing vitamin D exposure over time relative to prostate cancer risk, high-quality evidence from observational studies was limited. The results of the HPFS, the only large, prospective cohort study identified for calcium, are not supported by evidence from available RCTs. Therefore, the evidence from human studies is insufficient to permit the committee to draw conclusions about a role for vitamin D and/or calcium in reducing prostate cancer risk.

Concluding statement Overall experimental data indicating that cultured prostate epithelial and prostate cancer cells respond to vitamin D via the VDR suggest a role for vitamin D in prostate cancer. However, associational studies of vitamin D status and risk of prostate cancer have provided mixed results, and randomized controlled clinical trials of substantial quality examining incidence or mortality have not been reported. Thus, there are insufficient data to permit the committee to draw a conclusion about the utility of the evidence for this indicator to support DRI development.

Cardiovascular Diseases and Hypertension

CVD broadly describes a range of diseases affecting the heart and blood vessels. Diseases that fall under the umbrella of CVD comprise coronary artery disease, myocardial infarction, stroke/cerebrovascular disease, peripheral artery disease, atherosclerosis, hypertension, arrhythmias, heart failure, and other vascular disorders. CVD is a public health concern because it is associated with an enormous burden of illness, disability, and mortality. CVD and hypertension were considered as potential indicators

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based on proposed hypotheses that vitamin D alone or in combination with calcium may help to prevent CVD or hypertension. Calcium has also been implicated independently as a nutrient related to reducing risk for development of CVD. Limited data were available for this indicator in the 1997 DRI report (IOM, 1997); however, additional experimental animal and observational studies for both vitamin D and calcium and CVD have been published in the interim.

Biological Plausibility

Vitamin D has been linked to decreased risk for CVD. Ecological studies suggest that there is higher cardiovascular mortality during the winter and in regions with less average exposure to UVB radiation from sunlight (Zittermann et al., 2005). Various biological mechanisms have been proposed in support of this hypothesis. Experimental animal studies failed to demonstrate an effect of vitamin D on risk for hypertension (Li et al., 2004) and increased thrombogenicity (Aihara et al., 2004). In rodents, administration of calcitriol or its analogues enhances vascular reactivity (Hatton et al., 1994). In support of the hypothesis that biological activation of vitamin D is relevant to cardiovascular function, the *Vdr*-null mouse has been used to model CVD.

High dietary calcium intake may help to reduce CVD risk through its roles in decreasing intestinal absorption of lipids and increasing lipid excretion, lowering blood cholesterol levels, and promoting calcium influx into cells.

Systematic Reviews and Meta-Analyses

The AHRQ-Tufts report identified one RCT and four relevant observational studies for vitamin D and cardiovascular outcomes. The RCT (Trivedi et al., 2003) found no statistically significant difference in incidence of cardiovascular events and deaths for subjects treated with 100,000 IU of vitamin D every 4 months over 5 years of follow-up. Among the observational studies reviewed, the Framingham Offspring Study found a significant association between low serum 25OHD levels and incident CVD (T. J. Wang et al., 2008). However, a closer look at the individuals with the highest serum 25OHD levels suggests that there was no additional reduction in risk at levels greater than 75 nmol/L and that the dose–response relationship may be U-shaped above 75 nmol/L. In the HPFS, Giovannucci et al. (2008), using a nested case–control design, found a significant association between low (< 37.5 nmol/L) serum 25OHD levels and incident myocardial infarction. A study using data from NHANES III, however,

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found no significant association between serum 25OHD levels and cardiovascular mortality overall, although individuals with the lowest 25OHD levels experienced a significant increase in total mortality compared with those with the highest levels. Echoing the findings for incident CVD in the Framingham Offspring Study, a closer examination of the highest 25OHD levels suggested a U-shaped dose–response relationship, with increased total mortality at both the lowest and highest 25OHD levels in this cohort (Melamed et al., 2008). The fourth observational study reported in AHRQ-Tufts (Marniemi et al., 2005) also failed to find an association between serum 25OHD levels and total CVD incidence, although it did find that vitamin D intake predicted a decreased risk for stroke. With the exception of one case–control study, the overall findings from the observational studies reviewed reaffirm a lack of significant association between 25OHD level and CVD risk and that higher 25OHD levels may incur an increased risk for CVD. From this, AHRQ-Tufts concluded that the evidence was insufficient to support a relationship between vitamin D or calcium and risk for CVD.

A recent meta-analysis of randomized trials using calcium supplements (without vitamin D) suggested that calcium supplementation was associated with an increase in the risk of myocardial infarction (Bolland et al., 2010a). However, another recent meta-analysis that included CVD as a secondary outcome found a slightly reduced, but not significant, risk for CVD with vitamin D supplementation, no association with calcium supplementation, and no association with a combination of vitamin D plus calcium supplementation (Wang et al., 2010).

Additional Evidence from Randomized Controlled Trials

No new RCTs were identified that examined CVD as a pre-specified primary outcome although several trials analyzed CVD as a secondary treatment outcome, and the findings of secondary outcome studies were not supportive of a reduction in CVD risk for either vitamin D or calcium. Among the additional RCTs reviewed outside the AHRQ reviews examining CVD as a secondary outcome (Hsia et al., 2007 [400 IU vitamin D_3/1,000 mg calcium]; Major et al., 2007 [400 IU vitamin D/1,200 mg calcium]; Margolis et al., 2008 [400 IU vitamin D_3/1,000 mg calcium]; Prince et al., 2008 [1,000 IU vitamin D_2/1,000 mg calcium]; Manson et al., 2010 [400 IU vitamin D_3/1,000 mg calcium]), none found a significant treatment-related effect of vitamin D on risk of CVD (see Evidence Map in Appendix F). In a 5-year study of calcium intake and risk for CVD in New Zealand, Bolland et al. (2008) found that women taking 1,000 mg of elemental calcium had a significantly higher risk (compared to placebo) for myocardial infarction and a composite CVD endpoint of myocardial infarction, stroke, and sud-

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den death. However, when unreported events identified from a national database were added to the analysis, the increased cardiovascular risks in the calcium group were no longer statistically significant. A bone density trial, also conducted in New Zealand, assessing self-reported composite vascular events among men was also not significant for an interaction between 1,000 mg of calcium daily and CVD outcomes (Reid et al., 2008). The results of these trials are in agreement with the null findings of the AHRQ-Tufts review described above. The additional clinical trials reviewed did not show a statistically significant causal relationship between either vitamin D or calcium and decreased cardiovascular risk, and reductions in risk that were noted in some trials were not well supported by data analyses. Therefore, the totality of the evidence does not support an interaction between either vitamin or calcium and risk for CVD. Adverse cardiovascular effects associated with excess calcium intake were also noted, and these are discussed further in Chapter 6.

Observational Studies

In addition to the clinical trials reviewed, including those from AHRQ, several observational studies were identified that examined a role for vitamin D and/or calcium in reducing CVD risk. Two large, prospective cohort studies were identified. In one study of individuals at high risk of CVD, among coronary angiography patients followed for more than 7 years, those with the lowest serum 25OHD levels had significantly higher total mortality and cardiovascular mortality compared with those with the highest levels (Dobnig et al., 2008). Melamed et al. (2008) assessed 25OHD levels and prevalence of peripheral artery disease using data from NHANES 2001 to 2004. This study found a graded association between levels of 25OHD up to 29.1 nmol/L and levels of 29.2 nmol/L and above. In a trend analysis, a statistically significant difference was found between the lower 25OHD levels compared with the higher levels.

A number of small cohort studies were identified that evaluated serum 25OHD or calcitriol levels in patients at risk for various CVD indicators compared with control subjects who were free of CVD indicators. Watson et al. (1997) assessed calcitriol levels in subjects at high risk for developing coronary heart disease compared with asymptomatic individuals and found a significant inverse association between calcitriol and amount of vascular calcification in both groups, although the difference was greater in the at-risk group. Poole et al. (2006) compared serum 25OHD levels in a small group of patients admitted for a first stroke with those of healthy controls and found that serum 25OHD levels were significantly lower among stroke patients. Zittermann et al. (2003) compared both 25OHD and calcitriol levels against serum levels of biomarkers indicative of congestive heart

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failure in a small group of patients admitted for treatment and in free-living controls. The study found a significant difference in biomarker levels between treated patients compared with controls for both 25OHD and calcitriol levels.

One small case–control study was identified that determined the relationship between serum 25OHD levels and risk for myocardial infarction in at-risk patients compared with normal controls. In this study, Scragg et al. (1990) found that serum 25OHD levels were significantly lower in myocardial infarction cases than in controls and that the difference was greater (but not significantly so) during the winter.

Although these studies together provide evidence for lower serum 25OHD levels in individuals with CVD, whether the low serum 25OHD levels are sufficient to predict risk for CVD has not been clearly established. Additional evidence indicates that low serum 25OHD levels are associated with risk factors for CVD—specifically, increased carotid arterial thickness (Targher et al., 2006)—and apparent CVD in patients with type 2 diabetes (Cigolini et al., 2006; Chonchol et al., 2008). Additionally, some studies suggest a positive association between vitamin D intake and CVD risk factors associated with other chronic conditions, including hypertension (Krause et al., 1998; Pfeifer et al., 2001; Forman et al., 2007; L. Wang et al., 2008; Wang et al., 2010), impaired glucose tolerance or type 2 diabetes (Liu et al., 2005; Pittas et al., 2006, 2007a; Mattila et al., 2007), and inflammation (Timms et al., 2002; Schleithoff et al., 2006; Shea et al., 2008).

Risk of incident hypertension in relation to dietary vitamin D intake has been evaluated in three large prospective study cohorts; NHS 1, NHS 2, and the HPFS for 8 years and longer. Women in NHS 1 and NHS 2 (a younger cohort) showed no association between vitamin D intake and risk for incident hypertension. Likewise, among men from the HPFS no association was found between vitamin D intake and risk for incident hypertension. Al-Delaimy et al. (2003) also found no association between calcium intake, vitamin D intake, or total dairy intake and risk for total ischemic heart disease in men enrolled in the HPFS. Similarly, no association was found when the cohort was analyzed for calcium supplement intake, although an inverse association was identified between calcium intake among supplement users compared with nonusers and fatal ischemic heart disease only.

In contrast to the intake studies, in a prospective study, Forman et al. (2007) found inverse associations between incidence of hypertension and measured serum 25OHD levels in a larger cohort in the HPFS and in women from a larger cohort in NHS.

In summary, three of four large, prospective cohort studies reviewed found associations between serum 25OHD levels and risk for CVD. Among the many smaller observational studies of lower quality that were identified,

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most did not find a significant positive association between vitamin D and calcium intake and risk for CVD. Taken together, this observational evidence was strong enough to support a relationship between serum 25OHD levels and incident disease, but not a conclusion that higher serum 25OHD levels were associated with a lower risk for CVD. Additionally, the review of randomized trial evidence does not support a causal relationship between vitamin D intake and risk for CVD.

Concluding Statement

Review of the available evidence, from both RCTs and observational studies on associations between vitamin D and calcium intake and risk for CVD shows that although observational evidence supports a relationship between serum 25OHD levels and the presence of CVD, it does not show a relationship with risk for developing CVD, and evidence was not found for a causal relationship between vitamin D intake and development of disease. Given the lack of statistically significant evidence supporting associations between vitamin D intake or serum 25OHD level and risk for CVD and the lack of evidence on CVD as a primary outcome of treatment in RCTs with vitamin D and/or calcium, the committee could not draw an inference about the efficacy of this indicator to support DRI development.

Diabetes and Metabolic Syndrome

Type 2 diabetes is a blood glucose disorder characterized by insulin resistance and relative insulin deficiency. Metabolic changes that accompany chronic elevated blood glucose levels frequently lead to functional impairment in many organ systems, particularly the cardiovascular system, which contributes to substantially increased risk of morbidity and mortality.

Metabolic syndrome is a condition characterized by a constellation of metabolic risk factors, including abdominal obesity, atherogenic dyslipidemias, elevated blood pressure, insulin resistance, prothrombotic state, and proinflammatory state (e.g., elevated C-reactive protein).

Individuals with metabolic syndrome are at increased risk of coronary heart disease, stroke, peripheral vascular disease, and type 2 diabetes. Adiposity is a component of both type 2 diabetes and metabolic syndrome, which may have an impact on vitamin D status. Since the release of the 1997 DRIs (IOM, 1997), a number of studies have been published on relationships between vitamin D with or without calcium and type 2 diabetes and metabolic syndrome. The committee recognized that obesity can be a confounder to vitamin D analysis. However, as it is a component of the health outcome and because of the prevalence of both obesity and meta-

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bolic syndrome in the general population, this indicator was considered as a candidate for DRI development.

Biological Plausibility

Vitamin D was first implicated as a modulator of pancreatic endocrine function and insulin synthesis and secretion in studies using rodent models more than three decades ago (Norman et al., 1980; Clark et al., 1981; Chertow et al., 1983). Since then, the role of calcitriol in the synthesis and secretion of insulin and regulation of calcium trafficking in β-islet cells as well as its effects on insulin action have been established in both rodent models and in vitro cell culture models (Frankel et al., 1985; Cade and Norman, 1986; Faure et al., 1991; Sergeev and Rhoten, 1995; Billaudel et al., 1998; Bourlon et al., 1999). These findings stimulated observational and intervention studies examining the role of vitamin D and calcium in type 2 diabetes and metabolic syndrome in humans.

Systematic Reviews and Meta-Analyses

Neither AHRQ-Ottawa nor AHRQ-Tufts included type 2 diabetes or metabolic syndrome in its systematic review, although AHRQ-Tufts did include body weight as a health outcome and found no effect of vitamin D or calcium on changes in body weight. A systematic review and meta-analysis by Pittas et al. (2007b) included a large body of observational evidence and six intervention studies (four small short-term and two long-term studies) of vitamin D supplementation, one study using combined vitamin D and calcium supplementation and five studies using calcium alone or dairy supplementation. The results from these trials were largely negative; among the short-duration vitamin D trials, three studies reported no effect, and one reported enhanced insulin secretion but no improvement in glucose tolerance following vitamin D supplementation. In one study included in the review, however, the relationship was statistically significant only when non-Hispanic blacks were excluded from the meta-analysis.

Overall, the evidence reviewed from the intervention studies did not support a role for vitamin D alone, although vitamin D in combination with calcium supplementation may have a role in preventing type 2 diabetes in populations already at risk. The observational evidence in the review included cross–sectional and case–control studies in which serum vitamin D and calcium levels were determined from individuals in a population with established glucose intolerance. Similar confounding and a lack of adjustment for confounders limited the cohort studies. Thus, the one meta-analysis that included both observational and intervention studies could

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not be considered as supportive for a relationship between either vitamin D or calcium and the health outcomes of diabetes or metabolic syndrome.

Additional Evidence from Randomized Controlled Trials

Two randomized trials were identified that evaluated the effect of vitamin D supplementation with or without supplemental calcium on markers of glucose tolerance as a primary outcome and four additional trials were identified that evaluated glucose metabolism as a secondary outcome. A trial in New Zealand that examined the effect of supplementation with 4,000 IU of vitamin D_3 per day for 6 months on insulin resistance in non-diabetic overweight South Asian women found a significant improvement in insulin sensitivity compared with those in the placebo group after 6 months (von Hurst et al., 2010). Among women who had low serum 25OHD levels at the beginning of the study, those who achieved a serum 25OHD level above 80 nmol/L at 6 months had significant improvement in insulin sensitivity. In contrast, sub-analysis of data from the Randomised Evaluation of Calcium and/Or vitamin D (RECORD) trial examining the association between incidence of self-reported development of type 2 diabetes or initiation of treatment for type 2 diabetes and supplementation with 800 IU of vitamin D_3 and 1,000 mg of calcium in an elderly population found no association (Avenell et al., 2009a). Zittermann et al. (2009), in a weight loss trial evaluating the effect of supplemental vitamin D on markers of CVD in overweight adults as a primary outcome, found no significant difference for an effect on glucose metabolism. Jorde et al. (2010), in a 1-year trial in Norway with overweight or obese subjects, found no change in measures of blood glucose in vitamin D–supplemented subjects compared with control subjects, but they did identify an unexpected and significant increase in systolic blood pressure in the supplemented group compared with controls. Without further analysis, however, it is not possible to determine whether the increase in blood pressure was related to 25OHD levels in blood. A trial in India evaluated the effect of short-term vitamin D supplementation on homeostasis model assessment and oral glucose insulin sensitivity in healthy, centrally obese men (Nagpal et al., 2009). In an intention-to-treat analysis, the difference was not significant. Overall, higher waist-to-hip ratios and lower baseline serum 25OHD levels were significant predictors of improvement in oral glucose insulin sensitivity. A posthoc analysis of a trial testing the effects of long-term supplementation with 700 IU of vitamin D and 500 mg of calcium daily on health, including associations between combined supplementation and changes in fasting glucose levels, found that subjects with impaired fasting glucose who followed the supplementation regimen for 3 years had a significantly lower rise in fasting glucose levels and less insulin resistance compared with

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placebo controls (Pittas et al., 2007a). Although the findings of this study are in agreement with a previous secondary analysis of data from the NHS cohort (see below: Pittas et al., 2006), the study is limited by the small number of outcomes measured compared with the total cohort; thus, an unintended bias cannot be ruled out. In addition, the study was designed for skeletal outcomes as the primary analysis. When the totality of the evidence was considered, the negative findings from the clinical trials for an effect of vitamin D or calcium on risk for type 2 diabetes together with the lack of significant evidence from either the AHRQ reviews or the meta-analysis by Pittas et al. (2007b) compelled the committee to conclude that there was not sufficient evidence to establish a causal relationship.

Observational Studies

Low serum 25OHD levels have been implicated in metabolic syndrome, abdominal obesity, and hyperglycemia.

In a prospective cohort analysis of data from NHS, women were followed for 20 years to examine associations between vitamin D and calcium intake and risk for type 2 diabetes (Pittas et al., 2006). A significant inverse association was found between total vitamin D intake and calcium intake and risk for type 2 diabetes. A separate analysis of the association between risk for type 2 diabetes and dairy food consumption found that women who consumed three or more dairy servings per day were at lower risk compared with those who consumed less than one dairy serving per day. These findings suggest that risk for type 2 diabetes is associated with vitamin D or dairy food intake. A small cohort study in obese and overweight individuals found that in addition to a significant inverse association between serum 25OHD level and weight and waist circumference there was a weak inverse relationship with hemoglobin A1c. However, no association between serum 25OHD level and any other indicators of type 2 diabetes or metabolic syndrome were observed (McGill et al., 2008).

In other observational evidence reviewed, a cross–sectional survey of Polynesian and white adult populations in New Zealand found a significantly lower serum 25OHD level in subjects with newly diagnosed diabetes and impaired glucose tolerance compared with controls. In addition, among the control groups, the native New Zealand populations (Maori and Pacific Islanders) were found to have significantly lower serum 25OHD levels compared with Europeans. The authors speculated that the low serum 25OHD level in the native populations explained, in part, the higher prevalence of diabetes in those groups (Scragg et al., 1995). Isaia et al. (2001), in a cross–sectional study in Italy, found that postmenopausal women diagnosed with type 2 diabetes had significantly higher body mass indexes (BMIs), lower activity scores, higher prevalence of serum 25OHD

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levels below 12.5 nmol/L, and lower dietary calcium intake compared with controls. In summary, these observational studies fail to provide conclusive support of a relationship between vitamin D intake and risk for either type 2 diabetes or metabolic syndrome because of the lack of consistency among studies, the paucity of high-quality large cohort studies, and the lack of strength for an association between vitamin D status and incidence of type 2 diabetes or metabolic syndrome.

Concluding Statement

The available evidence from observational studies of the associations between vitamin D and calcium and risk for type 2 diabetes or metabolic syndrome and secondary analyses from RCTs on markers of glucose tolerance proved insufficiently strong to support DRI development. The association studies linking lower serum 25OHD levels to increased risk for type 2 diabetes may be confounded by overweight and obesity, which not only predispose individuals to type 2 diabetes, but also cause lower serum 25OHD levels as a result of sequestration in fat and possibly other mechanisms. Although both retrospective and prospective studies tend to support an inverse association between serum 25OHD levels and type 2 diabetes, these studies are limited by the study design and cannot show a causal relationship. Evidence from RCTs on the effect of vitamin D supplements on incident diabetes or markers of glucose homeostasis is variable, and few RCTs showing significant results were identified. Taken together, the evidence in support of a role for vitamin D as a modulator of pancreatic endocrine function and insulin synthesis and secretion is not conclusive and therefore is not sufficient to support glucose tolerance as an indicator for DRI development.

Falls and Physical Performance

The committee considered falls and physical performance as independent indicators. However, because of the integration of these indicators in the literature reviewed by the committee, the evidence for both indicators is examined together in this section.

The risk of falling is a major concern among the elderly, because falls can lead to fracture and long-term disability or death in this population. Vitamin D is necessary for normal development and growth of muscle fibers, and vitamin D deficiency may adversely affect muscle strength. Muscle weakness and pain (myopathy) are characteristics of rickets and osteomalacia and contribute to poor physical performance (Prineas et al., 1965; Skaria et al., 1975; Yoshikawa et al., 1979). Thus vitamin D-deficiency muscle weakness and the implications of poor muscle tone suggest a re-

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lationship between serum 25OHD level and risk for falling and/or poor physical performance in susceptible populations.

Biological Plausibility

Experimental evidence suggests that vitamin D exerts its effect on muscle tissue via the VDR, but it may also use other pathways. In vitro and in vivo experiments provide evidence to support calcitriol regulation of calcium uptake by muscle, which, in turn, controls muscle contraction and relaxation, synthesis of muscle cytoskeletal proteins involved in muscle contraction, and muscle cell proliferation and differentiation (reviewed in Ceglia, 2008). Because intracellular calcium levels control the contraction and relaxation of muscle, thus affecting muscle function, it is possible that calcium intake may also affect risk for falls and poor physical performance (reviewed in Ceglia, 2008). However, the topic is not considered in more detail here because of the lack of observational and RCT data on the relationship between calcium intake and physical performance.

Systematic Reviews and Meta-Analyses

The AHRQ-Ottawa systematic review identified a total of 14 RCTs in addition to five prospective cohort studies and one case–control study that examined vitamin D and risk for falls in postmenopausal women and elderly men. The evidence between the RCTs and observational studies was discordant. Overall the review reported that the evidence for an association between low serum 25OHD levels and risk of falls and measures of physical performance among postmenopausal women and elderly men was inconsistent and rated the evidence as "fair." The AHRQ-Tufts systematic review identified three additional RCTs (Bunout et al., 2006; Burleigh et al., 2007; Lyons et al., 2007), but these studies did not find a significant effect of vitamin D supplementation on reducing risk of falls or poor performance in the elderly and were given a "C" rating. No additional observational evidence was found for this indicator in the AHRQ-Tufts review.

A meta-analysis reported in AHRQ-Tufts, which included the AHRQ-Ottawa RCTs, highlighted the inconsistency of findings from RCTs on the effect of vitamin D treatment on reduction in risk or prevention of falls. A smaller meta-analysis by Bischoff-Ferrari et al. (2004a) examined RCTs in elderly populations for evidence of a reduction in risk for falls with "vitamin D"; however, only three studies used vitamin D, and the other two studies used calcitriol/1α-hydroxycholecalciferol. Some of the studies identified in this meta-analysis were also included in the AHRQ-Tuft analysis. In contrast to the AHRQ-Tufts analysis, Bischoff-Ferrari et al. (2004a) found, from pooled results, a significant reduction in risk of falling among sub-

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jects treated with vitamin D compared with those treated with calcium or placebo. This disparity in findings is best explained by the small numbers in the Bischoff-Ferrari et al. (2004a) analysis and the fact that none of the vitamin D studies pooled by Bischoff-Ferrari et al. (2004a) was individually significant.

A meta-analysis published in 2009 by Bischoff-Ferrari et al. (2009a) examined fall prevention based on supplemental intake and serum 25OHD concentrations. From this analysis of the eight RCTs (n = 2,426 subjects) that met the inclusion criteria, the authors concluded that supplemental vitamin D intake (700 to 1,000 IU/day) reduced the risk of falling among older subjects by 19 percent and that serum 25OHD concentrations less than 60 nmol/L may not reduce the risk of falling. This meta-analysis as conducted has major limitations.

First, the stated inclusion/exclusion criteria and their application are problematic. As stated by the authors, to be included in the primary analysis, the trial design had to be double-blinded and the assessment of falls had to be a primary or secondary endpoint defined at the onset of the trial. The study had to include a definition of falls and how they were assessed, and falls had to be assessed for the entire trial period. Studies using patients with Parkinson's disease, organ transplant recipients, or stroke patients were excluded as were trials using intramuscular injection of vitamin D. Of concern is the fact that some studies that met the inclusion/exclusion criteria were omitted, and at least one study that failed to meet the criteria was included. The Broe et al. (2007) study, did not have a secondary analysis that pre-specified falls as an outcome, was never powered to examine the incidence of falls; with the wide confidence interval due to small sample size the results are questionable. This study influenced the analysis considerably; other than the work of Pfeifer et al. (2009), it was the single largest contributor to the effect. The work of Law et al. (2006) was excluded because it was a cluster randomization design instead of individual randomization; however, such a design does not appear to violate the authors' stated criteria. It was also excluded because the dose of oral vitamin D (50,000 IU) was given every 3 months; however, the serum 25OHD increased from 45 to 75 nmol/L, indicating an adequate therapeutic level. Had the Law et al. (2006) study been included, in which 44 percent of the vitamin D–treated group and 43 percent of the control group were fallers (not significantly different), the overall results would have been negative.

Second and more importantly, Figure 3 as reported in Bischoff-Ferrari et al. (2009a) is inappropriately presented. The figure is intended to demonstrate fall prevention with dose of vitamin D and achieved serum 25OHD concentrations. Specifically, the figure is a meta-regression analysis of the relative risk (RR) against vitamin D dose or serum 25OHD concentration. However, the meta-regression appears to be incorrectly carried out, or the

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authors used assumptions that were not specified in the methods section of their publication. In their analysis, the dependent variable appearing in the graph is RR (linear scale of 0 to 2.5); however, log(RR) is typically used in this type of meta-regression, which is a weighted linear regression with each study being the unit of analysis. Even when RR is to be reported, a meta-regression of log(RR) against the predictor variable should be carried out and then retransformed back to the RR scale, in which case the line will be curvilinear instead of straight. Carrying out a meta-regression analysis using untransformed RR in the linear scale assumes an exponential relationship of the dose with effect. Moreover, the predictor variable (x-axis) is equally spaced for data points, but the data points are not equally spaced according to the vitamin D doses (or serum 25OHD concentrations). In the top panel of Figure 3 in Bischoff-Ferrari et al. (2009a), the dose intervals between the data points range from 0 IU (two 400 IU studies; two 800 IU studies) to 100 IU (between 600 IU and 700 IU) to 200 IU (between 200 IU and 400 IU, 400 IU and 600 IU, 800 IU and 1,000 IU). Two data points were also composed of multiple trials "collapsed" into a single data point for two levels (800 IU of vitamin D_3; 1,000 IU of vitamin D_2) of the predictor variables. This introduces considerable uncertainty as to the appropriateness of the location of the regression line. If the measurement intervals had been appropriately and evenly spaced, it is very likely that the conclusion of the analysis would have been that no significant relationship was demonstrated.

The importance of the limitations of this study becomes clear when the data are reanalyzed in the appropriate statistical manner. As shown in Figures 4-2 and 4-3, no dose–response relationship between vitamin D intake and risk of falls is evident. For this analysis, which used the STATA program, analyses were repeated by fitting a random effects meta-regression with the log(RR) of sustaining at least one fall as the response variable and the daily dose of vitamin D supplementation or the mean achieved 25OHD serum concentration in the vitamin D supplementation arm as the predictor variable (both predictor variables are continuous variables). Specifically, the results do not show a significant dose–response relationship between the risk of sustaining at least one fall and the daily dose of vitamin D supplementation or achieved 25OHD serum concentration (beta coefficient = −0.0005 ± 0.0003 and = −0.0087 ± 0.0056 standard error [SE], respectively; relative risk reduction = 0.95 for risk of falls per 100 IU/day and increased in dose of vitamin D, $p = 0.13$; relative risk reduction = 0.92 for risk of falls for every 10 nmol/L increase in 25OHD level, $p = 0.17$). Both analyses had significant heterogeneity across studies ($I^2 = 47$ percent, $p = 0.05$; $I^2 = 54$ percent, $p = 0.03$, respectively). Further, a non-linear dose–response relationship was explored by adding a quadratic term of the predictor variable to the model. The result suggests that a U-shaped curve

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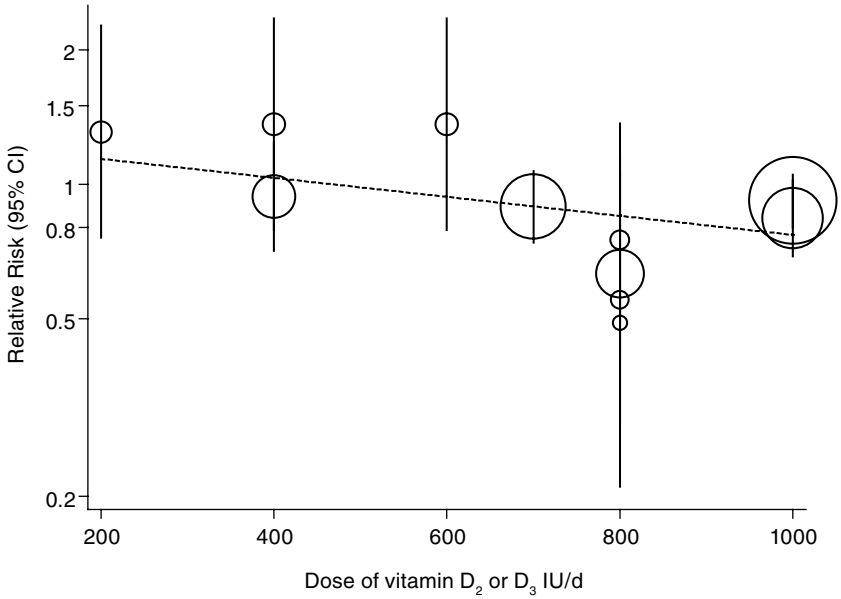


FIGURE 4-2 Relative risk of falls and vitamin D supplementation doses: Correct meta-regressions with continuous predictors showing non-significance.
NOTE: Relative risk reduction is 0.95 (95% confidence interval [CI] 0.89 to 1.02; $p = 0.13$) per 100 IU/day difference (increase) in dose.

better describes the relationship between the risk of sustaining at least one fall and the achieved serum 25OHD concentrations.

Additional Evidence from Randomized Controlled Trials

As discussed above, among RCTs that tested for effects of vitamin D with and without calcium on reduction in risk for falls, no consistent outcome was found. As described above, the data related to falls are questionable, and among muscle performance studies one included 12 subjects who were post-stroke patients and a second included 16 subjects for whom the study was only 8 weeks in length.

Two recent studies published after the AHRQ-Tufts analysis was completed have failed to show efficacy in reducing falls. A randomized but not placebo-controlled trial examined the effect of either 800 or 2,000 IU of vitamin D per day combined with enhanced or standard physiotherapy on the rate of falls and hospital re-admission following hip fracture in free-living adults with a mean age of 84 years (Bischoff-Ferrari et al., 2010). Neither of the two dosages of vitamin D_3 reduced the rate of falls or improved

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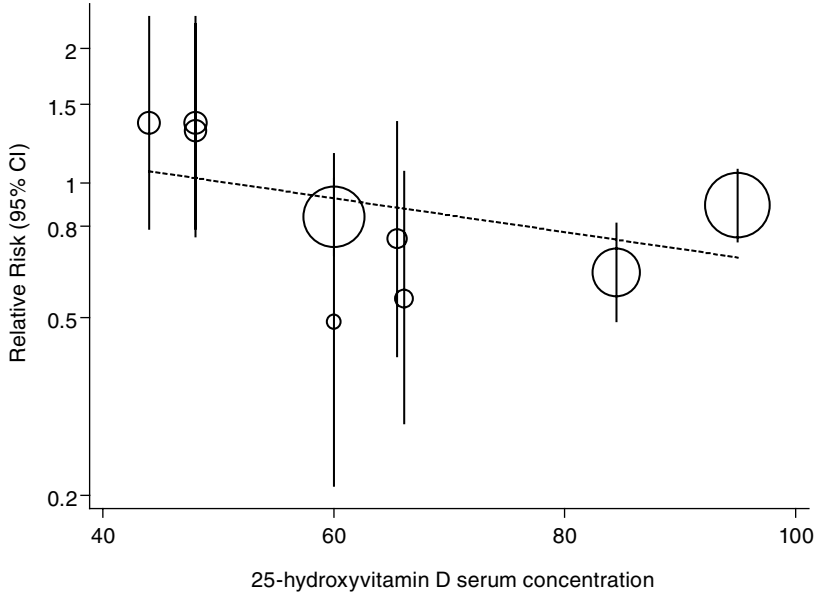


FIGURE 4-3 Relative risk of falls and mean achieved serum 25OHD concentrations: Correct meta-regressions with continuous predictors showing non-significance.
NOTE: Relative risk reduction is 0.92 (95% confidence interval [CI] 0.80 to 1.05; $p = 0.17$) per 10 nmol/L difference (increase) in mean achieved 25OHD concentration.

strength or function compared with physiotherapy. Another study (Sanders et al., 2010) that examined the incidence of falls and fractures in elderly women treated with 500,000 IU of vitamin D_3 annually for 3 years found a significant increase in falls and fractures in the treatment group compared with the placebo group. Notably, the increased incidence of falls was significant in the treatment group by 3 months following administration of the supplemental vitamin D. Further, as described in Chapter 6, the authors of this study concluded that levels of 65 nmol/L were not consistent with reduced rates of fall or fractures.

When this committee considered the totality of evidence for causality pertinent to the relationship between vitamin D and incidence of or risk for falls, it became clear that the greater part of the causal evidence indicated no significant reduction in fall risk related to vitamin D intake or achieved level in blood. Table 4-4 illustrates the range of clinical trial data assessing changes in fall incidence or risk for falls with varying levels of vitamin D treatment that were taken into account. Of the 18 studies considered, including several studies identified in Bischoff-Ferrari et al. (2009a), only 4 (Pfeifer et al., 2000; Harwood et al., 2004; Flicker et al.,

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TABLE 4-4 Outcome Measures for Falls: Summary of Evidence from Randomized Trials with Vitamin D and Calcium

Reference; Study Duration; Outcome	*N*	Vitamin D Dose	Calcium Dose	Serum 25OHD level (nmol/L)		Falls	Fallers	RR/OR (95% CI)/ (*p*-value)
				Baseline	Achieved			
Studies using oral doses								
Bischoff et al., 2003[a]	122	Placebo	1,200 mg/d	29.0 (23.0–25.0)	28.0 (24.5–41.5)	✓		0.68 (0.30–1.54)
12 weeks follow-up		800 IU/d	1,200 mg/d	30.8 (23.0–55.0)	65.5 (49.8–82.8)		✓	0.70 (0.30–1.50)
(primary analysis)								
Bischoff-Ferrari et al.,	Women:	Women:	Women:	Women:	Women:		Women:	Women:
2006b	246	Placebo	Placebo	63.0 (± 30.3)	68.0 (± 32.5)		✓	0.44 (0.21–0.90)
3-year trial		700 IU/d	500 mg/d	70.0 (± 33.0)	104.0 (± 41.8)			
(secondary outcome of	Men:	Men:	Men:	Men:	Men:		Men:	Men:
primary analysis)	199	Placebo	Placebo	83.0 (± 33.5)	76.5 (± 27.3)		✓	0.84 (0.42–1.66)
		700 IU/d	500 mg/d	82.0 (± 37.5)	110.0 (± 34.0)			
Bischoff-Ferrari et al., 2010	173	800 IU/d	500 mg/d	30.8 (± 19.3)	88.5 (± 25.3)	✓		28 (–4.0–68.0)
12-month trial		2,000 IU/d	500 mg/d	33.0 (± 20.3)	111.8 (± 26.0)			
(primary outcome)								
Broe et al., 2007	124	Placebo		53.0 (± 28.5)		✓		
5-month study period		200 IU/d	None	44.5 (± 23.0)				1.10 (0.49–2.50)
(secondary analysis)		400 IU/d	None	51.8 (± 29.0)				1.05 (0.48–2.28)
		600 IU/d	None	41.3 (± 18.5)				1.21 (0.55–2.61)
		800 IU/d	None	53.5 (± 23.0)				**0.28 (0.10–0.75)**[b]
Burleigh et al., 2007[a]	203	Placebo	1,200 mg/d	24.7 (± 10.0)		✓		0.82 (0.59–1.16)
30 days		800 IU/d	1,200 mg/d	21.7 (± 7.1)			✓	
(primary outcome)								

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Chapuy et al., 2002 2-year trial (secondary analysis)	583	Placebo	none	22.8 (± 17.3)	15.0		✓	1.08 (0.75–1.55) treatment groups combined
		800 IU/d or Ca combined	1,200 mg/d	22.5 (± 16.5)	80.0			
		800 IU/d + Ca given separately	1,200 mg/d	21.3 (± 13.3)	75.0			
Flicker et al., 2005 2-year follow-up (primary outcome)	540	Placebo	600 mg/d	42.5		✓		**0.73 (0.57–0.95)**[b]
		10,000 IU/wk later 1,000IU/d	600 mg/d	40.0			✓	0.82 (0.59–1.12)
Graafmans et al., 1996 28-week follow-up (primary outcome)		Placebo	none			✓		1.00 (0.60–1.80)
		400 IU/d	none	65.0			✓	1.00 (0.60–1.50)
Grant et al., 2005[c] 24- to 62-month follow-up (secondary analysis)	5,292	Placebo	none	38.0	45.4		✓	Subjects on Vit D vs no vit D
		Calcium	1,000 mg/d		42.0			
		800 IU/d	none		62.5			0.97 (0.84–1.12)
		800 IU/d + Ca	1,000 mg/d		62.0			
Larsen et al., 2005 42-month trial (primary outcome)	2,426	No intervention	none			✓		Women: 0.89 (0.79–1.03) Men: 1.07 (0.90–1.27)
		400 IU/d	1,000 mg/d	35.0				

continued

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TABLE 4-4 Continued

Reference; Study Duration; Outcome	*N*	Vitamin D Dose	Calcium Dose	Serum 25OHD level (nmol/L)		Falls	Fallers	RR/OR (95% CI)/ (*p*-value)
				Baseline	Achieved			
Law et al., 2006 10-month trial (primary outcome)	3,717	No pills 1,100 IU/d	none none	 47.0 (35.0–102.0)	 74.0 (52.0–110.0)	✓	 ✓	1.09 (0.95–1.25) 1.36 (0.80–2.34)
Pfeifer et al., 2009 12-month trial; follow-up at 20 months (primary outcome)	242	Placebo 800 IU/d	1,000 mg/d 1,000 mg/d	54.0 (± 18.0) 55.0 (± 18.0)	57.0 (± 20.0) 84.0 (± 18.0)	✓	 ✓	**0.61 (0.34–0.76)**[b] **0.40 ($p < 0.001$)**
Pfeifer et al., 2000 8-week trial (primary outcome)	137	Placebo 800 IU/d	1,200 mg/d 1,200 mg/d	24.6 (± 12.1) 25.7 (± 13.6)	42.9 (± 20.1) 66.2 (± 27.0)	✓	 ✓	 **0.55 (0.29–1.06)**[d]
Prince et al., 2008 1-year trial (primary outcome)	302	Placebo 1,000 IU/d	1,000 mg/d 1,000 mg/d	44.3 (± 12.8) 42.3 (± 12.5)	44.3 60.0		✓	0.66 (0.41–1.06)
Trivedi et al., 2003[c] 5-year trial (secondary analysis)	2,686	Placebo 100,000 IU 3 doses/y	none none		53.4 (±21.1) 74.3 (± 20.7)		✓	Women: 1.03 (0.72–1.48) Men: 0.87 (0.68–1.12)
Studies using injected doses								
Dhesi et al., 2004[a] 6-month trial (secondary outcome)	123	Placebo 600,000 IU IM injection once	none none	25.0 (23.8–26.3) 26.8 (25.5–28.0)	31.5 (28.5–34.5) 43.8 (41.3–46.3)	✓	 ✓	0.24 ($p = 0.28$) 11 ($p = 0.52$)

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Harwood et al., 2004[c] 1-year trial (secondary analysis)	150	No treatment 300,000 IU IM once 300,000 IU IM once 800 IU/d	0 mg/d 0 mg/d 1,000 mg/d 1,000 mg/d	30.0 (12.0–64.0) 28.0 (10.0–67.0) 30.0 (12.0–85.0) 30.0 (12.0–64.0)	27.0 40.0 44.0 50.0		✓	0.48 (0.3–0.9)
Latham et al., 2003 6-month follow-up (secondary outcome)	122	Placebo 300,000 IU once	none none	47.5 40.0	47.5 62.5	✓		1.12 (0.79–1.59)
Sanders et al., 2010 3-year trial (primary outcome)	2,256	Placebo 500,000 IU/y	none none	45.0 (40.0–57.5) 53.0 (40.0–65.0)	Median 55.0–75.0	✓	 ✓	1.15 (1.02–1.30) 0.74 ($p = 0.03$)
Smith et al., 2007 3-year trial (secondary analysis)	9,440	Placebo 300,000 IU IM injection/y	none none	56.5 56.5	56.5 68.5	✓		 0.98 (0.94–1.04)

NOTE: CI = confidence interval; IM = intramuscular; IU = International Units; OR = odds ratio; RR = relative risk; y = year.

[a]Included in AHRQ-Ottawa (Cranney et al., 2007) and/or AHRQ-Tufts (Chung et al., 2009).

[b]**Bolding** indicates significant difference and is presented for assessment of causality.

[c]Discussed in the "Skeletal Health" section below.

[d]Data provided in Bischoff-Ferrari et al. (2009a).

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2005; Broe et al., 2007) found a significant effect of vitamin D on fall incidence. The only two significant studies for fallers are Pfeifer et al. (2000, 2009), although Pfeifer et al. (2000) was a 2-month study and administered calcium with the vitamin D placebo.

Observational Studies

Observational studies have long suggested an association between a higher serum 25OHD level and a lower risk of falls in elderly persons; however, when analyzed as a whole in the AHRQ reviews, there was no consistency between study findings. Snijder et al. (2006), a study of elderly subjects participating in the Longitudinal Aging Study Amsterdam, a prospective cohort study, was not included in the AHRQ reviews. This study found that a low serum 25OHD level (< 25 nmol/L) was independently associated with an increased risk of falling for subjects who experienced two or more falls compared with those who did not fall or fell once; however the study outcome does not affect the discordant findings among observational studies identified in the AHRQ reviews.

Most observational studies of associations between serum 25OHD levels and physical performance have been cross–sectional, which limits causal inference. A cross–sectional study of 4,100 older adults from NHANES III found higher serum 25OHD concentrations associated with better lower-extremity function (Bischoff-Ferrari et al., 2004b). Much of the improvement occurred at concentrations ranging from 22.5 nmol/L to approximately 40 nmol/L, but some improvement was also seen from 40 to 94 nmol/L (the top of the reference range). Results were similar in men and women, three racial/ethnic groups (whites, African Americans, and Mexican Americans), active and inactive persons, and those with high and low calcium intakes. A study of Dutch adults 65 years of age and older found that serum 25OHD concentrations below 20 nmol/L were significantly associated with poorer physical performance at baseline and a greater decline in physical performance over a 3-year period (Wicherts et al., 2007). Another cross–sectional study of healthy post-menopausal women found that serum 25OHD level was significantly associated with physical fitness indexes, including balance, handgrip strength, androidal fat mass, and lean mass (Stewart et al., 2009). Finally, a cross–sectional study of 60 men and women with heart failure (mean age of 77 years) found a significant association between serum 25OHD level and 6-minute walk distance and frailty status (Boxer et al., 2008). Taken together, however, this evidence is weakened by the cross–sectional study design, does not provide strong support for an association between serum 25OHD level and physical performance, and does not contradict the findings of the AHRQ reviews.

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Concluding Statement

A problem in a number of the RCTs is that falls rather than fallers are analyzed; consequently, individuals who fell more than once were also counted more than once in the primary outcome analysis. The studies generally did not have the statistical power to detect a significant difference in the number of fallers but relied on repeat fallers to achieve the desired number of total falls. Moreover, the meta-analyses described above combined data from the few trials in which fallers were counted with data from trials in which falls were the outcome. By comparison, the U.S. Food and Drug Administration (FDA) mandated primary outcomes in osteoporosis and cardiovascular trials are the number of individuals with fractures or cardiovascular events rather than the number of events. It remains uncertain whether a reduction in the number of falls can be used to infer that the number of fallers would be significantly reduced.

The committee's review of the available evidence, including the results from RCTs and observational associations between vitamin D with or without calcium and risk for falls and poor physical performance, indicates a lack of sufficiently strong evidence to support DRI development. A limited review of observational data outside of the AHRQ reviews found some support for an association between 25OHD levels and physical performance. However, high-quality observational evidence from large cohort studies was lacking. Additionally, although the cross–sectional studies were more supportive of an association between high serum 25OHD levels and reduced risk for falls, evidence from RCTs in particular showed outcomes that varied in significance and thus did not support the observational findings or a causal relationship. The evidence was also not consistently supportive for a role for vitamin D combined with calcium in reduction of risk for falls.

Overall, data from RCTs suggest that vitamin D dosages of at least 800 IU/day, either alone or in combination with calcium, may confer benefits for physical performance measures. Although high doses of vitamin D (i.e., ≥ 800 IU/day) appear to provide greater benefit for physical performance than low doses (i.e., 400 IU/day), evidence is insufficient to define the shape of the dose–response curve for higher levels of intake. Thus, the outcome of physical performance is appropriate for identifying Estimated Average Requirements (EARs) of vitamin D, with or without calcium, in adults above the age of 50, but cannot be used to define the shape of the dose–response curve at higher levels of intake.

Immune Responses

Vitamin D has been reported to modulate immune functioning in cell culture and animal models. Vitamin D, specifically its active form, calcitriol,

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is a regulator of both adaptive and innate immune responses. However, its role is complex and not fully understood. Many factors influence the specific effect of vitamin D on immune function including its target cells; the nature of the immune challenge and response (either autoimmune or anti-infective); the status and availability of calcium, depending on the tissue or cell type; the physiological, differentiated or activated stage of the tissue or cell type; and the expression and polymorphisms of the genes for the *Vdr* and 1α-hydroxylase.

Asthma

Asthma is a chronic lung disease that manifests as inflammation in bronchial tissue. The disease is characterized by recurrent periods of wheezing, chest tightness, shortness of breath, and coughing and may be accompanied by comorbidities, such as eczema or atopic dermatitis. Diet has long been linked to asthma and allergic disease. Dietary sodium and magnesium intakes were implicated as risk factors for asthma in the 1980s and 1990s (Burney, 1987; Britton et al., 1994a, b). Dietary lipids have also been hypothesized to contribute to increased prevalence of asthma (Black and Sharpe, 1997). More recently, vitamin D has been linked to asthma incidence in the developing fetus and in young children (Litonjua and Weiss, 2007).

Biological plausibility Genetic studies mapping the *Vdr* gene in animal models of asthma suggest that *Vdr* polymorphism is linked with expression of asthma. In humans, Poon et al. (2004) compared *Vdr* genetic variants between members of a family-based cohort (223 families of 1,139 individuals) with and without asthma. Their analysis found significant associations between six polymorphisms in the *Vdr* gene and clinical diagnosis of asthma. Wjst (2005) conducted genotyping on 951 individuals from pedigrees that had at least two asthmatic children to determine whether transmission of *Vdr* polymorphism was associated with asthma in the children. Preferential transmission of candidate polymorphisms in asthmatic children could not be confirmed; however, the authors did hypothesize the possibility of transmitting a protective effect to unaffected offspring based on their finding of a low probability of an unaffected phenotype in an affected cohort.

Systematic reviews and meta-analyses No systematic reviews or meta-analyses were identified for this indicator.

Additional evidence from randomized controlled trials No RCTs were identified for this indicator.

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Observational studies A few studies were identified that examined a genetic linkage between vitamin D and risk for asthma or related conditions, and these were discussed above. Additional observational studies examined the relationship between perinatal serum 25OHD levels and risk of asthma in offspring. Devereux et al. (2007) examined associations between vitamin D intake during pregnancy and risk for childhood wheezing in a large prospective cohort study; a significant inverse association was found between maternal intake of vitamin D from diet and supplements and symptoms of wheezing at 2 and 5 years of age, although there was no significant association with diagnosed asthma at 5 years of age. In another study, Camargo et al. (2007) assessed the relationship between maternal dietary intake of vitamin D during pregnancy and risk of wheezing in children in Project Viva, a large prospective cohort study examining prenatal factors and pregnancy and child health outcomes. Overall, higher maternal intake of vitamin D during pregnancy was significantly associated with a lower risk for recurrent wheezing in the offspring at 3 years of age when compared with the lowest maternal vitamin D intake. Other associated symptoms of respiratory infection and eczema, however, were not significantly associated with maternal vitamin D intake. Similarly, Hypponen et al. (2004) found in a large prospective cohort study in Finland, that the prevalence of atopy and allergic rhinitis in subjects at age 31 years was higher among those who received regular vitamin D supplementation as infants than among those who did not; however, this study relied on retrospective recall of supplementation by the mother.

These large prospective cohort studies support an association between maternal or infant vitamin D intake and risk related to symptoms of asthma, particularly wheezing, but not with diagnosed disease. Several other observational studies have examined associations between 25OHD level in blood and risk for asthma. Gale et al. (2008), in a small prospective cohort study, found a five times increased risk for asthma at 9 years among children whose mothers' serum 25OHD level was below 27.5 nmol/L, compared with those whose mothers had levels above 75 nmol/L. The small size of the cohort that was followed to 9 years of age (178 subjects) was a limitation to the reported finding. In contrast, a larger analysis of NHANES data found no association between serum 25OHD level and sensitization to allergens (Wjst and Hypponen, 2007). The study did identify an increased prevalence of allergic rhinitis across levels of 25OHD, although unrecognized confounding may account for the association.

In a cross–sectional study examining associations between vitamin D status and markers of allergic or asthmatic response, Brehm et al. (2009) found that serum vitamin D levels below 75 nmol/L were identified in 28 percent of children and that inflammatory markers (immunoglobulin E

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[IgE] and eosinophil count) were significantly and inversely associated with vitamin D status. These lower quality observational studies largely support the associations with symptoms of asthma identified in the larger cohort studies but do not support an association between 25OHD level in blood and diagnosed asthma.

Although genetic studies support a possible biological mechanism for a functional role of vitamin D in development of asthma there are no RCTs to demonstrate a causal role.

Autoimmune Diseases

Autoimmune diseases such as multiple sclerosis (MS), rheumatoid arthritis (RA), inflammatory bowel disease (IBD), and lupus are characterized by abnormal T cell response to self, resulting in inflammatory reactions in peripheral tissues. Models of autoimmune diseases support a role for vitamin D in regulating the T helper 1 (Th1) immune response, an integral component of immune tolerance with regard to recognition of self (reviewed in Cantorna and Mahon, 2005; Szodoray et al., 2008). Recent genomic analyses for polymorphisms in the *Vdr* gene suggest that single nucleotide polymorphisms identified in individuals with type 1 diabetes could negatively modulate calcitriol synthesis and thereby play a detrimental role in autoimmune response and subsequent manifestation of the disease (Israni et al., 2009).

Diabetes (type 1) Type 1 diabetes is a chronic disease resulting from loss of β-cell function in the pancreas. The disease is characterized by diminished or absent insulin production and loss of control of blood glucose. Emerging evidence for an association between low vitamin D status and increased risk for type 1 diabetes comes from experimental animal, ecological, and observational studies; however, no intervention trials using supplemental vitamin D (not analogues) were identified to provide causal support for a relationship.

Biological plausibility Experimental animal, ecological, and observational evidence support a relationship between vitamin D status and risk for type 1 diabetes, although treatment protocols and dosages vary. Ecological evidence has suggested a link between type 1 diabetes risk and limited erythemal UVB exposure in Newfoundland (Sloka et al., 2009, 2010). Mohr et al. (2008) plotted incidence rates for type 1 diabetes by latitude in an ecologic study comparing geographical distribution, estimated UVB exposure and disease incidence and, using a polynomial analysis to best fit the data points, determined that the incidence of type 1 diabetes was greater at higher latitudes.

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In nonobese diabetic (NOD) mice, which are genetically predisposed to develop insulitis and type 1 diabetes, disease developed earlier when the mice were fed vitamin D–deficient diet and reared in the absence of UV light (Giulietti et al., 2004). However, type 1 diabetes was not prevented when NOD mice were treated with a supraphysiological dose of vitamin D, beginning from conception and continuing to 10 weeks of age (Hawa et al., 2004). Additionally, in a study in which NOD mice were cross bred with mice null for the *Vdr* gene, the rate of disease presentation did not differ from that in mice carrying only the NOD mutation, even though immune abnormalities were aggravated by the absence of the VDR (Gysemans, 2008). These results indicate that severe vitamin D and UV deficiency can increase the risk of type I diabetes in a genetically predisposed animal, yet neither vitamin D nor the absence of the *Vdr* gene affects the onset of type 1 diabetes.

Systematic reviews and meta-analyses Neither AHRQ-Ottawa nor AHRQ-Tufts included type 1 diabetes as a health outcome in its systematic reviews. Another recent systematic review and meta-analysis of five observational, four case–control and one cohort study (no RCTs were found) assessed whether vitamin D supplementation of infants reduced risk for type 1 diabetes later in life (Zipitis and Akobeng, 2008). The meta-analysis of data from the four case–control studies revealed a significant 29 percent reduction in risk for type 1 diabetes among vitamin D–supplemented infants compared with controls, which was further supported by the cohort study. The authors also cited evidence for a dose–response effect based on studies indicating reduced likelihood for developing diabetes among subjects who received regular vitamin D supplements, whereas subjects who developed rickets early in life were more likely to develop diabetes. A limitation of this meta-analysis is that two of the studies included had study designs that relied on delayed retrospective recalls by the mothers of vitamin D–supplemented infants. Additionally, no other meta-analyses were identified that either support or refute the findings of Zipitis and Akobeng (2008).

Additional evidence from randomized controlled trials No RCTs were identified for this indicator.

Observational studies No additional observational evidence that was not included in the systematic reviews and meta-analysis was identified for consideration.

Inflammatory bowel and Crohn's disease IBD is a group of conditions of chronic inflammation that usually involve the distal portion of the ileum. In Crohn's disease, inflammation spreads to the colon and upper gastrointestinal tract and causes local abscesses, scarring, and bowel obstruction; the condition is also characterized by diarrhea, cramping, and loss of ap-

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petite and weight. Vitamin D status has been linked to IBD in association studies of sun exposure and in genetic studies through down-regulation of the Th1-mediated immune response.

Biological plausibility Ecological studies have linked vitamin D, particularly 25OHD levels, to a number of autoimmune diseases. A connection between seasonal vitamin D status and risk for Crohn's disease was proposed by Peyrin-Biroulet et al. (2009), based largely on ecological evidence for an association between low 25OHD levels in blood and other autoimmune diseases. The effect of seasonal variation on serum 25OHD levels in patients with Crohn's disease, compared to matched controls found that mean serum 25OHD was lower in Crohn's patients despite having vitamin D intake from foods and supplements and sunlight exposure similar to those of matched controls (McCarthy et al., 2005). Genetic evidence in humans and in animal models provides some support for a biological association between polymorphisms in the *Vdr* and susceptibility to IBD and Crohn's disease. In a human study, a linkage analysis, used to identify the *TaqI* polymorphism in the *Vdr* gene, suggested that the variant may be a candidate for conferring susceptibility to IBD (Simmons et al., 2000). Animal model studies in both vitamin D–deficient and *Vdr* null mice suggested that the risk of developing IBD is increased in several respects: spontaneous occurrences are increased, the disease is more severe, and the disease is more easily provoked in response to agents that induce IBD or bacterial infections transferred from an affected animal (reviewed in Bouillon et al., 2008).

Systematic reviews and meta-analyses The AHRQ-Tufts systematic review found no RCTs for immune function clinical outcomes and no evidence for IBD or Crohn's disease. Thus, the evidence was insufficient for further analysis in the systematic review. No meta-analyses were identified for this indicator.

Additional evidence from randomized controlled trials No RCTs were identified for this indicator.

Observational studies Two observational studies were identified that evaluated 25OHD levels in patients with Crohn's disease and/or IBD. A cross–sectional assessment of serum 25OHD levels in children and young adults with IBD living in Boston found that prevalence of low 25OHD status (≤ 38 nmol/L) averaged 34.6 percent overall, with higher prevalence in winter compared with summer (Pappa et al., 2006). A small population-based cohort of patients with Crohn's disease and ulcerative colitis in Scandinavia found a prevalence of 25OHD levels below 30 nmol/L in 27 percent of those with Crohn's disease and 15 percent of those with ulcerative colitis. In addition, patients with Crohn's disease had lower mean serum 25OHD levels compared with those with ulcerative colitis or the reference population (Jahnsen et al., 2002). The study design and poor con-

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trols characteristic of these observational studies diminish the reliability of their findings. Another confounding problem is that vitamin D is absorbed with fat in the terminal ileum, and this is the area that is most inflamed in Crohn's disease (and can become inflamed in ulcerative colitis). Consequently, low 25OHD levels can be expected to occur as a consequence of the inflammatory condition. The question not answered by these studies is whether low 25OHD levels can predispose individuals to the conditions.

Multiple sclerosis MS is a chronic disease of the central nervous system that manifests as numbness in the limbs or, in more severe cases, paralysis or loss of vision. The progress, severity, and specific symptoms of MS are unpredictable and vary among individuals. The disease is an autoimmune response directed against myelin. Damaged myelin forms scar tissue (sclerosis), which impairs nerve impulse conduction, producing the variety of symptoms associated with the disease.

Biological plausibility Similar to findings with other autoimmune-related diseases, low solar exposure, latitude, and polymorphisms in the *Vdr* gene have been implicated in susceptibility to MS (Partridge et al., 2004; Dwyer et al., 2008; Sloka et al., 2008; Dickinson et al., 2009). However, whether a lack of sun exposure is causally related to MS cannot be shown. Findings from animal models are not consistent. In a mouse model, vitamin D deficiency accelerated development of autoimmune encephalomyelitis (the murine model of MS in humans), whereas treatment with calcitriol reduced it (Cantorna et al., 1996). In contrast, a subsequent study, using a mouse model null for the *Vdr* gene, found that the *Vdr* null mice were protected from development of the disease compared with wild-type mice (Meehan and DeLuca, 2002). A recent genetic study in humans evaluating associations between specific *Vdr* gene polymorphisms (*Apal* and *Taq1*) and serum 25OHD levels in healthy adults compared with those with MS, found no relationship between mutations in *Apal* and *Taq1* and incidence of MS (Smolders et al., 2009). Taken together, neither ecological studies nor genetic studies in animal models and humans show consistency in finding a significant relationship between serum 25OHD level and presence of MS.

Systematic reviews and meta-analyses The AHRQ-Tufts systematic review found no RCTs for immune function clinical outcomes and no evidence for MS related to vitamin D. In a recent review paper of observational studies on the effects of vitamin D on incidence and severity of MS, Smolders et al. (2008) concluded that there was no strong direct evidence supporting the ability of vitamin D to modulate MS or influence risk for the disease. Their review included observational evidence in humans linking low serum 25OHD levels with incidence of MS in white American adolescents; associations between lower circulating levels of 25OHD after onset of MS;

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associations between skin pigmentation and lower disability scores in females; congruence of geographical distribution of MS with geographical distribution of low vitamin D levels; associations between seasonal variation in birth and in disease severity with the seasonal variation of low vitamin D levels; associations between remission and pregnancy (when calcitriol levels increase); and variations in risk associated with polymorphisms in the *Vdr* gene. In addition to the lack of positive evidence, the authors raised concerns about the safety of calcitriol treatment for MS because of the dose-dependent risk for hypercalcemia identified with calcitriol treatment in animal models. No meta-analyses were identified for this indicator.

Additional evidence from randomized controlled trials No RCTs were identified for this indicator.

Observational studies Observational studies in humans have also failed to show a consistent association between serum 25OHD levels and MS. A small longitudinal study of 23 MS patients and 23 controls found no differences in circulating 25OHD levels, no difference in seasonal variation, and comparable rates of vitamin D deficiency or insufficiency based on serum 25OHD levels between MS patients and controls (Soilu-Hanninen et al., 2008). Interestingly, in this study, serum 25OHD levels were significantly lower during relapse episodes, whereas serum levels of intact PTH were significantly higher than in remission periods in MS patients. A prospective nested case–control study in military personnel reported that, for white subjects, serum 25OHD levels were inversely related to the risk of MS and that effect was even greater when serum 25OHD levels were low in individuals under 20 years of age (Munger et al., 2006). Overall, serum 25OHD levels were lower in black and Hispanic compared to white subjects and 25OHD levels were more frequently in the range of 25 to 40 nmol/L in MS patients compared to controls. These findings, however, were unrelated to risk for MS (Munger et al., 2006). In a small population-based case–control study of individuals living at latitudes of 41 to 43°S (similar to New York City and Boston), van der Mei et al. (2007) found that serum 25OHD levels below 25 nmol/L were moderately associated with MS, compared with levels above 40 nmol/L. With more consistent serum 25OHD levels and less seasonal variability, there was an association with less disability.

Taken together, these observational studies show widely variable outcomes for associations between serum 25OHD levels and MS and such associations are not supported by meta-analyses. In addition, the lack of causal evidence further diminishes the likelihood for a relationship between vitamin D and MS.

Rheumatoid arthritis RA is a chronic disease characterized by systemic inflammation that may affect many tissues and organs, but particularly the joints. In RA inflammatory synovitis of the joints can progress to destruction of the articular cartilage and ankylosis. RA can also produce diffuse

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inflammation in the lungs, pericardium, pleura, and sclera, as well as nodular lesions under the skin. This progressive disease can result in chronic pain, loss of function, and eventual disability.

Biological plausibility In experimental studies, Tetlow and Wooley (1999) found that the VDR was strongly expressed in cells associated with rheumatoid lesions, including macrophages, synovial fibroblasts, and chondrocytes, but weakly or not at all in normal articular cartilage tissue, suggesting an up-regulation of VDR-mediated activity in tissues affected by RA. Smith et al. (1999) found that cultured human synovial fibroblasts, but not human articular chondrocytes, when treated with the inflammatory cytokine, interleukin 1 (IL-1), followed by calcitriol, indicated inhibition of expression of the matrix metalloproteinases associated with RA. In a mouse model of RA, treatment with calcitriol decreased arthritis symptoms induced by injection with bovine collagen and halted the progression of arthritis after arthritic lesions were apparent (Cantorna et al., 1998). Together, this evidence is suggestive of an immunomodulatory role for vitamin D in expression of arthritic changes in some, but not all, cell types associated with RA.

Systematic reviews and meta-analyses The AHRQ systematic reviews found no RCTs for immune function clinical outcomes related to RA and no evidence that RA was related to vitamin D. No meta-analyses were identified for this indicator.

Additional evidence from randomized controlled trials No RCTs were identified for an effect of vitamin D and/or calcium on risk for RA.

Observational studies A number of studies have been conducted to determine whether serum 25OHD level and incidence of RA are associated. In a prospective cohort study, a small subset of subjects from the Iowa Women's Health Study were followed to determine if dietary vitamin D intake (primary outcome) and/or calcium intake (secondary outcome) were associated with incident RA (Merlino et al., 2004). No significant associations were found for dietary (not supplemented) vitamin D intake and risk for RA, although the association was significant for daily supplemental intakes of 400 IU or more compared with less than 400 IU. No association was found between calcium intake and risk for incident RA. A cross–sectional analysis of women with RA living in Brazil, found a significant correlation between higher mean serum calcium level and normal BMD compared with calcium levels in women with osteopenia, although no significant difference was found between calcium and vitamin D intake and BMD (Sarkis et al., 2009).

With no large prospective cohort studies and no clinical trials to support a relationship between vitamin D and/or calcium and RA, along with a paucity of other observational evidence, the committee could not conclude that either vitamin D or calcium is related to risk for RA.

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Systemic lupus erythematosus Systemic lupus erythematosus (SLE) is a chronic generalized connective tissue disorder characterized by skin eruptions, arthralgia, arthritis, leukopenia, anemia, visceral lesions, neurological manifestations, and lymphadenopathy. It has been proposed that vitamin D plays a role in maintenance of immune homeostasis, and recent studies have linked SLE to vitamin D deficiency although a causal relationship has not been established.

Biological plausibility Mouse models of SLE have been reported to produce high levels of IgG2a immune cells that are implicated in the pathogenesis of lupus (Slack et al., 1984). However, owing to the complexity of the disease, experimental animal studies have not shown consistent outcomes on questions regarding the role for vitamin D in preventing or alleviating manifestations of the disease. Administration of calcitriol in a murine SLE model using a treatment protocol of daily dosing with a low-calcium diet for 4 weeks followed by dosing every other day for 18 weeks resulted in attenuation of symptoms of SLE, including reduced dermatological lesions. All SLE mice in the study developed proteinuria by 20 weeks; however, among those treated with vitamin D, lower urinary protein/creatinine ratios indicated reduced levels of proteinuria (Lemire et al., 1992). In contrast to these findings, when Vaisberg et al. (2000) injected SLE-prone mice with vitamin D_3, they found a worsening of the histopathological effects of SLE in the kidney. Upon examining serum levels of calcitriol and 25OHD in SLE patients compared with unaffected controls, Muller et al. (1995) found lower levels of calcitriol but not 25OHD, but were unable to speculate on a cause for the difference. In humans, vitamin D has been proposed to modulate maturation and induction of interferon alpha-(IFN-α)–mediated monocyte differentiation into dendritic cells that are activated in SLE. The findings of Ben-Zvi et al. (2010) suggest that such a role is likely via vitamin D–mediated inhibition of over-expression of IFN-regulated genes in cultured monocytes from both normal and SLE patients following exposure to an activation factor. Altogether, however, there is a lack of consistency in study outcomes between animal models and human experimental studies, and thus findings are not supportive of a biological role for vitamin D in SLE.

Systematic reviews and meta-analyses The AHRQ systematic reviews found no RCTs for immune function clinical outcomes related to SLE and no evidence for a relationship between SLE and vitamin D. No meta-analyses were identified for this indicator.

Additional evidence from randomized controlled trials No RCTs were identified that examined a role for vitamin D or calcium in reducing risk for or manifestations of symptoms of SLE.

Observational studies Epidemiological evidence to support an association between vitamin D status and incidence of SLE shows variability in

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the levels of 25OHD associated with SLE. Kamen et al. (2006), in a small subset of a larger population-based cohort, found that 22 out of 123 SLE patients across race, age, and gender groups had 25OHD levels below 25 nmol/L and that African Americans had levels significantly lower than whites (40 nmol/L compared with 78 nmol/L [$p = 0.04$]). In a small pilot study of 50 subjects (25 per group), Huisman et al. (2001) found that 50 percent of female SLE patients had 25OHD levels below 50 nmol/L. The associations between vitamin D status and incidence of SLE identified in these studies are not borne out by evidence from a prospective cohort study of dietary factors and risk for developing SLE. An analysis of a small subset of women participating in NHS over a period of 22 years found no association between vitamin D (or calcium) intake assessed with a food frequency questionnaire and risk for developing SLE or RA (Costenbader et al., 2008). In a cross–sectional survey, Ruiz-Irastorza et al. (2008) found that 75 percent of patients with SLE had serum 25OHD levels below 75 nmol/L and 15 percent had levels below 25 nmol/L, and 25OHD levels in blood in patients with SLE were not responsive to calcium and vitamin D treatment. Thus, it is not clear whether therapeutic treatment would have any effect on disease manifestation. The few relevant studies identified for review, and the lack of uniformly significant findings between studies, which may be a result of the small study populations (< 200 participants), are not sufficient to permit the committee to draw a conclusion about an association between SLE and vitamin D intake or 25OHD levels in blood.

Infectious Diseases

Tuberculosis Pulmonary tuberculosis (TB) is a granulomatous infection in which hypercalcemia occurs in a subset of patients (Sharma et al., 1972; Abbasi et al., 1979; Need and Phillips, 1979). The increased production of immune and inflammatory cells in patients with TB correlates with increased serum levels of calcitriol (Adams et al., 1989) and with calcitriol in pleural fluid (Cadranel et al., 1994). Treatment of alveolar macrophages with IFN-γ appears to stimulate synthesis of calcitriol (Koeffler et al., 1985; Reichel et al., 1987). Although vitamin D has been used as a therapeutic agent in the management of TB (Martineau et al., 2007a), treatment of individuals with active TB with supplemental vitamin D exacerbates or reveals hypercalcemia (Sharma, 1981).

Biological plausibility Vitamin D may be an important factor in innate immunity in the upper respiratory tract (reviewed in Bartley, 2010). Although calcitriol does not have direct anti-bacterial activity, it induces anti-tubercular actions in cultured monocytes and macrophages (Chan et al., 1994). Recent evidence, stemming from the molecular cloning of 1α-hydroxylase (Monkawa et al., 2000), supports macrophages as the source

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of 1α-hydroxylase that converts 25OHD to calcitriol and stimulates the mobilization of calcium seen in inflammatory diseases (Inui et al., 2001; Yokomura et al., 2003; Karakelides et al., 2006). Animal models provide additional evidence that calcitriol levels increase in tubercular infection. Rhodes et al. (2003) identified a transient increase in calcitriol levels following infection of cattle with bovine mycobacterium, but only among animals that went on to develop TB. This increase in activated vitamin D that accompanied infection suggests a role for vitamin D in the host immune reactivity to TB infection. In a mouse model, calcitriol increased production of nitric oxide, an endogenously produced anti-infective compound, suggesting a bactericidal mechanism for vitamin D in TB-infected animals (Waters et al., 2004). Other more recent studies have identified the peripheral cellular conversion of 25OHD to calcitriol as a mechanism for rapid local induction of anti-microbial peptides as a direct mechanism for killing TB and staphylococcal bacteria (Liu et al., 2006; Schauber et al., 2006). Immune responses to increased 25OHD levels may vary among individuals as a result of the genetic expression of *Vdr* polymorphisms. For example, Selvaraj et al. (2008) found that allelic variations in the Cdx-2 polymorphism were associated with either resistance or susceptibility to TB bacteria; however, more research is needed to better understand the genetic relationship between *Vdr* polymorphisms and TB susceptibility.

Systematic reviews and meta-analyses The AHRQ reviews did not identify relevant evidence for TB as an outcome. Nnoaham and Clarke (2008) systematically reviewed and meta-analyzed the relationship between low serum 25OHD levels and TB among diverse community- and hospital-based population groups in both developed and developing countries. The meta-analysis was restricted to studies that compared serum 25OHD levels in TB patients not on a treatment regimen with healthy matched controls. Seven observational studies were included in the meta-analysis, which found a 70 percent probability that a person without TB would have a higher serum 25OHD level than a person with TB. Whether this association predicts low serum 25OHD as a risk factor for active TB cannot be concluded from the analysis; however, the findings would increase the strength of similar findings from other high-quality observational studies or from a larger meta-analysis.

Additional evidence from randomized controlled trials Even though treatment of individuals with active TB with supplemental vitamin D can lead to hypercalcemia, vitamin D has been used as a therapeutic agent in the management of the disease. A small double-blind RCT of 131 individuals who had come into close contact with patients with TB (or TB contacts) in the United Kingdom (UK) measured the ability of a single oral dose of 100,000 IU of vitamin D to inhibit growth of recombinant mycobacteria,

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an indicator of TB exposure, grown in vitro and detected using the Barger-Lux (BCG-*lux*) assay, from the study subjects (Martineau et al., 2007b). Compared with placebo, subjects who received the vitamin D treatment showed a significant increase in serum 25OHD levels for 6 weeks without hypercalcemia, as well as inhibition of in vitro growth of mycobacteria. Another small intervention trial examined the effect of UVB exposure on TB in recent Asian immigrants to the UK who have a higher TB prevalence than indigenous populations (Yesudian et al., 2008). Anti-microbial activity, expressed as response to the BCG-*lux* assay, varied between the subjects, showing a small transient decrease but no significant change following UVB exposure. Another RCT testing the effect of vitamin D supplementation on the clinical course of patients with TB also found no significant change in clinical outcome or reduction in mortality in patients treated with 100,000 IU of vitamin D three times over 8 months (Wejse et al., 2009). Only one of the two studies reviewed showed a significant effect of vitamin D treatment on 25OHD levels in TB patients, and none of the clinical trials showed a significant effect of vitamin D on clinical outcome. Thus, evidence from RCTs does not support a reduction in TB infections with vitamin D treatment.

Observational studies Because TB is rare in industrialized countries, particularly the United States, most observational studies are on populations from developing countries that have immigrated to developed countries. Gibney et al. (2008), in a retrospective analysis of hospitalized patients, found that low serum 25OHD levels in patients born in sub-Saharan African countries who immigrated to Australia were predictive of any form of TB infection as well as current and past infection. Another analysis of a small study of patients reporting to a TB clinic prior to treatment (Sita-Lumsden et al., 2007) found a statistically significant difference in serum 25OHD levels between TB patients and their contacts and the greatest difference among those patients with the lowest 25OHD levels. Although there was no difference in dietary intake of vitamin D between TB patients and their contacts, the TB patients did demonstrate a stronger correlation between dietary intake and measures of vitamin D in serum. Sun exposure did not differ between patients and their contacts. Strachan et al. (1995), in a case–control study of Asian immigrants to the UK who had a diagnosis of active TB, found a trend of increasing risk of TB correlated with a decreasing frequency of consumption of meat or fish and an 8.5-fold increased risk for TB among lactovegetarians compared with daily meat or fish eaters. Many observational studies of an effect of vitamin D on susceptibility to TB are confounded by endogenous production of calcitriol in infected individuals (Adams et al., 1989; Cadranel et al., 1994) and thus must be cautiously interpreted. Nevertheless, the few small studies identified support the findings

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of the meta-analysis from Nnoaham and Clarke (2008) for higher serum 25OHD levels in TB patients; however, these studies did not uniformly find significant associations between vitamin D intake and risk for TB.

Influenza and upper respiratory infections Influenza is an acute contagious viral infection characterized by inflammation of the respiratory tract, fever, chills, and muscular pain. Upper respiratory infections are most commonly viral infections characterized by inflammation of the respiratory tract. Vitamin D has been hypothesized to act through the immune system to prevent influenza infections.

Biological plausibility Environmental observations of seasonal variation in serum 25OHD levels and occurrence of influenza have been proposed as an indicator to show a correlation between vitamin D and risk for influenza (Cannell et al., 2006; Hayes, 2009). In an animal study (Underdahl and Young, 1956), mice deficient in vitamins A, D, and E and inoculated with influenza had the same intensity of influenza infection as those mice replete in vitamins A, D, and E, showing no effect of vitamin D status on reducing influenza infection. This finding contrasts with earlier work by Young et al. (1949), which suggested that vitamin D could reduce the susceptibility of mice to influenza.

Systematic reviews and meta-analyses The AHRQ studies did not identify any relevant studies for influenza as a health outcome. There were no meta-analyses identified for this indicator.

Additional evidence from randomized controlled trials Available data from RCTs do not provide strong support for a role for vitamin D in reducing susceptibility to influenza infection. A small RCT testing the effect of 1,200 IU of vitamin D supplementation per day for 4 months found that for influenza A as the primary outcome, occurrence between days 1 and 30 was not significantly different between the vitamin D group and placebo, but between days 31 and 60, influenza A occurred significantly less often in the vitamin D than in the placebo group; between day 61 and the end of the study, the occurrence of influenza A was not significantly different between the vitamin D and placebo groups (Urashima et al., 2010). Analysis of other related secondary outcomes showed no significant difference for influenza B, influenza-like illness (negative in rapid influenza diagnostic tests), non-specific fever, gastroenteritis, pneumonia, hospital admission, or absence from school. Overall, the absolute reduction in influenza A cases was offset by a similar increase in the number of influenza B cases. These may be chance findings, however, as a result of confounding by the loss of subjects. In another 3-month prospective double-blind RCT (Li-Ng et al., 2009), even though 73 percent of subjects supplemented with 2,000 IU of vitamin D_3 daily achieved a serum 25OHD level above 75 nmol/L, no benefit of supplementation was seen for either prevention of self-reported

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upper respiratory infections or a decrease in their severity. This evidence from RCTs in both children and adults shows no causal role for vitamin D in either reducing or preventing influenza.

Observational studies Only one study of 16 patients was identified in which plasma 25OHD levels were measured in children undergoing tympanosotomy tube placement. The study showed that 50 percent of the children had 25OHD levels less than 50 nmol/L and 31 percent had levels between 52.5 and 72.5 nmol/L (Linday et al., 2008). The authors concluded from this finding that there was a possible relationship between vitamin D and susceptibility to bacterial infection and influenza. This small observational study is not adequate to support a relationship between vitamin D and one outcome related to influenza infection and no additional evidence was found to verify any causal or associative relationship between vitamin D and influenza.

Concluding Statement

The committee's review of the results of large cohort studies showed support for a positive association between vitamin D intake and reduction in symptoms associated with asthma but not with diagnosed disease. Other observational evidence of lower quality was found to largely support an association between risk for asthma and 25OHD level in blood, but associations have not been shown for diagnosed asthma. The lack of causal evidence and the lack of observational data demonstrating a relationship between vitamin D and diagnosed asthma led the committee to conclude that development of a DRI for this indicator is not supported by the totality of the evidence reviewed.

Emerging observational evidence in humans and experimental studies in animals inversely links vitamin D measures to risk of autoimmune disorders such as type 1 diabetes, MS, and IBD as well as infectious diseases such as TB (Maruotti and Cantatore, 2010). However, even though animal models indicate plausibility for a mechanistic role for vitamin D in autoimmune or anti-microbial function, results from RCTs as well as from observational associations between vitamin D and calcium and risk for either autoimmune or infectious diseases show a lack of consistency. Although both retrospective and prospective studies tend to support an inverse association between serum 25OHD levels and autoimmune and infectious diseases, these studies are limited in their interpretation owing to confounding effects that require further verification. The evidence available from RCTs is of limited utility because of the small size of the trials, inconsistency in measured outcomes, and lack of dose–response data. Overall, the evidence was not consistently supportive of a causal role for vitamin D combined with calcium or for vitamin D alone in reducing risk for developing auto-

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immune or infectious diseases. In the absence of verifiable dose–response data from RCTs a conclusion about asthma, autoimmune, or infectious diseases as indicators for DRI development cannot be reached.

Neuropsychological Functioning

Emerging evidence is suggestive of a role for vitamin D in neuropsychological functioning, including a range of diseases from autism to Alzheimer's.

Autism

Autism is a neurodevelopmental disorder of unknown etiology that manifests as repetitive behaviors, social withdrawal, and communication deficits. A number of factors are implicated in development of the disorder, including genetic (Abrahams and Geschwind, 2008) and environmental (Deth et al., 2008) factors.

Biological plausibility Although mechanistic studies using animal models tend to support an association between vitamin D intake during pregnancy and subsequent development of autism, these studies are limited in their interpretation and extrapolation to humans. There are some animal models suggesting a mechanism whereby vitamin D may influence the development of autism (reviewed in McGrath et al., 2004). These experiments demonstrate that pre-natal deprivation of vitamin D_3 results in gross abnormalities in fetal rat brains at birth. Feron et al. (2005) subsequently reported that vitamin D deprivation and associated disruptions in brain development seen in rat pups at birth persisted into adulthood.

In humans, *Vdr* gene polymorphisms have been proposed as a possible link to psychiatric diseases, including autism. Yan et al. (2005) analyzed the coding sequences and splice junctions of 100 patients with schizophrenia and, in a pilot study within the same population, 24 patients with autism. The frequency of the sequence variants identified, however, was not significantly different from that of sequence variants found in control subjects.

Systematic reviews and meta-analyses The AHRQ reviews did not identify evidence to support autism as a relevant health outcome for vitamin D. No meta-analyses were identified for this indicator.

Additional evidence from randomized controlled trials No RCTs were identified for this indicator.

Observational studies No large, prospective cohort studies were identified that examined associations between either vitamin D intake or 25OHD

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levels in blood and risk for autism. Three lower-quality observational studies were identified for further consideration: one retrospective chart review, one small cohort from a developing country, and one cross–sectional study. A recent study in Sweden that retrospectively reviewed serum 25OHD levels from medical records of out-patients receiving psychiatric care, including autism, suggested a high prevalence of vitamin D insufficiency in psychiatric outpatients (Humble et al., 2010). Although the study did not include matched controls, comparisons made with previously published samples from healthy Swedish populations suggested that the prevalence of vitamin D insufficiency was greater in the population receiving psychiatric care. The study, however, did not take into account dietary intake of vitamin D. Fernell and Gillberg (2010) tested the association between serum 25OHD levels and prevalence of autism in an analysis of a small cohort of mothers of children with autism from Somalia and Sweden, but they found no statistically significant differences in serum 25OHD levels between either group of mothers and controls.

Herndon et al. (2009), in a cross–sectional study, examined associations between vitamin D and calcium in dairy foods and autism. This study found that children with autism spectrum disorders consumed less calcium and fewer servings of dairy foods compared with children with typical development. Interpretation of this evidence for an association between vitamin D measures and risk for autism, however, is confounded by other potential factors that could influence vitamin D measures.

Concluding statement Owing to the lack of causal evidence from RCTs and a paucity of evidence, as well as a lack of data from large, prospective cohort studies and inconsistent findings for an association between vitamin D and incidence of autism from largely cross–sectional observational studies, autism was not considered further as an indicator for DRI development.

Cognitive Function

Loss of cognitive function in the form of dementia is frequently associated with aging. Between the ages of 60 and 85, the prevalence of dementia in the general population increases from 1 to 40 percent (Bolla et al., 2000). Dementia is classified into four major subtypes: Alzheimer's disease, Lewy body dementia, frontotemporal dementia, and vascular dementia (Bolla et al., 2000; Grossman et al., 2006). Vitamin D has been hypothesized to confer neuroprotective effects and reduce the risk for developing dementia (Buell and Dawson-Hughes, 2008; McCann and Ames, 2008).

Biological plausibility Vitamin D has been proposed to prevent cognitive decline, and plausible biological mechanisms support this hypothesis. Vitamin D may protect against cognitive decline by promoting vascular health

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through anti-inflammatory or other pathways, but may also have direct neuroprotective effects (Buell and Dawson-Hughes, 2008; McCann and Ames, 2008). Rodent models show morphological and biochemical effects of vitamin D on brain tissue. Early experiments on rat brain revealed that vitamin D-deficiency reduced vitamin D–dependent enzyme activity in the cerebral context, including non-sodium-mediated glucose transport, that was restored when rats were treated with calcitriol (Stio et al., 1993). Subsequent work in mouse models demonstrated that developmental vitamin D deficiency had a negative effect on brain development, as manifested by changes in brain size and shape and ventricular size and reduced nerve growth factor expression (McGrath et al., 2004; Feron et al., 2005), as well as effects on brain function and exploratory behavior (Harms et al., 2008). When adult offspring of dams deprived of vitamin D during pregnancy underwent learning tests, they displayed impaired learning at 30 weeks of age but not at 60 weeks (de Abreu et al., 2010).

VDR and 1α-hydroxylase are found throughout the brain. Vitamin D affects gene and protein expression in brain tissue, including expression of neurotropins and glial cell–derived neurotrophic factor (Naveilhan et al., 1996; Sanchez et al., 2002). A battery of 40 different tests in *Vdr*-null mice showed them to have normal cognitive function but abnormal muscle and motor behavior, although the abnormal neuromuscular function may be due to hypocalcemia rather than a direct effect of loss of the VDR (reviewed in Bouillon et al., 2008). In neurological tissue, vitamin D modulates certain calcium-binding proteins, including calbindin-D28K, parvalbumin, and calretinin, which are important for brain function (de Viragh et al., 1989; Alexianu et al., 1998). In addition, calcitriol down-regulates the expression of calcium channel currents in rat hippocampal cells (Brewer et al., 2006), stimulates neurogenesis in human neuroblastoma cells (Moore et al., 1996; Taniura et al., 2006), and may affect other pathways (Garcion et al., 1997; Baas et al., 2000; Brown et al., 2003; Obradovic et al., 2006). Vitamin D restriction results in unfavorable structural and biochemical changes in the brain (Ko et al., 2004; Feron et al., 2005). However, experiments in rats rendered vitamin D deficient by dietary and UV radiation restriction and in *Vdr* null mice have not consistently shown learning impairments, although the data are sparse (Becker et al., 2005; Minasyan et al., 2007). Calcium independently of or in concert with vitamin D is involved in many physiological processes related to neural functioning, and disturbed calcium homeostasis is also characteristic of neurodegenerative disorders (Canzoniero and Snider, 2005; Mattson, 2007; Toescu and Verkhratsky, 2007).

Systematic reviews and meta-analyses The AHRQ reviews did not identify sufficient evidence to support cognition (or cognitive decline) as a relevant health outcome for vitamin D. No meta-analyses were identified for this indicator.

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Additional evidence from randomized controlled trials In the WHI trial, a subset of participants completed a cognitive test battery, but results of analyses examining the vitamin D intervention's effect on cognitive function are not yet available.

Observational studies Numerous observational studies that examined associations between vitamin D, serum 25OHD level, or calcium and cognitive function were identified as potentially relevant to DRI development. The greatest number of studies, however, were cross–sectional. No large prospective cohort studies were identified for review, although two analyses of data from large population-based annual surveys and several small cohort studies were included.

Low serum 25OHD levels have been associated with decreased cognitive function in various population groups. A cross–sectional analysis of 752 women 75 years of age and older in the Epidémiologie de l'Ostéoporose (EPIDOS) study found that participants with vitamin D deficiency (serum 25OHD level < 25 nmol/L) had twice the odds of cognitive impairment as other participants (Annweiler et al., 2010). In a population-based cross–sectional study, Lee et al. (2009) examined associations between serum 25OHD level and cognitive function and mood among adult men in a European population. In a spline regression model, significant associations were found between slower information processing and serum 25OHD levels below 35 nmol/L in men ages 40 years and older. In contrast to these findings, a more recent cross–sectional study found that among 1,604 men up to 65 years of age in the Osteoporotic Fractures in Men (MrOS) Study, there were no associations between serum 25OHD level and cognitive impairment, even after adjusting for age, race/ethnicity, education, and other potential confounders (Slinin et al., 2010). This study also examined vitamin D measures as a predictor of subsequent cognitive decline over a mean of 4.6 years of follow-up and found only a borderline significant trend across the first three quartiles of serum 25OHD levels, (≤ 49.75 nmol/L, 50.0 to < 62.75 nmol/L, and 62.75 to < 74.5 nmol/L, respectively), compared with the fourth quartile (≥ 74.5 nmol/L); serum 25OHD level did not predict decline on a timed test of executive function.

In a cross–sectional study of 318 older individuals (mean age 74 years) receiving home health care services, those who received a neurological exam and cranial magnetic resonance imaging (MRI), a lower serum 25OHD level (< 50 nmol/L) was associated with at least twice the odds for all-cause dementia, Alzheimer's disease, and stroke, as well as increased white-matter hyperintensity volume and prevalence of large-vessel infarcts (Buell et al., 2010). Among three age groups (adolescent, adult, and elderly) examined from NHANES III, no association was found between high serum 25OHD levels and learning or memory, and only the elderly population group was found to have an inverse association between 25OHD level

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and performance on a task of learning and memory. Within the elderly population group, those in the highest quintile for serum 25OHD level were also the most impaired; thus, the results fail to confirm the hypothesis that serum 25OHD level enhances performance in learning and memory (McGrath et al., 2007).

There are few observational studies on calcium and cognitive function. In a cross–sectional study on Korean adults 60 years of age and older, a positive association was found between calcium intake and score on the Mini-Mental State Examination for Koreans (MMSE-K) in women but not in men after adjustment for age (Lee et al., 2001). In contrast, another study in Portuguese adults more than 65 years of age found no association between calcium intake and MMSE score after 8.5 months of follow-up (Velho et al., 2008). Using a cross–sectional analysis, Wilkins et al. (2009) found, as expected, lower serum 25OHD levels among the African American population compared with the white population, and poorer cognitive performance among African Americans with the lowest 25OHD levels compared with those with higher levels. Similarly, in a cross–sectional analysis in a British population of adults ages 65 years or older with serum 25OHD levels reported in quartiles, Llewellyn et al. (2009) found a greater risk for impaired cognitive performance among persons in the lowest (8 to 30 nmol/L) compared with the highest quartile (66 to 170 nmol/L). Even though the committee identified a large number of observational studies that evaluated associations between vitamin D and calcium and cognitive function, these were predominantly lower quality cross–sectional studies or small cohort studies, and their results were mixed. No causal evidence was found to support experimental evidence for biological plausibility and the relatively weak observational evidence. The committee took into account the generally lower quality of the study designs in its interpretation of the findings and in drawing conclusions about outcomes associated with this indicator.

Depression

Depression is a disease with characteristic signs and symptoms that interfere with the ability to work, sleep, eat, and enjoy once-pleasurable activities. These signs and symptoms include loss of interest in activities; a persistently sad or anxious mood; feelings of hopelessness, pessimism, guilt, worthlessness, or helplessness; social withdrawal; fatigue; sleep disturbances; difficulty in concentrating or making decisions; unusual restlessness or irritability; persistent physical problems that do not respond to treatment; and thoughts of death or suicide or suicide attempts. Depressive disorders include major depressive disorder, dysthymic disorder, psychotic depression, postpartum depression, and seasonal affective disorder, with

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major depressive disorder and dysthymic disorder being the most common.[2] Whether there is a functional relationship between measures of serum vitamin D or intake and mood or depression has not been determined.

Biological plausibility Seasonal affective disorder occurs more often at northern latitudes, and the etiology is presumed to be due, at least in part, to lack of sunlight exposure. In turn, lack of sunlight exposure causes low serum 25OHD levels unless the diet is adequate in vitamin D. Investigators have pursued the hypothesis that the low serum 25OHD levels are a cause of seasonal affective disorder, although it must be considered that the lack of sunlight may independently cause both seasonal affective disorder and low serum 25OHD levels without a direct link between them.

Systematic reviews and meta-analyses The AHRQ reviews did not identify sufficient evidence to support depression as a relevant health outcome for vitamin D. No meta-analyses were identified for this indicator.

Additional evidence from randomized controlled trials One RCT was identified that evaluated effects of vitamin D supplementation on depressive symptoms. Jorde et al. (2008) gave either 20,000 or 40,000 IU of vitamin D_3 or a placebo treatment weekly for 1 year to men and women ages 21 to 70 years, living in Norway. Symptoms of depression were evaluated using the Beck Depression Inventory (BDI), and serum 25OHD level and BMI were measured. Participants whose serum 25OHD levels were below 40 nmol/L had significantly higher BDI scores, indicating a higher incidence of depressive disorder, compared with those whose serum 25OHD levels were 40 nmol/L and above after 1 year. Both treatment groups indicated significant improvement in BDI score compared with placebo.

Results of other randomized trials testing the effects of vitamin D on a subtype of depression that occurs during the winter months have been mixed. Three small, short-term trials examining effects of treatment with vitamin D for seasonal affective disorder reported that vitamin D improves mood (Lansdowne and Provost, 1998; Gloth et al., 1999; Vieth et al., 2004), but a larger, longer-term trial found no effect (Thys-Jacobs et al., 1998). Vieth et al. (2004) treated adults with serum 25OHD concentrations below 61 nmol/L with the equivalent of either 4,000 or 600 IU of vitamin D per day for 3 months over two consecutive winters and found evidence of a significant difference in measures of improved well-being at the higher compared with the lower dose. Lansdowne and Provost (1998) assigned healthy adults to 5 days of treatment with either 400 or 800 IU of vitamin

[2]Available online at http://www.nimh.nih.gov/health/publications/depression/what-are-the-different-forms-of-depression.shtml (accessed April 5, 2010).

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D or placebo and found that both vitamin D doses increased positive affect and decreased negative affect compared with placebo. Gloth et al. (1999) assigned 15 people to either 100,000 IU of vitamin D or broad-spectrum light therapy for 1 month and found an increase in serum 25OHD level was significantly associated with improvement in depressive symptoms. However, Harris and Dawson-Hughes (1993) randomized 250 middle-aged and older women to treatment with 400 IU of vitamin D per day of vitamin D plus 377 mg of calcium per day or to calcium alone for 1 year and found no treatment-related changes in seasonal mood as assessed by the Profile of Mood States (POMS) questionnaire.

Observational studies Among four cross–sectional studies on small population groups ($n < 50$) that evaluated associations between serum 25OHD level and evidence for clinical diagnosis of depression in women (Michelson et al., 1996; Herran et al., 2000; Eskandari et al., 2007) or men and women (Schneider et al., 2000), only Eskandari et al. (2007) found a significant association between serum 25OHD level and diagnosis of depression and Michelson et al. (1996) found a significant association with calcitriol level. Another large population-based cross–sectional study among middle-aged and elderly Chinese also found, after controlling for confounders and geographic location, no significant associations between serum 25OHD level (grouped by tertile) and symptoms of clinical depression (Pan et al., 2009). In contrast to the cross–sectional studies, Hoogendijk et al. (2008) found, in a cohort study in the Netherlands, a significantly lower mean serum 25OHD level (47.5 nmol/L) among individuals with both major and minor depression compared with a mean level of 55 nmol/L among those who did not have depression.

Concluding Statement

Although some observational studies support an association between low measures of vitamin D exposure and risk for cognitive impairment or changes in mood, results have been inconsistent, and the majority of studies were cross–sectional in study design, including possible selection bias or other confounding factors that diminish the quality ranking of the studies. In addition, few or no clinical trials were identified to support biological plausibility. As a result of the many shortcomings in study design and quality of observational evidence and the paucity of high-quality evidence from RCTs identified by the committee, the findings for neuropsychological indicators are inconclusive. The committee's review of the available evidence for either associations or a causal relationship between vitamin D and calcium and risk for cognitive disorders shows a lack of sufficient evidence to support DRI development.

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Preeclampsia, Pregnancy-Induced Hypertension, and Other Non-Skeletal Reproductive Outcomes

Preeclampsia is a serious condition in which hypertension and proteinuria arise in pregnancy. It can affect both the mother and the unborn child. Pregnancy-induced hypertension is a transient hypertension without proteinuria that occurs during pregnancy. Pregnancy is a type of immunological challenge, and women with some autoimmune diseases, particularly type 1 diabetes and RA, are at increased risk for developing preeclampsia (Evers et al., 2004; Wolfberg et al., 2004). Clinical observations have noted that urinary calcium excretion is low in women with preeclampsia, whereas it is elevated in women during normal pregnancy. Calcium intake has been examined relative to reducing the risk of preeclampsia.

Biological Plausibility: Preeclampsia and Pregnancy-Induced Hypertension

Vitamin D metabolism may be altered under conditions of preeclampsia (August et al., 1992), when calcitriol level is low and hypocalciuria is present, but it is unclear whether these are causes or consequences of preeclampsia. The placenta and deciduas both express 1α-hydroxylase and activate 25OHD in vitro. Calcitriol regulates immunomodulatory cytokine production in cultured decidual cells (Evans et al., 2006) and placental trophoblasts (Diaz et al., 2009). However, the specific role of vitamin D in vivo is less clear. Its actions in vitro may provide some clues as to its physiological relevance, but these hypotheses need to be examined rigorously in future studies.

As mentioned above, it has long been observed that urinary excretion of calcium is increased during pregnancy, and hypercalciuria may result. In contrast, women with preeclampsia often have hypocalciuria. This observation has prompted a number of investigations to test whether low calcium intake predisposes a pregnant woman to both hypocalciuria and preeclampsia, although a biological mechanism to explain how low calcium intake would cause the preeclampsia has not been clearly elucidated.

Systematic Reviews and Meta-Analyses: Preeclampsia and Pregnancy-Induced Hypertension

The AHRQ-Tufts analysis identified a single nested case–control study (rated B for methodological quality) that evaluated the association between serum 25OHD concentration and the risk of preeclampsia (Bodnar et al., 2007). The researchers found a significant association between preeclampsia and serum 25OHD concentrations when the serum values were less than 37.5 nmol/L early in pregnancy.

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A 2007 systematic review of evidence incorporated 12 RCTs (15,528 women) to examine the relationship between calcium supplementation and preeclampsia prevention (Hofmeyr et al., 2007). Calcium supplementation reduced overall hypertension in 11 of the studies reviewed and incidence of preeclampsia in 12 of them. There was also a significant effect for calcium among women at high risk, which was greatest among those with lower baseline calcium intakes.

Additional Evidence from Randomized Controlled Trials: Preeclampsia and Pregnancy-Induced Hypertension

Additional RCTs not included in the review from Hofmeyr et al. (2007), although of lower-quality study design, also reported similar results suggesting that there may be no effect from daily calcium supplementation when dietary calcium intake is already adequate (Hofmeyr et al., 2006 [1,000 mg/day]; Villar et al., 2006 [2,000 mg/day]; Hiller et al., 2007 [1,800 mg/day]; Kumar et al., 2009 [500 mg/day]).

A prospective non-randomized clinical trial of the effect of vitamin D (0.5 mg/day) and calcium (312 mg/day) supplementation in women at risk for preeclampsia found that the incidence of preeclampsia was 10.9 percent lower in treated women than in controls (Ito et al., 1994). However, women who had the highest level of angiotensin II, a marker for preeclampsia, also had the lowest incidence of preeclampsia; thus, the role of calcium and vitamin D supplementation in preventing of preeclampsia is not clear from this trial (Ito et al., 1994). A small randomized trial of pregnant women in India supplemented with 600,000 IU of vitamin D in the seventh and eighth months of pregnancy compared with controls found no significant difference in incidence of preeclampsia, although a significant reduction in systolic blood pressure of 8 mmHg was seen in the vitamin D–supplemented group (Marya et al., 1987). The relationship of this small decrease in blood pressure to pregnancy-induced hypertension is unclear.

Observational Studies: Preeclampsia and Pregnancy-Induced Hypertension

Findings from observational studies have shown mixed results. In addition to the nested case–control study reviewed in AHRQ-Tufts, the committee identified one large prospective cohort study, a large retrospective cohort study, and two small case–control studies examining vitamin D intake or serum 25OHD level and risk for preeclampsia, as well as one small case–control study of serum calcitriol level and pregnancy-induced hypertension (Lalau et al., 1993). In the large prospective study, Haugen et al. (2009) examined associations between risk for preeclampsia and intake of vitamin D from diet and supplements. This study found that women

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who developed preeclampsia did not have a lower intake of vitamin D from foods compared with women without preeclampsia, but they did have a significantly lower intake of vitamin D from supplements, although the dose of the supplement was not correlated with prevalence of preeclampsia. Risk for preeclampsia was reduced by 31 percent in women who achieved a total vitamin D intake from food plus supplements between 300 and 400 IU/day, and the minimum combined intake of vitamin D needed for a protective effect was 200 IU/day. Hypponen et al. (2007) retrospectively examined the use of vitamin D supplements in infants of women with previously diagnosed preeclampsia in the Northern Finland Birth Cohort of 1966. The female children of mothers who had preeclampsia had a greater prevalence of preeclampsia in their own pregnancies, but vitamin D supplementation was significantly associated with a lower subsequent risk of developing preeclampsia. In contrast to these findings, two case–control studies, one in the United States (Seely et al., 1992) and one in Denmark (Frolich et al., 1992), found no significant difference in serum 25OHD levels between women with preeclampsia and those without, even though serum calcium levels were significantly lower in the women with pregnancy-induced hypertension or preeclampsia, respectively. In a small case–control study, women with pregnancy-induced hypertension had lower total and free serum calcitriol levels than did normotensive women during pregnancy (Lalau et al., 1993), but serum 25OHD levels and vitamin D intake were not measured.

Concluding Statement: Preeclampsia and Pregnancy-Induced Hypertension

Overall, two observational studies identified associations between supplementary vitamin D and incidence of preeclampsia, but data on associations between serum 25OHD level and preeclampsia were not conclusive. Similarly, only one observational study reported an association of pregnancy-induced hypertension with lower serum total and free calcitriol levels (Lalau et al., 1993), and no placebo-controlled RCTs were identified that examined a causal relationship between vitamin D and preeclampsia or pregnancy-induced hypertension.

Calcium supplementation has not been shown to have an effect on the incidence of preeclampsia in normal women meeting calcium requirements, but it may be of benefit in cases of low calcium intake. Associations between serum 25OHD level (as well as calcitriol level) and the onset of preeclampsia have not been well studied and a mechanism of action is unclear. Thus, because of the lack of a causal relationship and the inconsistent results in the observational studies for both vitamin D and calcium the committee concluded that neither preeclampsia nor pregnancy-induced hypertension can be considered as an indicator for DRI development.

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Other Non-Skeletal Reproductive Outcomes

Neither AHRQ-Ottawa nor AHRQ-Tufts addressed maternal non-skeletal outcomes beyond preeclampsia and pregnancy-induced hypertension. However, other non-skeletal outcomes may include maternal events such as cesarean section, obstructed labor, and vaginosis. Regarding fetal outcomes, so-called developmental programming of health outcomes in the offspring may focus on immune-related outcomes such as type 1 diabetes and atopic eczema, which has been included above in autoimmune response, as well as measures of BMD and skeletal development, discussed below in the Skeletal Health section. Infant birthweight is also of interest.

One observational study has reported an increased risk of approximately 65 percent and 26 percent of bacterial vaginosis in women with serum 25OHD levels below 20 nmol/L and below 50 nmol/L, respectively, compared with those with serum 25OHD levels of 75 nmol/L (Bodnar et al., 2009). Two observational studies reported conflicting results for the association of serum 25OHD levels with maternal delivery (cesarean/obstructed), with one finding an inverse association (Merewood et al., 2009) and the other finding no relationship with serum 25OHD levels. Overall, insufficient evidence makes maternal Cesarean delivery/obstructed labor uninformative for DRI development.

Regarding infant birthweight, AHRQ-Tufts discussed two RCTs (Mallet et al., 1986 and Maxwell et al., 1981; quality graded B and C, respectively) and reported no effect of supplemental vitamin D during pregnancy on offspring's birthweight or length, and also one RCT (Marya et al., 1988; quality graded C) that reported an increased birthweight in vitamin D–supplemented pregnant women with low dietary intakes of vitamin D. One additional RCT published after the AHRQ-Tufts report (Yu et al., 2009) also reported no effect of vitamin D on infant birthweight.

Brooke et al. (1980) reported on an RCT that involved 126 women treated with either a dose of vitamin D or a placebo during pregnancy. The intended dose for the treated group was 1,000 IU/day, but it appears that a higher dose was administered (10,000 IU/day) as the achieved cord level of 25OHD was 138 nmol/L for the treated group versus 10 nmol/L. In any case, no change in birthweight was evidenced. In a smaller study of 40 pregnant women, Delvin et al. (1986) showed no effect on birthweight comparing 1,000 IU/day versus a placebo.

Several observational studies have also examined this relationship, again with conflicting results. In a nested case–control study, a U-shaped relationship was found only in white women, with an increased probability (2.4 to 3.9) of small-for-gestational-age measures in those women in the lowest (21 to 58 nmol/L) and the highest (90.7 to 245.0 nmol/L) quartiles of serum 25OHD levels (Bodnar et al., 2010). In the same study, despite

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lower serum 25OHD levels in black women, no relationship was found between small-for-gestational-age measures and maternal serum 25OHD levels. In a prospective cohort observational study, mean birthweight was lowest in infants born to women with 25OHD levels below 30 nmol/L, intermediate in those born to women with 25OHD levels 30 to 50 nmol/L, and highest in those born to women with 25OHD levels above 50 nmol/L (Leffelaar et al., 2010). Birthweight was 60 g lower for the infants of women who consumed less than 200 IU of vitamin D per day during pregnancy compared to those who consumed 200 IU of vitamin D or more per day, and there was a significant linear trend for increased birthweight from lowest to highest quintile of intake (Scholl and Chen, 2009). Morley et al. (2006) enrolled 475 women in a study that compared maternal serum 25OHD levels during pregnancy to offspring birth size. No relationship was reported. Other observational studies reported no effect of vitamin D on birthweight (Brunvand et al., 1998; Gale et al., 2008; Farrant et al., 2009). The relationship, however, has not been tested in a sufficiently powered clinical trial. Corrections for differences in gestational length and other potentially confounding factors are not usually possible with associational studies. The available evidence for non-skeletal outcomes is limited and presently conflicting among both RCTs and observational studies, precluding the ability to find these data useful at this time for DRI development.

Skeletal Health

Skeletal health, referred to commonly as bone health, is manifested by desirable growth and maintenance of skeletal tissue, including bones and teeth. The use of bone health outcomes as reflective of calcium and vitamin D requirements is long-standing. Bone health served as an indicator for determining the calcium and vitamin D DRIs in 1997, when nutrient reference values for these nutrients were last reviewed (IOM, 1997). Since that time, additional studies have added to the scientific understanding of the relationships between calcium and vitamin D and bone health and are reflected to a large extent in AHRQ-Ottawa and AHRQ-Tufts, completed in 2007 and 2009, respectively.

Bone health is a concern throughout the life span. Initially, it comprises skeletal development during the times of gestational development and growth in infancy, childhood, and adolescence; this is followed by bone maintenance in adulthood. Menopause and aging result in bone loss. Various measures and health conditions are relevant to considerations of bone health; these include BMC/BMD, calcium balance, rickets/osteomalacia, and fracture risk. The latter is particularly germane to older adults. Further, while not health outcomes, measures of serum 25OHD concentrations and of circulating PTH levels have been incorporated into

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studies as intermediates related to bone health. Finally, although physical performance and the incidence of falls are defined by some as a component of bone health, these measures are reviewed separately in this report.

Compared with other potential indicators, this health outcome is characterized by a sizable number of RCTs as well as numerous observational studies from large cohorts. However, many of the studies evaluated calcium and vitamin D in combination, and there are relatively few studies that have evaluated the effects of calcium alone without vitamin D supplementation or vice versa. Given the nature of the available data and the need to integrate information to develop a set of measures reflective of bone health as a potential indicator for developing DRIs, this section is organized differently from those for other potential indicators. The understandings that link calcium and vitamin D to bone health and provide the basis for biological plausibility for an effect on bone health have been described in Chapters 2 and 3 and are therefore not repeated here. The many observational studies are briefly summarized.

Given the depth and breadth of the available AHRQ systematic analyses for the topic of bone health, the AHRQ analyses are considered in a single section. The two AHRQ analyses systematically reviewed, first, the published literature on the relationship between bone health and vitamin D (often in combination with calcium) (AHRQ-Ottawa) and, second, the relationship between bone health and vitamin D alone or vitamin D in combination with calcium (AHRQ-Tufts). Neither of the two AHRQ analyses considered calcium alone in relation to bone health. These two analyses have been described at the beginning of this chapter, and specific information about the studies included in AHRQ can be found in Appendixes C and D. Relevant information has been summarized and included in the tables presented below.

AHRQ included only minimal information about reproductive outcomes, and therefore the literature related to skeletal health during pregnancy and lactation is highlighted. AHRQ also included only minimal information about PTH level, a measure some investigators relate to bone health, so PTH level as a potential measure for bone health is examined separately.

The final component of this section integrates the available data on the basis of bone accretion, bone maintenance, and bone health. The preliminary step of specifying the utility of serum 25OHD level as a marker as well as the relationship between calcium absorption and serum 25OHD level provides the opening discussions for the integration section.

Summary of Observational Studies

The observational studies surrounding bone health are myriad. However, as is the case with the evidence hierarchy, the basis for the relation-

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ship between the two nutrients and bone health is more appropriately explored by examining evidence from controlled interventions, although data from observational studies can lend support and offer confirmatory input. Observational data regarding calcium intake and bone health are mixed regarding the finding that a range of increasing calcium intakes above deficiency levels are associated with improved bone mass and reduced fracture risk. These studies are confounded by an array of variables that have an impact on measures of bone density.

Regarding serum 25OHD concentrations and bone health, the AHRQ-Ottawa analysis concluded that observational studies suggested a correlation between higher serum 25OHD concentrations and increased BMC for older children and adolescents. For postmenopausal women and elderly men, observational studies reviewed in AHRQ-Ottawa provided *fair evidence* to support an association between serum 25OHD level and BMD or changes in BMD at the femoral neck. This analysis noted that the observational data overall were discordant with the results from available RCTs. Newer observational studies for the most part are consistent with older observational studies with respect to a relationship between low serum 25OHD levels and outcomes such as bone loss, fractures, or osteomalacia (Cauley et al., 2008; Looker and Mussolino, 2008; van Schoor et al., 2008; Ensrud et al., 2009; Bolland et al., 2010b; Cauley et al., 2010; Melhus et al., 2010). However, there are confounders related to such studies, including age, calcium intake, and social situation.

Bone mineral content/bone mineral density: Serum 25OHD AHRQ conducted its analyses for serum 25OHD concentrations on the basis of certain age and gender groups, as presented below.

Infants Overall, AHRQ-Ottawa, for which some studies included combinations of calcium and vitamin D, has reported that there is inconsistent evidence for an association between serum 25OHD concentrations and BMC measures in infants. Of the two RCTs examining BMC (Greer et al., 1982; Zeghoud et al., 1997), one demonstrated no significant benefit of higher serum 25OHD concentrations on radial bone mass, whereas the other showed a transient increase of BMC compared with the unsupplemented group at 12 weeks, but not at 26 weeks. Based on case–control studies (Okonofua et al., 1986; Bougle et al., 1998; Namgung et al., 1998; Park et al., 1998), greater whole-body BMC was related to higher serum 25OHD levels. Data are summarized in Table 4-5. AHRQ-Tufts found no additional RCTs for infants published in the period since the completion of the AHRQ-Ottawa review.

Children and adolescents For children and adolescents, there was fair evidence from AHRQ-Ottawa of an association between serum 25OHD levels and baseline BMD and change in BMD or BMD indexes. However, the results from the RCTs (Ala-Houhala et al., 1988; El-Hajj Fuleihan et al.,

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TABLE 4-5 Serum 25OHD Levels and Bone Health Outcomes for Infants: Summary from AHRQ-Ottawa Analyses[a]

Reference; Country; Jadad Score for RCTs[b]	Population Description	Intervention/ Duration	Bone Health Outcomes	Results
RCTs				
Greer et al., 1982 United States Jadad = 3	Healthy full-term infants; exclusively breast-fed n = 18 66% female 17 Caucasian 1 Asian-Indian	IG1: 400 IU vit D_2/d CG: placebo 12 wk (double-blind) At 6 mo, unblinded to mother, and placebo group began to receive 400 IU vit D_2/d Followed to 1 y	Distal L radius BMC (SPA) Measured at 3, 6, 12, 26, 40, and 52 wks	Serum 25OHD, mean (nmol/L) Baseline: no significant difference between groups 12 wk: IG1: 95* (graph) CG: 50 26 wk: IG1: 81.8 CG: 32.3 BMC, mean (SEM) (mg/cm) 12 wk: IG1 79 (3); CG 64 (3); $p < 0.003$ 26 wk: IG1 70 (6); CG 75 (5); NS 52 wk: IG1 108 (20); CG 120 (19) (CG receiving vit D for 6 mo)

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Greer and Marshall, 1989 United States Jadad = 4	Healthy full-term infants born to mothers willing to breastfeed for 6 mo n = 46 (+ 12 controls) 46% female All infants had Caucasian mothers (fathers included 1 black, 1 American Indian)	IG1: 400 IU vit D_2/d CG: placebo 6 mo, starting at birth	Distal L radius BMC (SPA) Measured at 1.5, 3.0, and 6.0 mo	Total serum 25OHD, mean (SD) (nmol/L) At birth: IG1: 59.7 (11.8) CG: 58.8 (19.1) 6 mo: IG1: 92.4 (29.7) CG: 58.8 (24.9), $p < 0.01$ BMC, mean (SD) (mg/cm) No significant difference between groups at 1.5 and 3.0 mo. At 6.0 mo, CG was significantly greater than IG1: IG1 89.5 (12.5) vs. CG 101.0 (17.9), $p < 0.05$ However, change in mean BMC from 1.5 to 6.0 mo was not different between groups

continued

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TABLE 4-5 Continued

Reference; Country; Jadad Score for RCTs[b]	Population Description	Intervention/ Duration	Bone Health Outcomes	Results
Zeghoud et al., 1997 France Jadad = 1	Healthy neonates and their mothers $n = 80$ European	IG1: 500 IU vit D_2/d IG2: 1,000 IU vit D_2/d Starting at 3–6 d after birth All infants fed formula with mean (SD) 426 (46) IU vit D_3/L	iPTH (RIA) Measured at 3–6 d, 1 mo, 3 mo	Serum 25OHD, mean (SD) Baseline total sample: 29.5 (13.8) nmol/L; range 10–80 nmol/L 51/80 (63.7%) ≤ 30 nmol/L iPTH was significantly higher in neonates with 25OHD < 16 nmol/L than in those born with 25OHD > 30 nmol/L: mean (SD) 70 (30) pmol/L Mean baseline 25OHD by group** Group 1 ($n = 14$): 25OHD ≤ 30 nmol/L and iPTH > 60 ng/L: 17.9 (7.8) Group 2 ($n = 36$): 25OHD ≤ 30 nmol/L and iPTH < 60 ng/L: 22.7 (6.5) Group 3 ($n = 29$): 25OHD > 30 nmol/L and iPTH < 60 ng/mL: 43.7 (10.6) At 1 mo, all 3 groups (pooled vit D doses): mean serum 25OHD was significantly increased, and there was no significant difference between groups Group 1: 53.1 (12.0) nmol/L Group 2: 59.8 (17.7) nmol/L Group 3: 59.2 (11.4) nmol/L At 3 mo, mean 25OHD for total sample (pooled doses) was 69.0 nmol/L; highest value 92.5 nmol/L IG1 (500 IU D_2/d): For group 1, at 1 mo (45.5 nmol/L) and 3 mo (56.1 nmol/L), serum 25OHD values were significantly lower than in the other 2 groups receiving same dose and lower than in all groups receiving 1,000 IU/d IG2 (1,000 IU vit D_2/d): Change in serum 25OHD (3 mo) was not significantly different between the 3 groups

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Case–control studies

Okonofua et al., 1986 UK	Healthy full-term infants $n = 21$ 47.6% Caucasian 52.4% Asian	NA	Fractures during birth	Serum 25OHD, mean (SD) (nmol/L): Lower in Asian vs. white full-term infants ($p < 0.01$) White: 15 (5) (range 9–39) Asian: 6 (4) (range < 5–20) Maternal 25OHD in white mothers was 30 (11) nmol/L and in Asian mothers was 15 (10) nmol/L; serum PTH was higher in Asian mothers 25OHD levels in mothers were significantly higher than neonatal levels; the two were correlated ($r = 0.60$) Fractures during birth: 0
Bougle et al., 1998 France	Healthy full-term infants $n = 82$ (also 44 preterm) Asian	NA	LS BMD and BMC (DXA)	Full-term infants: Serum 25OHD, mean (SD) nmol/L (range) 75 (52.5) (10.0–292.5) 25OHD negatively related to BMD ($r = -1.7$, $p = 0.02$) and to BMC ($r = -0.04$, $p = 0.02$) in a simple regression analysis, but not related to BMC or BMD in a multiple regression analysis

continued

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TABLE 4-5 Continued

Reference; Country; Jadad Score for RCTs[b]	Population Description	Intervention/ Duration	Bone Health Outcomes	Results
Namgung et al., 1998 Korea	Healthy full-term infants $n = 71$ (37 born in summer and 34 in winter) Summer 59% female Winter 38% female Korean	NA	Whole-body BMC measured before 3 d of age (DXA)	Serum 25OHD, mean (SD) (nmol/L): Winter-born infants had lower 25OHD than summer-born infants ($p < 0.001$) Winter born: 26.8 (19.0) Summer born: 75.0 (24.0) % of infants with levels < 27.5 nmol/L Winter born: 97% Summer born: 47% Winter-born infants had 8% lower whole-body BMC than summer-born infants ($p = 0.0002$) BMC LSM (SD) (g/cm): Winter born: 86.7 (7.7) Summer born: 93.9 (7.8) Whole-body BMC correlated positively with serum 25OHD ($r = 0.243$, $p = 0.047$) Maternal 25OHD was lower in winter than in summer: 24 (13) vs. 43 (18), $p < 0.001$

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Park et al., 1998 Korea	Healthy full-term infants born in winter (some exclusively breast-fed [n = 18] or formula-fed with 400 IU vit D [n = 17]) n = 35 Breast-fed 28% female Formula-fed 47% female Korean	NA	LS BMC and BMD (DXA)	Serum 25OHD, mean (SD) (nmol/L): Mean was lower in breast-fed vs. formula-fed infants, p = 0.001 Breast-fed: 39.9 (28.2) Formula-fed: 72.5 (22.2) % with 25OHD < 28 nmol/L Breast-fed: 8/18 (44%) Formula-fed: 1/17 (6%), p = 0.01 LS BMD no difference between breast-fed (n = 14/18) and formula-fed infants (n = 14/17) (data NR) LS BMC, mean (SD) (g/cm) No difference between groups Breast-fed: 0.62 (0.2) Formula-fed: 0.65 (0.2) 25OHD did not correlate with BMC (r = 0.173, p = 0.39, n = 28)

NOTE: *SEM provided in graph but not estimable; **1/80 infants did not clearly fit into any category and had findings suggestive of transient congenital hypoparathyroidism; BMC = bone mineral content; BMD = bone mineral density; CG = control group; d = day; DXA = dual-energy X-ray absorptiometry; IG = intervention group; iPTH = intact parathyroid hormone; IU = International Units; L = Left; LS = lumbar spine; LSM = least squares mean; mo = month(s); NA = not applicable; NR = not reported; NS = not significant; RCT = randomized controlled trial; RIA = radioimmunoassay; SD = standard deviation; SEM = standard error of the mean; SPA = single-photon absorptiometry; UK = United Kingdom; vit = vitamin; wk = week(s); y = year(s).

[a]This table has been truncated for the purposes of this chapter, but it can be found in its entirety in Appendix C.

[b]Jadad score is based on a scale of 1 to 5. See Box 4-1 for details on the scoring system.

SOURCE: Modified from Cranney et al. (2007).

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2006) did not confirm a consistent benefit on BMD or BMC across skeletal sites and age groups. Some studies included combinations of calcium and vitamin D.

There were seven studies in older children and adolescents (two RCTs, three cohort studies, one case–control study, and one before-and-after study) that evaluated the relationship between serum 25OHD concentrations and BMC or BMD (see Table 4-6). In older children, there was one RCT, one prospective cohort study, and one before-and-after study. The RCT (Ala-Houhala et al., 1988) did not find an association between serum 25OHD concentrations and distal radial BMC. Two of three non-RCT studies found a positive association between baseline serum 25OHD concentrations and BMC or BMD. The effect of bone size and muscle mass on these outcomes in relation to baseline serum 25OHD concentrations was not reported.

One RCT with children and adolescent girls (El-Hajj Fuleihan et al., 2006) demonstrated a significant relationship between baseline serum 25OHD concentrations and baseline BMD of the lumbar spine, femoral neck, and radius. However, only high dose supplementation with 14,000 IU of vitamin D_3 per week increased BMC of the total hip.

AHRQ-Tufts identified two RCTs available after the AHRQ-Ottawa analysis, both rated C, that compared the effect of vitamin D supplementation alone on BMC in healthy girls between 10 and 17 years of age (El-Hajj Fuleihan et al., 2006; Andersen et al., 2008). Both RCTs were rated C because the results were not adjusted for important potential confounders, such as height, bone area, lean mass, sun exposure, and pubertal status. One RCT (Andersen et al., 2008) analyzed 26 healthy girls, who were Pakistani immigrants primarily living in the Copenhagen area of Denmark (latitude 55°N). Girls were randomly assigned to receive either a daily dose of 400 or 800 IU of vitamin D_3 or placebo for 1 year. The mean baseline dietary calcium intake was 510 mg/day, and the serum 25OHD concentration was 11 nmol/L. At the end of the study, there were no significant differences in whole-body BMC changes between groups receiving the two doses of vitamin D_3 (400 or 800 IU/day) and the placebo group. A second RCT (El-Hajj Fuleihan et al., 2006) analyzed 168 healthy girls living in the Greater Beirut area, Lebanon (latitude 33°N). Girls were randomly assigned to receive either weekly oral vitamin D doses of 1,400 IU (equivalent to 200 IU/day) or 14,000 IU (equivalent to 2,000 IU/day) or placebo for 1 year. The mean baseline dietary calcium intake was 677 mg/day, and the 25OHD concentration was 35 nmol/L. At the end of the study, there were no significant differences in whole-body BMC changes between either the low-dose vitamin D group (200 IU/day) or the high-dose vitamin D group (2,000 IU/day) and the placebo group. The same findings were seen when analyses were restricted to either premenarcheal or postmenarcheal girls.

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TABLE 4-6 Serum 25OHD Levels and Bone Health Outcomes for Older Children and Adolescents: Summary from AHRQ-Ottawa Analyses[a]

Reference; Country; Jadad Score for RCTs[b]	Population Description	Intervention/ Duration	Bone Health Outcomes	Results
RCTs				
Ala-Houhala et al., 1988 Finland Jadad = 1	Children, 8–10 y old $n = 60$ IG1: 62% female CG: 48% female Caucasian	IG1: 400 IU vit D_2 5–7×/wk CG: placebo 13 mo	Distal radius BMC (SPA)	Serum 25OHD, mean (SD) (nmol/L) Baseline (winter): IG1: 49.3 (19.0) vs. CG: 46.0 (15.5) Mid-study (autumn): IG1: 78.0 (24.3) vs. CG 59.0 (17.8) End-of-study (winter): IG1: 71.3 (23.4) vs. CG 43.3 (19.5), $p < 0.01$ No difference between groups in distal radius BMC at 13 mo

continued

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TABLE 4-6 Continued

Reference; Country; Jadad Score for RCTs[b]	Population Description	Intervention/ Duration	Bone Health Outcomes	Results
El-Hajj Fuleihan et al., 2006 Lebanon Jadad = 4	Children and adolescent girls (premenarcheal and postmenarcheal), 10–17 y n = 179 Middle Eastern	IG1: 1,400 IU vit D/wk IG2: 14,000 IU vit D/wk CG: placebo 1 y	BMD and BMC LS, forearm, total body (DXA)	25OHD, mean (SD) (nmol/L) baseline: IG1: 35.0 (22.5) IG2: 35.0 (20.0) CG: 35.0 (17.5) 1 y: IG1: 42.5 (15.0) IG2: 95.0 (77.5) CG: 40.0 (20.0) Covariates: percentage change in bone area, percentage change in lean mass Significant association between baseline serum 25OHD and: LS BMD (r = 0.16, p = 0.033) Femoral neck (r = 0.17, p = 0.028) Radius BMD levels (r = 0.24, p = 0.002) Radius BMC levels (r = 0.16, p = 0.033) Largest increases in bone mass in IG2 (high dose) subjects with lowest 25OHD levels at baseline

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Prospective cohort studies

Guillemant et al., 1999 France	Healthy adolescent boys from a jockey training center; age range 13 y 5 mo to 16 y 1 mo n = 175 Caucasian	NA	iPTH (immunoradiometric assay, Nichols)	25OHD, mean (SD) (nmol/L) Post-summer 58.5 (10.0) Post-winter 20.6 (6.0), p = 0.0001 At serum 25OHD > 83 nmol/L, iPTH plateau occurred at 2.48 pmol/L
Javaid et al., 2006 UK	Children with known maternal 25OHD status in third trimester; 9 y old n = 198 Caucasian	NA	Total body and LS BMC and areal BMD, calculated volumetric BMD (DXA Lunar DPX-L)	Maternal serum 25OHD in late pregnancy: 18% had serum 25OHD levels < 27.5 nmol/L and 31% had levels 27.5–50.0 nmol/L Mothers with lower 25OHD during pregnancy had children with reduced total body (r = 0.21, p = 0.0088) and lumbar spine BMC (r = 0.17, p = 0.03). Adjustment for height did not weaken the relationship between total body BMC and 25OHD; volumetric LS BMD was not associated with maternal 25OHD. Adjusted for age of child

continued

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TABLE 4-6 Continued

Reference; Country; Jadad Score for RCTs[b]	Population Description	Intervention/ Duration	Bone Health Outcomes	Results
Lehtonen-Veromaa et al., 2002 Finland	Healthy adolescent girls; 12.9 (1.7) y, range 9–15 y $n = 191$ Caucasian	NA	LS BMD and BMAD FN BMD and BMAD (DXA)	25OHD, mean (SD) (nmol/L) Baseline: 34.0 (13.2) (winter) 1 y: 33.2 (11.1) 3 y: 40.6 (15.8) Baseline 25OHD correlated with Δ LS BMD ($r = 0.35$, $p < 0.001$) and Δ FN BMD ($r = 0.32$, $p < 0.001$) Baseline 25OHD correlated with Δ LS BMAD (0.35, $p < 0.001$) and Δ FN BMAD (0.24, $p < 0.002$) Adjusted for: baseline reproductive year, bone mineral values, increases in height and weight, mean intake of calcium, and mean amount of physical activity Significant correlation between baseline 25OHD and Δ 3-y adjusted LS or FN BMD and BMAD Difference in mean 3-y Δ LS BMD between group with baseline 25OHD < 20 nmol/L and group with baseline 25OHD ≥ 37.5 was 4%

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Case–control studies

Marwaha et al., 2005 India	Healthy school children (from LSES and USES); age range 10–18 y n = 5,137 (3,089 LSES; 2,048 USES) LSES 65.1% female USES 52.7% female Indian	NA	BMD (distal forearm and calcaneum) using DXA	Serum 25OHD, mean (SD) (nmol/L): 29.5 (18.0) LSES: 26 (1); USES: 34 (1) 25OHD < 22.5 nmol/L: 35.7%; LSES 42.3% vs. USES 27%, $p <$ 0.01 Prevalence of clinical vit D deficiency (defined by genu varum or genu valgum): LSES 11.6% vs. USES 9.7%, p = 0.07 Forearm mean BMD significantly higher ($p < 0.01$) in USES group compared with LSES BMD adjusted for height and weight Serum calcium not significantly different between groups, but dietary calcium intake lower in LSES group No significant correlation between BMD and serum 25OHD in either group

continued

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TABLE 4-6 Continued

Reference; Country; Jadad Score for RCTs[b]	Population Description	Intervention/ Duration	Bone Health Outcomes	Results
Before-and-after studies				
Rajakumar et al., 2005 United States	Healthy 6–10 y olds Tanner stage I/II (81% I) Skin type III/IV (81% IV); mean age 8.9 (1.2) y (range 6–10 y) Vit D dietary intake: mean (SD) 277 (146) IU/d 16/41 (39%) dietary intake < 200 IU/d n = 42 34% female African American	400 IU vit D/d (isoform not specified) 1 mo	iPTH (Immulite iPTH chemiluminescent assay)	Serum 25OHD, mean (SD) (nmol/L) Baseline: 60.0 (26.3) 49% < 50 71% < 75 Group 1 = 25OHD < 50 nmol/L at baseline: 38.5 (8.0) Group 2 = 25OHD > 50 nmol/L at baseline: 80.3 (20.5) 1 mo (total group): 68.8 (18.8) Group 1: 57.5 (16.0) Group 2: 79.5 (14.5) Increase in serum 25OHD was observed only in group 1 7/39 (18%) of group 1 continued to have a level < 50 nmol/L after 1 mo of supplementation Negative correlation of 25OHD with body weight (r = −0.378, p = 0.015) at baseline No significant differences at baseline or 1 mo in markers of bone turnover, $1,25(OH)_2D$ or PTH between groups with 25OHD < 50 nmol/L or > 50 nmol/L at baseline

NOTE: BMAD = bone mineral apparent density; BMC = bone mineral content; BMD = bone mineral density; CG = control group; DXA = dual-energy X-ray absorptiometry; FN = femoral neck; IG = intervention group; iPTH = intact parathyroid hormone; IU = International Units; LS = lumbar spine; LSES = lower socioeconomic status; mo = month(s); NA = not applicable; PTH = parathyroid hormone; RCT = randomized controlled trial; SD = standard deviation; SPA = single-photon absorptiometry; UK = United Kingdom; USES = upper socioeconomic status; vit = vitamin; wk = week(s); y = year(s).

[a]This table has been truncated for the purposes of this chapter, but it can be found in its entirety in Appendix C.

[b]Jadad score is based on a scale of 1 to 5. See Box 4-1 for details on the scoring system.

SOURCE: Modified from Cranney et al. (2007).

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Postmenopausal women and elderly men Overall, regarding serum 25OHD and bone density measures, AHRQ-Ottawa, which included some studies that combined calcium and vitamin D, reported discordance between the results from RCTs and the majority of observational studies; the authors attributed this as likely due to the impact of confounders relative to observational data as a general matter. Nineteen studies (see Table 4-7) evaluated the association between serum 25OHD levels and BMD. Of these, six studies were RCTs. One RCT (Ooms et al., 1995b) reported an association between serum 25OHD concentrations and BMD or bone loss, whereas the other five RCTs (Dawson-Hughes et al., 1995; Storm et al., 1998; Schaafsma et al., 2002; Cooper et al., 2003; Aloia et al., 2005) and three cohort studies did not. Four cohort studies found a significant association between 25OHD concentrations and bone loss, which was most evident at the hip sites, but the evidence for an association between 25OHD concentrations and lumbar spine BMD was weak. Six case–control studies suggested an association between 25OHD concentrations and BMD, and the association was most consistent at the femoral neck. A forest plot showing the effect of vitamin D plus calcium supplementation (versus placebo) for femoral neck BMD at 1 year is shown in Figure 4-4. Overall, significant increases at the femoral neck were observed with a combined estimate as reported in Table 4-7 of 1.37 percent (95% confidence interval [CI]: 0.24–2.50) from three trials after 1 year.

Based on the results from the observational studies, there is fair evidence to support an association between serum 25OHD levels and BMD or changes in BMD at the femoral neck. Specific circulating concentrations of 25OHD below which bone loss at the hip was increased ranged from 30 to 80 nmol/L.

AHRQ-Tufts identified two more recent RCTs, one that combined calcium with vitamin D and one that did not. The first, an A-quality RCT (Zhu et al., 2008a), compared the effect of vitamin D_2 supplementation on hip BMC in 256 elderly women between 70 and 90 years of age. All elderly women in this trial had normal physical functioning. They were randomly assigned to receive either vitamin D_2 (1,000 IU/day) plus calcium (1,200 mg/day) supplement or calcium (1,200 mg/day) supplement alone for 1 year. The mean baseline dietary calcium intake was 1,097 mg/day, and the mean serum 25OHD concentration was 44.3 nmol/L. Total hip BMD increased significantly in both groups, with no difference between the vitamin D_2 plus calcium and calcium alone groups (hip BMD change: vitamin D, +0.5 percent; control, +0.2 percent).

The second, a B-quality RCT (Andersen et al., 2008), analyzed 89 healthy adult women and 83 healthy adult men separately. The participants were Pakistani immigrants living in the Copenhagen area of Denmark (latitude 55°N). Women and men were randomly assigned to receive either a daily dose of 400 or 800 IU vitamin D_3 or placebo for 1 year. For women,

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TABLE 4-7 Serum 25OHD Levels and BMC/BMD in Postmenopausal Women and Older Men: Summary from AHRQ-Ottawa Analyses[a]

Reference; Country; Jadad Score for RCTs[b]	Population Description	Intervention/ Duration	Bone Health Outcomes	Results
RCTs				
Aloia et al., 2005 United States Jadad = 5	PM women; IG1: 59.9 (6.2) y CG: 61.2 (6.3) y *n* = 208 100% African American	IG: 800 IU vit D_3 for 2 y, then 2,000 IU for 1 y + 1,200–1,500 mg CaCG: 1,200–1,500 mg Ca 3 y	BMD: LS, total hip, total body, mid radius (DXA)	No association between serum 25OHD and Δ BMD Analyses examining those with low baseline 25OHD or high PTH showed no influence of 25OHD on Δ BMD
Cooper et al., 2003 Australia Jadad = 4	PM women not on HRT; IG1: 56.5 (4.2) y, CG: 56.1 (4.7) y *n* = 187 Caucasian	IG1: 10,000 IU vit D_2/wk + 1,000 mg Ca/d CG: 1,000 mg Ca/d 2 y	BMD: LS, FN, Ward's triangle, Tr, proximal forearm (DXA)	No significant correlation between baseline 25OHD concentration and Δ BMD at any site or between Δ 25OHD and Δ BMD at any site
Dawson-Hughes et al., 1995 United States Jadad = 3	Healthy, ambulatory PM women; IG1: 63.0 y CG: 64.0 y *n* = 247 Caucasian	IG1: 700 IU vit D_3 + 500 mg Ca citrate malate CG: 100 IU vit D_3 + 500 mg Ca daily 2 y	BMD: LS, FN, and total body (DXA)	25OHD concentrations during either season did not correlate with Δ BMD at any site

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Ooms et al., 1995b Netherlands Jadad = 4	Elderly women; IG1: 80.1 (5.6) y CG: 80.6 (5.5) y $n = 348$	IG1: 400 IU vit D_3/d CG: placebo 2 y	BMD: FN, Tr, and distal radius (DXA)	Effect of vitamin D supplementation was independent of baseline 25OHD as well as 25OHD corrected for season
Schaafsma et al., 2002 Netherlands Jadad = 4	Healthy, PM women 50–70 y $n = 85$ Caucasian	IG1: eggshell powder + 200 IU vit D_3 IG2: Ca carbonate + 200 IU vit D_3 CG: placebo 12 mo	BMD: LS, hip (DXA)	No significant correlation between 25OHD and BMD
Storm et al., 1998 Netherlands Jadad = 4	PM women without OP $n = 60$ Caucasian	IG1: 4 glasses of fortified milk (325 IU vit D/quart) daily IG2: Ca carbonate daily CG: placebo 2 y	BMD: Tr, FN, LS (DXA)	Serum 25OHD was not a significant determinant of FN BMD at baseline, during winter ($p = 0.23$), or over the entire study period
Prospective cohort studies				
Bischoff-Ferrari et al., 2005 United States	Individuals with knee OA; 74.4 (11.1) y $n = 228$ 64% female	1–2 y	BMD: FN (DXA Lunar DPX-L)	Significant positive association between 25OHD and BMD independent of age, gender, BMI, knee pain, physical activity, and disease severity Significant trend between being in a higher serum 25OHD group and having higher BMD ($p < 0.04$)

continued

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TABLE 4-7 Continued

Reference; Country; Jadad Score for RCTs[b]	Population Description	Intervention/ Duration	Bone Health Outcomes	Results
del Puente et al., 2002 Italy	Active, noninstitutionalized females (menopausal and premenopausal); 58 (9) y n = 139 Caucasian	2 y	BMD: LS and FN (DXA)	25OHD independent predictor of BMD change at FN and LS (FN Δ BMD [β = 0.26 (0.13), p = 0.04] and LS Δ BMD [β = 0.07 (0.03), p = 0.04]) In stepwise analysis discrimination models, only FN significant (partial R^2 = 0.26, p = 0.04)
Dennison et al., 1999 UK	Healthy adults age 60–75 y n = 316 45% female	4 y	BMD: LS and proximal femur (DXA)	No association between baseline 25OHD and BMD at LS and proximal hip (β = 0.002 spine, 0.001 hip) and no association between 25OHD and bone loss after adjustment for adiposity
Gerdhem et al., 2005 Sweden	Ambulatory independently living women; 75 (75–75.9) y n = 1,044	3 y	BMD: FN and LS (DXA)	No association between baseline 25OHD and BMD
Melin et al., 2001 Sweden	Healthy, independent elderly individuals; 83.7 y n = 64 81% female Caucasian	1 y	BMD: FN (DXA)	FN BMD associated with serum 25OHD after summer (r = 0.38, p = 0.003) and winter (r = 0.37, p = 0.003) After adjusting for BMI, 25OHD remained a significant determinant after winter (adjusted R^2 = 0.14, p = 0.005)

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Rosen et al., 1994 United States	Healthy independently living elderly women; 77 (2) y $n = 18$	2 y	BMD: LS and FN (DXA)	Δ 25OHD between summer and winter was associated with LS BMD in 2nd y ($r = 0.59$, $p = 0.04$), but not FN BMD
Stone et al., 1998 United States	Healthy elderly females > 65 y, random sample, subcohort of individuals not on HRT from Study of Osteoporotic Fractures $n = 261$ Caucasian	42–71 mo	BMD: total hip (DXA), calcaneal (SPA)	Significant association between lower 25OHD levels and total hip BMD loss Lower 25OHD levels associated with increased loss at total hip after adjusting for estradiol, testosterone, SHBG, season, and use of supplements 25OHD not associated with calcaneal BMD after adjusting for age and weight
Case–control studies				
Al-oanzi et al., 2006 UK	Men with idiopathic OP Cases: 59.6 (13.6) y Controls: 62.4 (10.4) y $n = 56$ (+ 114 controls) Caucasian	NA	BMD diagnosis of OP based on T-score FN and LS	No significant difference between plasma 25OHD in cases and controls, but mean free plasma 25OHD was about 33% lower in men with OP vs. controls ($p < 0.0001$)

continued

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TABLE 4-7 Continued

Reference; Country; Jadad Score for RCTs[b]	Population Description	Intervention/ Duration	Bone Health Outcomes	Results
Boonen et al., 1999 Belgium	PM women (hip fracture patients and controls) n = 100 Cases: 74.2 (7.8) y Controls: 75.8 (5.6) y	NA	BMD: FN and Tr (DXA) Fractures	Mean $25OHD_3$ was lower in cases vs. controls ($p < 0.001$) Vit D deficiency (< 30 nmol/L): 64% of cases vs. 8% controls within the same 4-mo sampling period (no relation between 25OHD and month of sample collection) FN and Tr BMD were significantly lower in cases than in controls. No significant relation found between the $25OHD_3$–PTH axis and BMD in cases and controls. In multiple regression of pooled data, models using $25OHD_3$ and PTH were highly predictive of FN BMD (R^2 = 32%, $p < 0.001$).
Landin-Wilhelmsen et al., 1999 Sweden	PM patients with OP and age-matched controls from outpatient clinic n = 128 (+ 227 age-matched controls) Cases: 59 (6) y Controls: 59 (5) y	NA	BMD and BMC: LS, total body, and FN (DXA) Fractures	25OHD significantly lower in OP patients vs. controls ($p < 0.05$) OP patients had lower body weight and BMI vs. controls ($p < 0.001$)

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Villareal et al., 1991 United States (Midwest)	Ambulatory, independently living PM women, women with low (< 38 nmol/L) 25OHD and controls $n = 98$ Cases: 64 y Controls: 63 y Caucasian	NA	BMD: (LS, T12-L3), QCT	Women with low 25OHD levels had a reduced LS BMD. In the low 25OHD group, LS BMD correlated with 25OHD ($r = 0.41$, $p < 0.01$). In multivariate analysis, iPTH was the major determinant of a decrease in LS BMD
Thiebaud et al., 1997 Switzerland	Hip fracture patients, hospital controls, and community controls $n = 179$ (+ 180 controls) Cases: 81.0 y (women) and 77.7 y (men); hospital controls: 80.9 y (women) and 76.9 y (men); community controls: 71.7 y (women) and 71.3 y (men)	NA	BMD: FN, total hip, and Tr (DXA) Fractures	Women and men with hip fractures significantly lower 25OHD levels vs. controls Fracture patients had lower hip BMD vs. controls ($p < 0.001$) Significant biochemical markers in the multivariate logistic regression model of the risk for hip fracture were serum albumin and PTH In women FN, Tr BMD weakly correlated with 25OHD, and the only significant association was at the Tr ($r = 0.13$, $p < 0.05$)

continued

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TABLE 4-7 Continued

Reference; Country; Jadad Score for RCTs[b]	Population Description	Intervention/ Duration	Bone Health Outcomes	Results
Yan et al., 2003 China 42°N and UK 52°N	Older individuals (60–83 y) n = 352 Chinese: 50% female British: 50% female 64% Chinese (Asian), 36% British (Caucasian)	NA	BMC: FN (DXA)	Significantly higher 25OHD levels in British subjects Weak association (r = 0.054, p = 0.05) between 25OHD and FN BMC in British subjects after adjusting for size, but not in Chinese subjects

NOTE: BMC = bone mineral content; BMD = bone mineral density; BMI = body mass index; CG = control group; DXA = dual-energy X-ray absorptiometry; FN = femoral neck; HRT = hormone replacement therapy; IG = intervention group; iPTH = intact parathyroid hormone; IU = International Units; LS = lumbar spine; mo = month(s); NA = not applicable; OA = osteoarthritis; OP = osteoporosis; PM = postmenopausal; PTH = parathyroid hormone; QCT = quantitative computed tomorgraphy ; RCT = randomized controlled trial; SHBG = sex hormone binding globulin; SPA = single-photon absorptiometry; Tr = trochanter; UK = United Kingdom; vit = vitamin; wk = week(s); y = year(s).

[a]This table has been truncated for the purposes of this chapter, but it can be found in its entirety in Appendix C.

[b]Jadad score is based on a scale of 1 to 5. See Box 4-1 for details on the scoring system.

SOURCE: Modified from Cranney et al. (2007).

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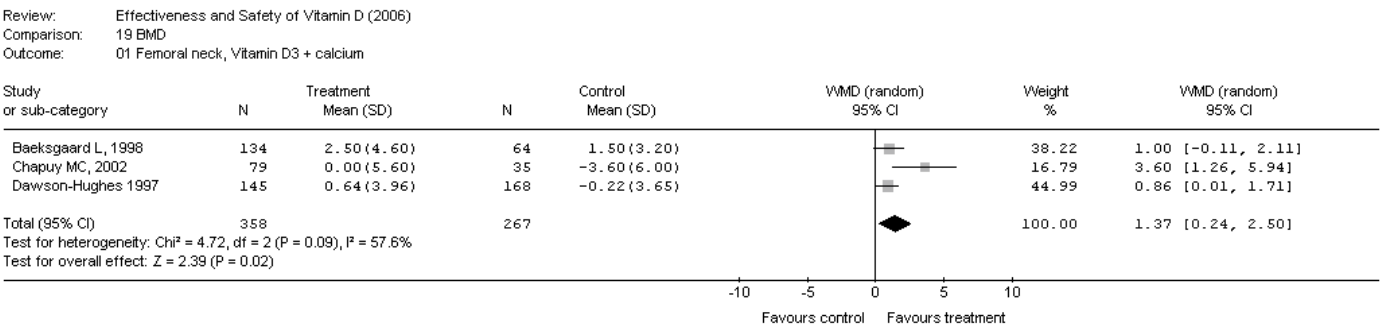


FIGURE 4-4 Forest plot: Effect of vitamin D_3 + calcium vs. placebo on femoral neck BMD at 1 year.
SOURCE: Cranney et al. (2007).

the mean baseline dietary calcium intake was 495 mg/day, and the mean serum 25OHD concentration was 12 nmol/L. For men, the mean baseline dietary calcium intake was 548 mg/day, and the mean serum 25OHD concentration was 21 nmol/L. At the end of the study, in both women and men, there were no significant differences in lumbar spine BMD changes between the groups receiving the two doses of vitamin D_3 (400 or 800 IU/day) and the placebo group.

Pregnant or lactating women Overall, from AHRQ-Ottawa, there was insufficient evidence on the association between 25OHD concentration and change in bone density during pregnancy. Four studies (no RCTs, three cohort studies, one before-and-after study) assessed vitamin D nutriture at various time points in pregnancy, with vitamin D deficiency being observed in 0 to 50 percent of subjects, but only one cohort study (n = 115) rated

BOX 4-3
AHRQ Findings by Life Stage for Serum 25OHD Measures and BMC/BMD*

0–6 months: Inconsistent evidence for an association between a specific serum 25OHD concentration and the bone health outcome BMC in infants.

7 months–2 years: Fair evidence of an association between 25OHD concentrations and baseline BMD and change in BMD or BMC indexes from the studies in older children and adolescents.

3–8 years: Fair evidence of an association between 25OHD concentrations and baseline BMD and change in BMD or BMC indexes from the studies in older children and adolescents.

9–18 years: Fair evidence of an association between 25OHD concentrations and baseline BMD and change in BMD or BMC indexes from the studies in older children and adolescents. Two new RCTs identified by AHRQ-Tufts enrolled only girls in this life stage. The results showed no significant differences in whole-body BMC changes between groups receiving either lower doses of vitamin D (200 or 400 IU/day) or higher doses of vitamin D (800 or 2,000 IU/day) and the placebo group.

19–50 years: Discordance between the results from RCTs and the majority of observational studies in postmenopausal women and elderly men. Based on results of the observational studies, there is fair evidence to support an association between serum 25OHD concentration and BMD or changes in BMD at the femoral neck. One new RCT identified by AHRQ-Tufts enrolled primarily men and women in this life stage. The

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as good quality included maternal BMD as an outcome, and there was no relationship between vitamin D status and postpartum changes in BMD. Information on the four studies can be found in Appendix C. AHRQ-Tufts found no new RCTs.

Summary Evidence regarding serum 25OHD concentrations and BMC/BMD measures varied by life stage. The findings from the AHRQ analyses are summarized by DRI-relevant life stage group in Box 4-3 below.

Bone mineral content/bone mineral density: Vitamin D supplementation with or without calcium AHRQ addressed data primarily for menopausal women. One RCT for girls was also identified. Overall, AHRQ-Ottawa concluded that there is good evidence that vitamin D_3 plus calcium supplementation resulted in small increases in BMD of the spine, total body,

results showed that there were no significant differences in lumbar spine BMD changes between the groups receiving two doses of vitamin D_3 (400 or 800 IU/day) and the placebo group.

51–70 years: Discordance between the results from RCTs and the majority of observational studies in postmenopausal women and elderly men. Based on results of the observational studies, there is fair evidence to support an association between serum 25OHD concentration and BMD or changes in BMD at the femoral neck. One new RCT identified by AHRQ-Tufts enrolled some men in this life stage. The results showed that there were no significant differences in lumbar spine BMD changes between the groups receiving two doses of vitamin D_3 (400 or 800 IU/day) and the placebo group.

≥71 years: Discordance between the results from RCTs and the majority of observational studies in postmenopausal women and elderly men. Based on results of the observational studies, there is fair evidence to support an association between serum 25OHD and BMD or changes in BMD at the femoral neck. One new RCT identified by AHRQ-Tufts enrolled only elderly women in this life stage. The results showed that vitamin D_2 supplementation (1,000 IU/day) had no additional effect on hip BMD compared with calcium supplementation alone.

Pregnant or lactating women: Insufficient evidence for an association between a specific serum 25OHD concentration and the bone health outcome BMC.

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*Evidence from AHRQ-Ottawa; information from AHRQ-Tufts as noted.
SOURCE: Modified from Chung et al. (2009).

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femoral neck and total hip. Based on included trials, it was less certain whether vitamin D_3 supplementation alone has a significant effect on BMD.

Seventeen RCTs evaluated the effect of supplemental vitamin D_2 or vitamin D_3 on BMD, predominantly in populations of late menopausal women (see Table 4-8). Only one small RCT included premenopausal women, and two trials included older men (> 60 years). Most trials were 2 to 3 years in duration and used vitamin D doses of up to 800 IU daily. Most trials used vitamin D_3 and also included 500 mg of calcium as a co-intervention.

Meta-analysis results of 17 RCTs comparing vitamin D_3 plus calcium with placebo (AHRQ-Tufts) were consistent with a small effect on lumbar spine, femoral neck, and total body BMD. The WHI trial found a significant benefit of supplementation with 400 IU of vitamin D_3 plus 1,000 mg of calcium on total hip BMD. However, when the effect of supplementation with vitamin D_3 plus calcium versus supplementation with calcium alone was assessed by AHRQ-Tufts, no significant increase in BMD was observed with either intervention, suggesting that vitamin D_3 may be of less benefit in calcium-replete postmenopausal women. It is noted, however, that the dose administered was 400 IU/day, which is a lower level than has been used commonly, although the authors of the report did measure background intakes of vitamin D for participants, which, when added to the 400 IU dose results in an average intake of approximately 750 IU/day. Vitamin D_3 alone versus placebo did not result in a significant increase in BMD in postmenopausal women, except in one trial that noted an increase in femoral neck BMD. Only a few trials reported the impact of baseline serum 25OHD concentrations on BMD; in all of these trials, baseline 25OHD concentration was not associated with increased BMD.

AHRQ-Tufts identified four RCTs that were made available after the completion of AHRQ-Ottawa, one of which focused on children (see Table 4-9). Two of the three new RCTs for women and elderly men indicated a significant increase in hip or total BMD in postmenopausal women, comparing vitamin D_3 or vitamin D_2 (300 or 1,000 IU/day, respectively) plus calcium (1,200 mg/day) with placebo. The RCT that focused on healthy girls, ages 10 to 12 years (Cheng et al., 2005) compared the effect of vitamin D_3 (200 IU/day) plus calcium (1,000 mg/day) supplementation on bone indexes with placebo. The mean background dietary calcium intake was 670 mg/day. The intention-to-treat analyses suggested that after 2 years of supplementation, there was no significant difference in the BMC changes between girls who received vitamin D plus calcium supplement or placebo. The methodological quality of this study was rated C, as a result of being underpowered and having low compliance rate. The findings from AHRQ are summarized by DRI-relevant life stage groups in Box 4-4.

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TABLE 4-8 Effect of Vitamin D_2 or Vitamin D_3 on BMD by Site in Individual Trials (for Women of Reproductive Age, Postmenopausal Women, and Older Men): Summary from AHRQ-Ottawa Analyses[a]

Reference	Duration; Sample Size (*n*/Total *N*)	Vitamin D Dose (IU/day); Mean Dietary Vitamin D Intake (Tx/Control)	Lumbar Spine BMD % change (SD)		Femoral Neck BMD % change (SD)		Total Body BMD % change (SD)	
			Tx	Control (e.g., placebo, calcium, or lower dose of vit D)	Tx	Control	Tx	Control
Aloia et al., 2005	3 years 208	800 D_3 for 2 y, then 2,000 D_3 for 1 y + calcium 184 IU/d	0.25 (1.82)	0.30 (1.82)	NR	NR	−0.35 (1.60)	−0.30 (1.50)
Baeksgaard et al., 1998	2 years 240	560 D_3 + 1,000 mg calcium 158/140 IU/d	1.6	−0.2	1	0.4	NR	NR
Chapuy et al., 1992	1.5 years 56 (56/3270)	800 D_3 + 1,200 mg calcium NR	NR	NR	2.90 (6.40)	1.80 (9.40)	NR	NR
Chapuy et al., 2002	2 years 114 (114/583)	800 D_3 + 1,200 mg calcium 40/42 IU/d	NR	NR	−1.20 (7.40)	−4.50 (7.10)	NR	NR
Cooper et al., 2003	2 years 276 (187/187)	10,000 D_2/wk + 1,000 mg calcium NR	0.21 (4.89)	1.66 (5.27)	0.87 (4.95)	3.32 (5.10)	NR	NR

continued

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TABLE 4-8 Continued

Reference	Duration; Sample Size (n/Total N)	Vitamin D Dose (IU/day); Mean Dietary Vitamin D Intake (Tx/Control)	Lumbar Spine BMD % change (SD)		Femoral Neck BMD % change (SD)		Total Body BMD % change (SD)	
			Tx	Control (e.g., placebo, calcium, or lower dose of vit D)	Tx	Control	Tx	Control
Dawson-Hughes et al., 1991	1 year 261 (220–246/276)	400 D_3 + 377 mg calcium During treatment, 106/87 IU/d, August–November	0.85 (2.41)	0.15 (2.62)	NR	NR	0.03 (1.35)	–0.08 (1.25)
Dawson-Hughes et al., 1995	2 years 215 (215–246/261)	700 D_3 + 500 mg calcium 120/107 IU/d	–0.31 (2.87)	–0.11 (3.15)	–1.06 (3.76)	–2.54 (4.07)	–0.20 (1.66)	–0.35 (1.56)
Dawson-Hughes et al., 1997b	3 years 389	700 D_3 + 500 mg calcium Women: 174/184 IU/d Men: 202/197 IU/d	2.12 (4.06)	1.22 (4.25)	0.50 (4.80)	–0.70 (5.03)	0.06 (1.83)	–1.09 (1.71)
Grados et al., 2003a Companions: Grados et al., 2003b; Brazier et al., 2005	1 year 192 (67–72/192)	800 D_3 + 1,000 mg calcium 84.9/83.9 IU/d	2.98*	–0.21*	1.19*	–0.83*	0.99*	0.11*

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Harwood et al., 2004	1 year 150 (40/150)	800 D_3 + 1,000 mg calcium 300,000 D_2 single injection 300,000 D_2 single injection + 1,000 mg calcium NR	−1.6	8.2	−1.9	−0.9	NR	NR
Hunter et al., 2000	2 years 128 Comparison of 64 pairs of twins	800 D_3 135/134 IU/d	0.00 (5.62)	0.00 (5.56)	—	—	—	—
Jackson et al., 2006	7 years 2,431 of total sample	400 D_3 + 1,000 mg calcium Total vit D intake diet and supplements: 365/368 IU	Graph	Graph	Graph	Graph	Graph	Graph
Jensen et al., 2002	3 years 68/83	400 D_3 + 1,450 mg calcium NR	1.20 (4.32)	0.73 (4.08)	NR	NR	−1.10 (1.78)	−1.78 (1.56)
Komulainen et al., 1998	5 years 206/425	300 D_3 + 500 mg calcium NR	−4.6 (5.08)	−4.5 (4.90)	−4.3 (5.03)	−4.3 (4.9)	NR	NR

continued

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TABLE 4-8 Continued

Reference	Duration; Sample Size (n/Total N)	Vitamin D Dose (IU/day); Mean Dietary Vitamin D Intake (Tx/Control)	Lumbar Spine BMD % change (SD)		Femoral Neck BMD % change (SD)		Total Body BMD % change (SD)	
			Tx	Control (e.g., placebo, calcium, or lower dose of vit D)	Tx	Control	Tx	Control
Meier et al., 2004	2 years 55 (43/55)	500 D_3 + 500 mg calcium NR	0.8	NR	0.1	NR	NR	NR
Ooms et al., 1995b	2 years 348	400 D_3 NR	NR	NR	1.47 (6.13) left femoral neck	−0.21 (6.12)	NR	NR
Patel et al., 2001	2 years 70	800 D_3 NR	NA crossover trial					

NOTE: * Median % change; Dawson-Hughes et al. (1997b) included 176/389 men (45% of participants) and Meier et al. (2004) included 19/55 men (35% of participants). All other studies included women only. BMD = bone mineral density; IU = International Units; NA = not applicable; NR = not reported; SD = standard deviation; Tx = treatment; vit = vitamin; wk = week(s).

[a]This table has been truncated for the purposes of this chapter, but it can be found in its entirety in Appendix C.

SOURCE: Modified from Cranney et al. (2007).

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TABLE 4-9 Combined Vitamin D and Calcium and Bone Mineral Density/Bone Mineral Content: Characteristics of RCTs Published after AHRQ-Ottawa Report: Summary from AHRQ-Tufts Analyses[a]

Reference; Location (Latitude)	Population Description	Background Calcium Intake and Vitamin D Data	Comparisons	Compliance	Comments
Cheng et al., 2005 Jyvaskyla, Finland (62°24′N)	Health status: Healthy Mean age (range), y: 11.2 (10–12) Male (%): 0	Diet vit D: 100 IU/d Ca: 670 mg/d	200 IU vit D_3/d + 1,000 mg Ca carbonate/d vs. placebo	65% completed intervention with > 50% compliance	
Bolton-Smith et al., 2007 UK (54°N)	Health status: Healthy (assumed postmenopausal) Mean age (range), y: 68 (≥ 60) Male (%): 0	25OHD: 59.4 nmol/L Ca: 1,548 mg/d	400 IU vit D_3/d + 100 mg elemental Ca/d vs. placebo	Good supplement adherence based on pill count (median, 99; IQE 97.3–99.8%)	Noncompliant women were excluded
Zhu et al., 2008b Western Australia	Health status: nd (assumed postmenopausal) Mean age (SD), y: 74.8 (2.6) Male (%): 0	25OHD: 68 nmol/L Ca: 1,010 mg/d	1,000 IU vit D_2/d + 1,200 mg Ca citrate/d vs. placebo	No differences in adherence among groups (81–89% by tablet counting)	
Moschonis and Manios, 2006 Greece (31°N)	Health status: Postmenopausal Mean age (range), y: 61 (55–65) Male (%): 0	Diet vit D: 23.6 IU/d Ca 680.0 mg/d	300 IU vit D_3/d + 1,200 mg Ca/d (from low-fat dairy products) vs. control (usual diet)	Dairy group 93% (assessed via information obtained at the biweekly sessions)	Control group had no intervention (or usual diet), so compliance issue not applicable

NOTE: IU = International Units; nd = not determined; SD = standard deviation; UK = United Kingdom; vit = vitamin; y = year(s).

[a]This table has been truncated for the purposes of this chapter, but it can be found in its entirety in Appendix D.

SOURCE: Chung et al. (2009).

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BOX 4-4
AHRQ Findings by Life Stage for Vitamin D and Calcium and BMC/BMD*

0–6 months: No data

7 months–2 years: No data

3–8 years: No data

9–18 years: One RCT showed that, compared with placebo, there was no significant effect of vitamin D_3 (200 IU/day) plus calcium (1,000 mg/day) on BMC changes in healthy girls between 10 and 12 years of age.

19–50 years: No data

51–70 years: No new data were identified in AHRQ-Tufts

≥ 71 years: No new data were identified in AHRQ-Tufts

Postmenopause: Findings from the AHRQ-Ottawa report showed that vitamin D_3 (≤ 800 IU/day) plus calcium (~500 mg/day) supplementation resulted in small increases in BMD of the spine, total body, femoral neck, and total hip in predominantly populations of late-menopausal women. Two of the three new RCTs showed a significant increase in hip or total BMD in postmenopausal women, comparing vitamin D_3 or vitamin D_2 (300 or 1,000 IU/day, respectively) plus calcium (1,200 mg/day) with placebo.

Pregnant and lactating women: No new data were identified in AHRQ-Tufts

*Evidence from AHRQ-Ottawa; information from AHRQ-Tufts as noted.
SOURCE: Modified from Chung et al. (2009).

Fractures and BMD in postmenopausal women and older men: Serum 25OHD The association between risk of fractures and vitamin D in combination with calcium, as well as vitamin D alone, was addressed by AHRQ. Neither analysis focused on fracture risk and calcium intake alone.

AHRQ-Ottawa, which included some studies that combined calcium and vitamin D, identified observational studies (ranging from poor to fair quality) that reported on the association between serum 25OHD concentrations and fractures. The studies are identified in Table 4-10. The analysis concludes that there is inconsistent evidence to support an association between serum 25OHD concentration and an increased risk of fracture. Five studies of good quality evaluated the association between serum 25OHD concentration and risk of falls (see discussion in section above on Falls and

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TABLE 4-10 Serum 25OHD Levels and Fractures in Postmenopausal Women and Older Men: Summary from AHRQ-Ottawa Analyses[a]

Reference; Country	Population Description	Duration (or Matching Variables)	Bone Health Outcomes	Covariates; Summary of Results
Prospective cohort studies				
Cummings et al., 1998 United States	Subset of a cohort of ambulatory community-dwelling women ≥ 65 years of age (nested case–control study); 72.6 y (subset) n = 9,704 Caucasian	5.9 y	Hip fractures Vertebral fractures BMD calcaneus (SPA)	Adjusted for age, weight, and calcaneal BMD (SPA) There were no statistically significant unadjusted or adjusted (age, weight, season, use of vit D supplements) associations between serum 25OHD or PTH and the risk of hip or vertebral fracture For women in the lowest quintile of serum 25OHD levels, there was no increased risk for hip or vertebral fracture Women in the lowest quintile of serum $1,25(OH)_2D$ had a significant increase in hip fracture risk (RR 2.1, 95% CI 1.2–3.5), but not vertebral fracture risk
Gerdhem et al., 2005 Sweden	Ambulatory independently living women, 75 y (range 75–75.9 y) n = 1,044	3 y	Fractures (low energy)	119/986 (12%) had a total of 159 low-energy fractures (29 hip, 28 wrist, 12 proximal humerus, 43 vertebral, and 47 other) 9/43 (21%) with 25OHD < 50 nmol/L had one or more fractures vs. 110/943 (12%) with 25OHD > 50 nmol/L: HR 2.04 (95% CI 1.04–4.04) Fracture association was independent of season, although a seasonal difference was noted in mean level of 25OHD (September 101 nmol/L vs. February 89.8 nmol/L)

continued

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TABLE 4-10 Continued

Reference; Country	Population Description	Duration (or Matching Variables)	Bone Health Outcomes	Covariates; Summary of Results
Woo et al., 1990 Hong Kong	Elderly ≥ 60 y living independently in sheltered housing $n = 470$ 60% females Asian (Chinese)	30 mo	Fractures	Adjusted for age, gender, drinking, smoking, and BMI Subjects with lower serum 25OHD (males < 79 nmol/L and females < 66 nmol/L) had a nonsignificant increase in adjusted RR for fracture
Case–control studies				
Bakhtiyarova et al., 2006 Russian Federation	Hip fracture cases (spontaneous or low trauma) and controls admitted to ophthalmology department Cases: 68.8 (9.5) y Controls: 70.2 (8.3) y $n = 64$ (+ 97 controls) Cases: 69% female Controls: 55% female Caucasian	NR	Hip fractures	Median serum 25OHD levels significantly lower in hip fracture cases vs. controls (graph only) Hip fracture patients more likely to have serum 25OHD < 25 nmol/L than controls (65% vs. 47%, $p = 0.006$)

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Boonen et al., 1997 Belgium	Elderly women with hip fractures and community-dwelling controls n = 117 (+ 117 controls) Caucasian	Age, PM status, gender, ethnicity	Hip fractures BMD (FN and Tr) (DXA)	Serum 25OHD significantly lower in cases vs. controls (p = 0.001) Hip BMD (FN and Tr) significantly lower in cases vs. controls (p < 0.001)
Boonen et al., 1999 Belgium	PM women (osteoporotic hip fracture patients and independently living controls) Cases: 79.2 y Controls: 77.7 y n = 50 (+ 50 controls)	Age, gender, PM status, sampled at the same time of year	Fractures BMD (FN and Tr) (DXA)	Adjusted for age Mean $25OHD_3$ was significantly lower in cases vs. controls 25OHD < 30 nmol/L: 64% of cases vs. 8% controls within the same 4-mo sampling period (no relation between 25OHD and month of sample collection) FN and Tr BMD were significantly lower in cases than in controls
Cooper et al., 1989 UK	Hip fractures and healthy controls Cases: 77.4 (8.6) y Controls: 73.3 (10.5) (inpatients) and 66.9 (11.8) y (outpatients) n = 41 (+ 40 controls)	Age (cases and one of the two control groups similar), gender	Hip fractures	Age and albumin Mean 25OHD was significantly lower in cases vs. controls (p < 0.01). When age and albumin were used as covariates in the analysis, there was no residual difference in serum 25OHD levels. More hip fracture cases (49%) had 25OHD levels < 25 nmol/L vs. 15% of inpatient and 10% of outpatient controls

continued

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TABLE 4-10 Continued

Reference; Country	Population Description	Duration (or Matching Variables)	Bone Health Outcomes	Covariates; Summary of Results
Diamond et al., 1998 Australia	Men with hip fracture and healthy controls Cases: 79.6 y Controls: 78.7 y and 77 y $n = 41$ (+ 82 controls)	Age, gender	Hip fractures	Age, body weight, comorbidity score, smoking history, alcohol intake, serum calcium, albumin, 25OHD, and free testosterone Men with hip fractures had significantly lower 25OHD levels vs. controls ($p = 0.007$) 25OHD < 50 nmol/L: 63% of fracture patients vs. 25% of combined controls, OR 3.9 (95% CI 1.74–8.78) Multiple regression analysis showed that serum 25OHD level < 50 nmol/L was strongest predictor of hip fracture ($r = 0.34$ [0.19], $p = 0.013$) Age was the best determinant of a serum 25OHD level < 50 nmol/L, $p = 0.028$
Erem et al., 2002 Turkey	Women with hip fractures and healthy PM women, all independent community-dwellers Cases: 76.7 (6.5) y Controls: 75.4 (6.3) y $n = 21$ (+ 20 controls) Far Eastern	Age, gender, PM status	Hip fractures	NR Nonsignificant difference in 25OHD levels in hip fracture patients vs. controls 25OHD levels in all groups < 37.5 nmol/L

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Landin-Wilhelmsen et al., 1999 Sweden	PM women with OP, controls from outpatient clinic Osteoporotic women: 59 (6) y Controls: 59 (5) y n = 128 (+ 227 controls)	Age, gender, PM status	Fractures BMD and BMC: LS, TB, and FN (DXA)	NR 25OHD significantly lower in osteoporotic women vs. controls (p < 0.05); PTH significantly higher in osteoporotic women vs. controls (p < 0.001) Fracture history in 56% of osteoporotic women vs. 4% of controls (p < 0.001) Osteoporotic women had lower body weight and BMI vs. controls (p < 0.001)
Lau et al., 1989 Hong Kong	Hip fracture patients in hospital and community-living controls Age range: 49–93 y (cases), 60–90 y (controls) n = 200 (+ 427 controls) Asian	Ethnicity	Hip fractures	NR 25OHD levels were significantly lower in cases vs. controls (p < 0.01) Hip fracture patients with low 25OHD (male < 36.5 nmol/L, female < 34.3 nmol/L, defined by lower limit of 95% CI for controls) were less mobile than those with normal 25OHD; 33% with low 25OHD could walk outdoors without an aid vs. 61% of those with a normal 25OHD level
LeBoff et al., 1999 United States	Community-dwelling women [30 with hip fracture and OP (group 1); 68 women admitted for elective joint replacement with (17) or without (51) OP (group 2)] Group 1: 77.9 y Group 2: OP 69.9 y; non-OP 64.4 y n = 98	Gender, PM status, setting, surgical procedure OP in group 1 and subset of group 2	Hip fractures BMD: LS, FN, Tr, TB (DXA)	Adjusted for age and estrogen replacement therapy Women with hip fracture and OP had significantly lower 25OHD vs. women with OP admitted for surgery (p = 0.01) and vs. women without OP admitted for surgery (p = 0.02) % of women with 25OHD < 30 nmol/L: Significantly more in group 1 (50%) vs. OP or non-OP group 2 (graph only ~ 5% for OP and 10% for non-OP) (p < 0.002) Mean BMD (LS, FN, Tr) was significantly less in women with acute hip fracture/OP vs. elective surgery non-OP controls

continued

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TABLE 4-10 Continued

Reference; Country	Population Description	Duration (or Matching Variables)	Bone Health Outcomes	Covariates; Summary of Results
Lips et al., 1983, 1987 Netherlands	Consecutive patients with FN fracture and 74 healthy community controls Cases: 75.9 (11) y Controls: 75.6 (4.2) $n = 125$ (+ 74 controls) Cases: 67% female Controls: 73% female	Age	Hip fractures	Adjusted for age and sex Serum 25OHD levels lower in cases vs. controls ($p < 0.001$)
Lund et al., 1975 Denmark	67 consecutive cases of proximal femur fractures Controls: middle-aged (30–59 y, $n = 27$) and elderly healthy individuals (60–95 y, $n = 67$) at same time of year	Age	Proximal femur fractures	There was no statistically significant difference in serum 25OHD levels vs. either controls
Punnonen et al., 1986 Finland	Cases of hip fracture and controls (from gynecological clinic) Cases: 77.1 (8.6) y Controls: 73.8 (8.4) y $n = 40$ (+ 25 controls)	Age, gender, setting	Hip fractures (FN)	NR 25OHD levels were significantly lower in cases vs. controls ($p < 0.01$)

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Thiebaud et al., 1997 Switzerland	179 hip fracture patients; 180 hospital controls; 55 community controls Cases: women 81.0 y; men 77.7 y Hospital controls: women 80.9 y, men 76.9 y Community controls: women 71.7 y, men 71.3 y Cases: 76% female Hospital controls: 75% female Community controls: 85% female	Age, setting (for cases and one control group)	Fractures BMD: FN, TH, Tr (DXA)	Adjusted for age, sex, and creatinine Women and men with hip fractures had significantly lower 25OHD levels vs. controls. Fracture patients had lower hip (TH, FN) BMD vs. either control group ($p < 0.001$). In multivariate logistic regression of the risk for hip fracture, serum albumin and PTH were significant. In women, BMD was weakly correlated with 25OHD, and the only significant association was at the Tr ($r = 0.13$, $p < 0.05$).

NOTE: Total 25OHD or either isoform of 25OHD (isoform not specified); BMC = bone mineral content; BMD = bone mineral density; BMI = body mass index; CI = confidence interval; DXA = dual-energy X-ray absorptiometry; FN = femoral neck; HR = hazard ratio; mo = month(s); LS = lumbar spine; NR = not reported; OP = osteoporosis; OR = odds ratio; PM = postmenopausal; PTH = parathyroid hormone; RR = relative risk; SPA = single-photon absorptiometry; TB = total body; TH = total hip; Tr = trochanter; vit = vitamin; y = year(s).

[a]This table has been truncated for the purposes of this chapter, but it can be found in its entirety in Appendix C.

SOURCE: Modified from Cranney et al. (2007).

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Physical Performance). Nineteen studies assessed the association between serum 25OHD concentrations and BMD, and there is fair evidence from observational studies for an association between serum 25OHD concentrations and changes in hip BMD sites. Some studies identified specific serum concentrations of 25OHD below which falls, fractures, or bone loss increased; these values ranged from approximately 40 to 80 nmol/L.

The findings from AHRQ are summarized by DRI-relevant life stage groups in Box 4-5.

Fractures in postmenopausal women and older men: Vitamin D supplementation with or without calcium[3] Overall, AHRQ-Ottawa concluded that supplementation with vitamin D (most studies used vitamin D_3) plus calcium is effective in reducing fractures in institutionalized older populations. AHRQ-Tufts did not identify new RCTs examining the combined effect of vitamin D plus calcium supplementation on fractures in postmenopausal women and older men.

As reported by AHRQ-Ottawa, 15 RCTs evaluated the effect of vitamin D_2 or vitamin D_3 (with or without calcium supplementation) on fractures in postmenopausal women and older men (Table 4-11). The majority of the trials used vitamin D_3 preparations (300 to 800 IU/day). Ten trials were of higher quality, although high losses to follow-up and inadequate reporting of allocation concealment were limitations of a number of trials. Vertebral fractures were not included as an outcome in most trials. Vitamin D_3 (700 to 800 IU/day) combined with calcium supplements (500 to 1,200 mg/day) significantly reduced non-vertebral and hip fractures although the benefit was predominantly in older subjects living in institutionalized settings (hip fractures: odds ratio [OR] = 0.69; 95% CI 0.53–0.90). The benefit of vitamin D and calcium on fractures in community-dwelling individuals was inconsistent across trials.

Specifically, AHRQ-Ottawa conducted a meta-analysis of 13 of the RCTs (omitting Anderson et al. [2004], which is an abstract only, and Larsen et al. [2004], which included no placebo control). Included in the 13 RCTs was the report from Jackson et al. (2006), which reflected data from the WHI trials based on 36,282 subjects. Reproduced in Figures 4-5 through 4-7 are the relevant forest plots for the outcomes related to total fractures from studies that used either oral vitamin D_3 or vitamin D_2 plus or minus calcium versus calcium or placebo, total fractures for studies that used vitamin D_3 plus calcium versus placebo, and hip fractures (by setting)

[3]As an aside, it was noted that one RCT of premenopausal women, ages 17 to 35 years, showed that 800 IU/day of vitamin D in combination with 2,000 mg/day of calcium supplementation can reduce the risk of stress fracture from military training compared with placebo (Lappe et al., 2008).

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BOX 4-5
AHRQ Findings by Life Stage for 25OHD and Fractures*

0–6 months: Not reviewed

7 months–2 years: Not reviewed

3–8 years: Not reviewed

9–18 years: Not reviewed

19–50 years: No data

51–70 years: The AHRQ-Ottawa report concluded that the associations between serum 25OHD concentrations and risk of fractures are inconsistent. No new data were identified in the AHRQ-Tufts report.

≥ 71 years: Findings from three new RCTs did not show significant effects of either vitamin D_2 or vitamin D_3 supplementation (daily doses ranged from 400 to 822 IU) in reducing the risk of total fractures among men and women in this life stage.

Postmenopause: The AHRQ-Ottawa report concluded that the associations between serum 25OHD concentrations and risk of fractures are inconsistent. No new data were identified in the AHRQ-Tufts report.

Pregnant and lactating women: Not reviewed

*Evidence from AHRQ-Ottawa; information from AHRQ-Tufts as noted.
SOURCE: Modified from Chung et al. (2009).

for studies that used vitamin D_3 plus or minus calcium versus placebo. As highlighted above, the benefit in community-dwelling individuals was inconsistent, but benefit was evidenced for institutionalized individuals.

As reported by AHRQ-Tufts, findings from three RCTs that postdated AHRQ-Ottawa (Bunout et al., 2006; Burleigh et al., 2007; Lyons et al., 2007) did not show significant effects of either vitamin D_2 or vitamin D_3 supplementation (daily doses of 400 to 822 IU) in reducing the risk of total fractures (Table 4-12). The findings from AHRQ are summarized by DRI-relevant life stage groups in Box 4-6.

Rickets in children Rickets was explored by AHRQ-Ottawa relative to serum 25OHD measures only. Overall, there was fair evidence for an association between low serum 25OHD concentrations and confirmed rickets, regardless of the types of assays used to measure serum 25OHD concentrations. There is inconsistent evidence to determine whether there is a

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TABLE 4-11 Odds Ratio (95% Confidence Interval) for Total Fractures from Individual RCTs of Vitamin D: Summary from AHRQ-Ottawa Analyses[a]

Reference; Jadad Score for RCTs[b]	Duration (y)	Sample Size (*n*)	Vitamin D (IU/day) Follow-up	Mean Baseline 25OHD for IG (nmol/L)	End of trial 25OHD for IG (nmol/L)	OR (95% CI)
Chapuy et al., 2002 Jadad = 3	2	583	800 D_3 + 1,200 mg Ca	22	75 (graph)	0.79 (0.54–1.17)
Chapuy et al., 1992 Jadad = 2	1.5	3,270	800 D_3 + 1,200 mg Ca	40	105	0.72 (0.58–0.90)
Lips et al., 1996 Jadad = 5	4	2,578	400 D_3	27	62	1.12 (0.86–1.44)
Dawson-Hughes et al., 1997b Jadad = 4	3	389	700 D_3 + 500 mg Ca	82.7 M, 67.5 F	112	0.42 (0.20–0.88)
Law et al., 2006 Jadad = 2	1	3,717	1,100 D_2	59	77	1.4 (0.9–2.0)
Pfeifer et al., 2000 Jadad = 3	1	148	800 D_3 + 1,200 mg Ca	25.6	66.1	0.48 (0.12–1.99)
Komulainen et al., 1998 Jadad = 3	5	232	300 D_3 + 500 mg Ca	28.6	37.5	0.71 (0.31–1.61)
Grant et al., 2005 Jadad = 5	5	5,292	800 D_3 with or without 1,000 mg Ca	39	62.2	1.02 (0.84–1.22)
Flicker et al., 2005 Jadad = 4	2	625	1,100 D_2 + 1,000 mg Ca	NR	NR	0.69 (0.4–1.18)

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TABLE 4-11 Continued

Reference; Jadad Score for RCTs[b]	Duration (y)	Sample Size (*n*)	Vitamin D (IU/day) Follow-up	Mean Baseline 25OHD for IG (nmol/L)	End of trial 25OHD for IG (nmol/L)	OR (95% CI)
Jackson et al., 2006 Jadad = 4	7	36,282	400 D_3 + 1,000 mg Ca	46	NR	0.97 (0.91–1.03)
Porthouse et al., 2005 Jadad = 3	2	3,314	800 D_3 + 1,000 mg Ca	—	—	0.96 (0.65–1.46) Unequal 1.09 (0.60–1.96) Equal
Trivedi et al., 2003 Jadad = 3	5	2,686	100,000 D_3 4 mo	NR	74.3	0.78 (0.60–1.00)
Harwood et al., 2004 Jadad = 3	1	150	800 D_3 + 1,000 mg Ca	28–30	40–50	0.58 (0.13–2.64)

NOTE: CI = confidence interval; F = female; IG = intervention group; IU = International Units; OR = odds ratio; M = male; mo = month(s); NR = not reported; y = year(s).

[a]This table has been truncated for the purposes of this chapter, but it can be found in its entirety in Appendix C.

[b]Jadad score is based on a scale of 1 to 5. See Box 4-1 for details on the scoring system.

SOURCE: Modified from Cranney et al. (2007).

threshold concentration of serum 25OHD above which rickets does not occur.

Six studies (one RCT, three before-and-after studies, and two case–control studies) reported mean or median serum 25OHD concentrations below 30 nmol/L in children with rickets, whereas the other studies reported that the mean or median serum 25OHD concentrations were above 30 nmol/L (and up to 50 nmol/L). In seven of eight case–control studies, serum 25OHD concentrations were lower in children with rickets compared with controls. Information on the 13 studies is shown in Table 4-13.

AHRQ-Tufts identified no new RCTs concerning rickets since the completion of AHRQ-Ottawa.

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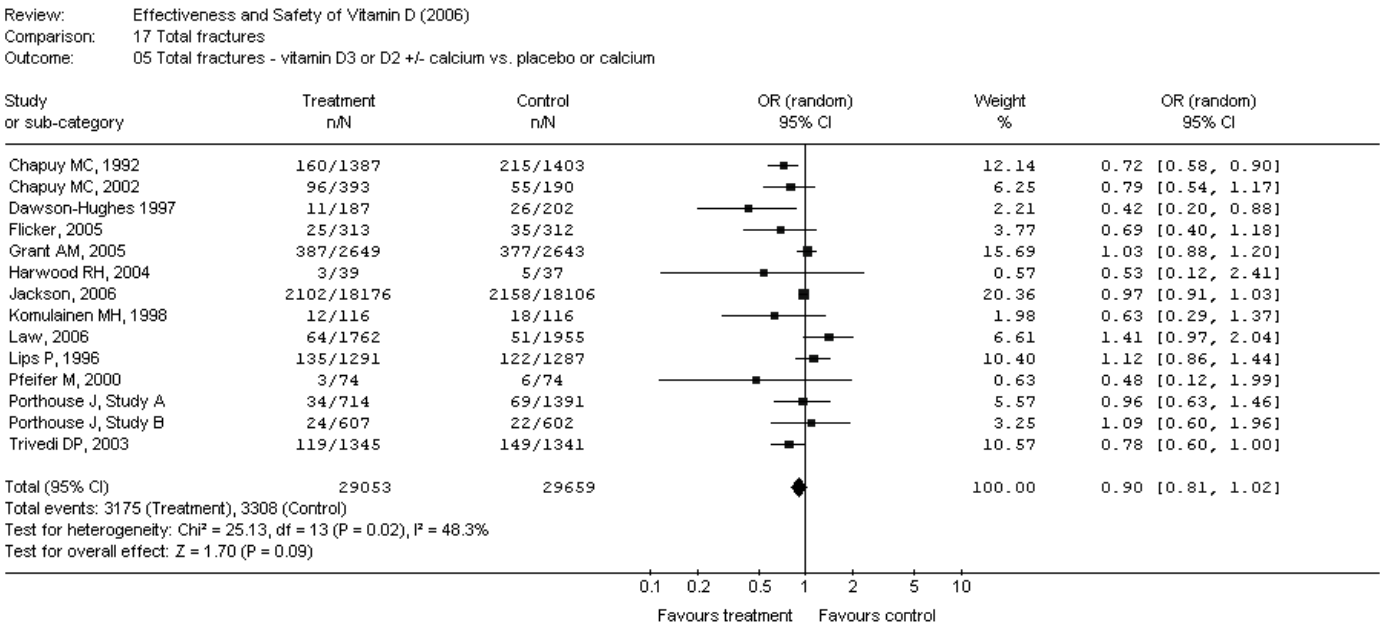


FIGURE 4-5 Forest plot comparing risk of total fractures with vitamin D_2 or vitamin D_3 with or without calcium vs. placebo or calcium.
SOURCE: Cranney et al. (2007).

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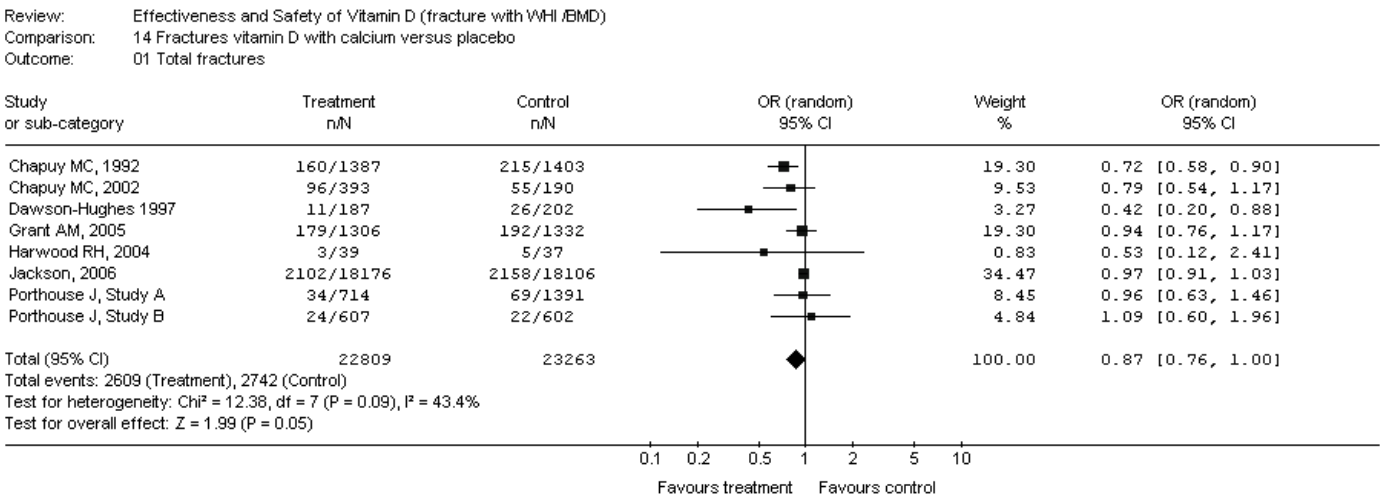


FIGURE 4-6 Forest plot comparing the risk of total fractures with vitamin D_3 combined with calcium vs. placebo.
SOURCE: Cranney et al. (2007).

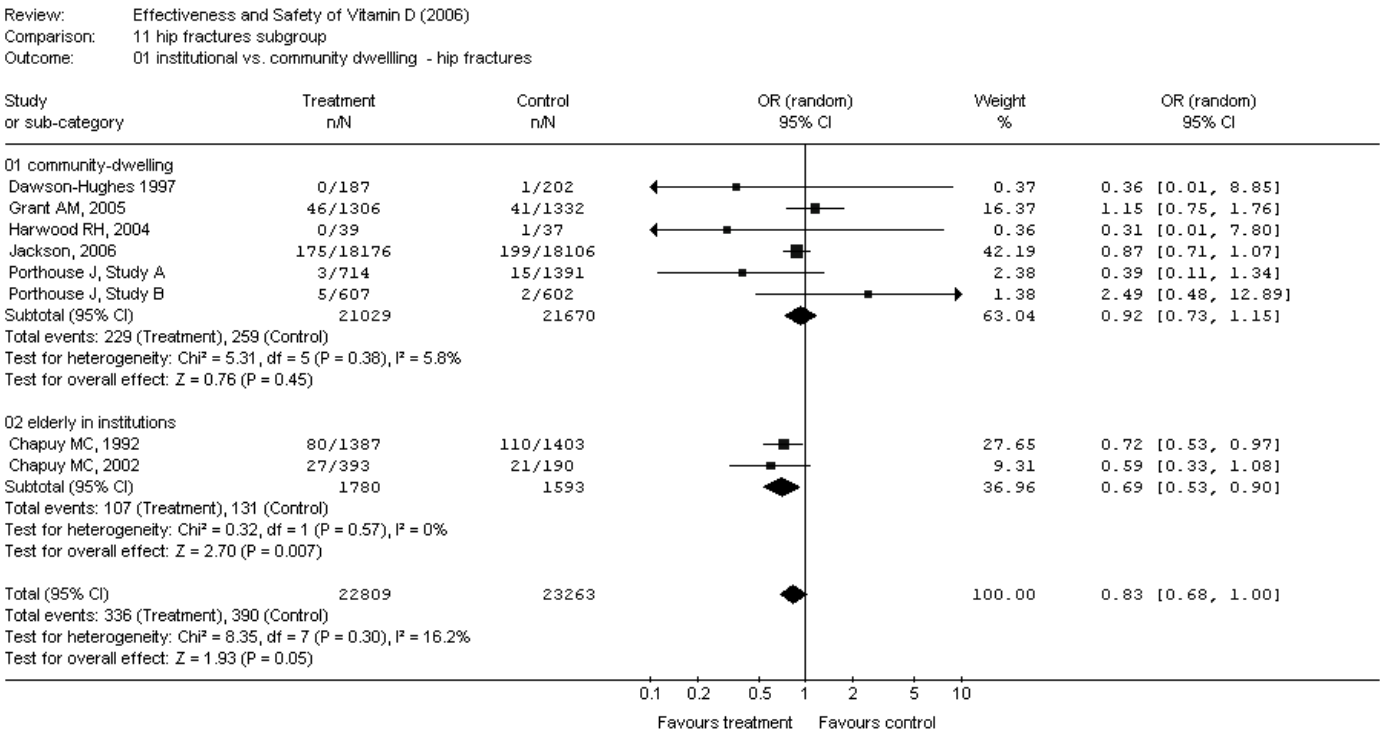


FIGURE 4-7 Forest plot comparing risk of hip fractures with vitamin D_3 with or without calcium vs. placebo by setting. SOURCE: Cranney et al. (2007).

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TABLE 4-12 Vitamin D and Bone Health: Characteristics of RCTs Published after AHRQ-Ottawa[a]

Reference; Location (Latitude)	Population Description		Background Calcium Intake and Vitamin D Data	Comparisons	Compliance
Lyons et al., 2007 South Wales, UK (52°N)	Health status	Living in care facilities including some elderly with mobility, cognitive, visual, hearing, or communication impairments	nd	100,000 IU vit D_2 4× monthly vs. placebo	80% (percentage of occasions observed to take tablets)
	Mean age (range), y	84 (62–107)			
	Male (%)	23.7			
Burleigh et al., 2007 Scotland (55°57′N)	Health status	Inpatient with high levels of comorbidity, mortality, and polypharmacy	25OHD: 22 nmol/L	800 IU vit D_3/d + 1,200 mg Ca carbonate/d vs. 1,200 mg Ca carbonate/d	Ca group = 87%, vit D + Ca group = 89% (total study drug taken/total study drug prescribed, as recorded in drug prescription charts)
	Mean age (SD), y	83 (7.6)			
	Male (%)	40			
Bunout et al., 2006 Chile (32°S)	Health status	Healthy	25OHD: ≤ 40 nmol/L	800 mg Ca/d vs. 800 mg Ca/d + 400 IU vit D/d (with and without exercise training)	92% (tablet counting)
	Mean age (SD), y	76 (4)			
	Male (%)	11.6			

NOTE: IU = International Units; nd = not determined; SD = standard deviation; UK = United Kingdom; vit = vitamin; y = year(s)

[a]This table has been truncated for the purposes of this chapter, but it can be found in its entirety in Appendix D.

SOURCE: Modified from Chung et al. (2009).

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BOX 4-6
AHRQ Findings by Life Stage for Vitamin D and Calcium for Clinical Outcomes of Bone Health*

0–6 months: Not reviewed

7 months–2 years: Not reviewed

3–8 years: Not reviewed

9–18 years: Not reviewed

19–50 years: The AHRQ-Ottawa report concluded that supplementation with vitamin D (most studies used vitamin D_3) plus calcium is effective in reducing the risk of fractures in institutionalized populations. One RCT of female Navy recruits ages 17 to 35 years showed that vitamin D (800 IU/day) in combination with calcium (2,000 mg/day) supplementation can reduce the risk of stress fractures from military training compared with placebo.

51–70 years: No new data were identified in the AHRQ-Tufts report

≥ 71 years: No new data were identified in the AHRQ-Tufts report

Pregnant and lactating women: No data

*Evidence from AHRQ-Ottawa; information from AHRQ-Tufts as noted.
SOURCE: Modified from Chung et al. (2009).

Pregnancy, Fetal Development, and Lactation

Pregnancy and lactation constitute specific, unique life stages that are of current interest regarding calcium and vitamin D functions and nutritional requirements. The body of evidence concerning skeletal health as it relates to the calcium and vitamin D nutriture of pregnancy, lactation, and fetal development is integrated below so as to provide context for the selection of indicators for DRI development.

Pregnancy: Calcium The developing fetus requires calcium, especially during the third trimester when the skeleton is undergoing mineralization. Direct measurements of the calcium content of the newborn skeleton have indicated that 25 to 30 g of calcium is transferred to the fetus by the end of gestation (Givens and Macy, 1933; Trotter and Hixon, 1974). Maternal intestinal calcium absorption doubles beginning early in pregnancy even though little calcium is transferred to the embryo at this stage (Heaney

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TABLE 4-13 Serum 25OHD Levels in Infants and Young Children with Established Rickets: Summary from AHRQ-Ottawa[a]

Reference; Country	Population Description	Intervention; Duration	Bone Health Outcomes	Results
RCTs				
Cesur et al., 2003 Turkey	Infants with nutritional rickets 10.7 (6.1) mo (range 3–36 mo) 36% female $n = 56$	IG1: 150,000 IU vit D IG2: 300,000 IU vit D IG3: 600,000 IU vit D (single dose) 2 mo	Rickets	$25OHD_3$, mean (SD) (nmol/L): Stage I: 15.8 (6.4) Stage II: 15.4 (4.8) Stage III: 14.7 (3.9) Ca, mean (SD) (mmol/L): All patients 1.9 (0.33)
Before-and-after studies				
Bhimma et al., 1995 South Africa	Children with rickets: vit D deficiency rickets (25OHD < 25 nmol/L); Ca deficiency rickets; phosphopenic rickets; healing/healed rickets Age range 1–12 y Vit D deficiency rickets 56% female $n = 23$	5,000–10,000 IU vit D_3/d (plus 500–1,000 mg Ca/d) 12 mo	Rickets	25OHD, mean (SD) (nmol/L): Vit D–deficient rickets: 9.3 (8.8) Ca-deficient rickets: 45.5 (10.0) Ca, mean (SD) (mmol/L): Vit D–deficient rickets: 2.09 (0.27) Ca-deficient rickets: 2.16 (0.28)

continued

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TABLE 4-13 Continued

Reference; Country	Population Description	Intervention; Duration	Bone Health Outcomes	Results
Elzouki et al., 1989 Libya	Children < 2 y admitted for treatment of rickets n = 22 37.5% female African black	1–3 h/d of sunshine followed by single IM injection of 600,000 IU vit D_2 Follow-up median 17 d	Rickets	25OHD: At diagnosis, 50% of patients had 25OHD > 20 nmol/L Range 4–65 nmol/L (graph) Ca: ND
Garabedian et al., 1983 France/Belgium	Infants and children with rickets and controls Infants and young children: range 4–26 mo Older children: range 4–12 y n = 20 (+ 60 controls) 80% immigrants from North Africa, Black Africa, Turkey, Portugal, Pakistan	IG1: 2,000 IU vit D_2/d IG2: 400 IU vit D_3/kg (single dose) 6 mo	Rickets	25OHD mean (SD) (nmol/L): All patients: 11.5 (8.0) Ca, mean (SD) (mmol/L): All patients: 1.8 (0.27)
Markestad et al., 1984 Norway	Children with rickets 11 (64.7%) immigrants from Pakistan, Cape Verde Islands, Turkey, Morocco, Sri Lanka, and West Africa; 6 (35.3%) Norwegians	1,700–4,000 IU vit D_2/d (reduced to 500–1,000 IU in 3 children at 2–4 wk) 10 wk	Rickets	25OHD median (range) (nmol/L): n = 9 diagnosed in summer: 21.0 (4.1–30.6) n = 8 diagnosed in winter: 12.1 (3.8–19.4) Ca: ND

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Case–control studies

Arnaud et al., 1976 Canada/Midwest United States	Children with mild, moderate, and severe rickets and controls; 2 mo–3.5 y $n = 9$ (+ 9 controls) Rickets: 22% female Canadian (First Nations, West Indian black, Portuguese) and American (mid-northwestern United States)	5,000 IU vit D/d 4 wk	Rickets	25OHD, mean (SD) (range) (nmol/L): Mild rickets: 45.0 (7.5) (range 40.0–52.5) Moderate rickets: 30 (5) Severe rickets: 20 (NR) Controls: 90 (30) Ca, mean (SD) (mmol/L): ND for mild, moderate, severe subgroups Stage II rickets: 2.4 (0.15) Age-matched controls: 2.53 (0.1)
Balasubramanian et al., 2003 India	Children and adolescents with rickets/osteomalacia and controls Children: Rickets: median 33 mo (range 11–120 mo) Controls: median 27 mo (range 6–84 mo) Adolescents: Rickets: median 198 mo (range 168–240 mo) Controls: median 156 mo (range 120–228 mo) $n = 40$ (+ 53 controls) Rickets: 54.1% female Controls: 47% female Hindu/Muslim	Cases: 6,000 IU vit D/d or single dose of 600,000 IU 3 mo	Rickets	25OHD, mean (SD) (nmol/L): Children: Rickets: 50.0 (38.9) Controls: 61.3 (35.9), NS Adolescents: Rickets: 12.6 (7.1) all but one < LLN Controls: 46.0 (45.4), $p < 0.001$ Ca, mean (SD) (mmol/L): Children: Rickets: 2.2 (0.3) Controls: 2.4 (0.3), NS Adolescents: Rickets: 2.1 (0.2) Controls: 2.3 (0.2), $p = 0.008$

continued

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TABLE 4-13 Continued

Reference; Country	Population Description	Intervention; Duration	Bone Health Outcomes	Results
Dawodu et al., 2005 United Arab Emirates	Children with rickets and historical controls Rickets: 13.5 mo Controls: 13.0 mo n = 38 (+ 50 controls) Rickets: 50% female Controls: 40% female Arab	NA	iPTH (rickets group only)	25OHD, median (IQR) (nmol/L): Rickets: 8.0 (3.8–15.3) Controls: 43.8 (25–64.3), p = 0.001 PTH showed a trend toward negative correlation with 25OHD (data NR) Ca, median (IQR) (mmol/L): Rickets: 2.22 (1.88–2.35) Controls: 2.4 (2.25–2.5), p = 0.001
Graff et al., 2004 Nigeria	Children with rickets and controls (unrelated) Rickets: 46 (22) mo Controls: 47 (22) mo n = 15 (+ 15 controls) 60% female Rickets: 7 Muslim and 8 Christian Controls: 4 Muslim and 11 Christian	Cases: 1,000 mg Ca/d (no vit D supplement) Treatment duration: 6 mo Follow-up: 12 mo	Rickets	25OHD, mean (SD) (nmol/L): Significantly lower in children with rickets Rickets: 37.5 (13.5) Controls: 72.5 (11.5), p < 0.001 Ca, mean (SD) (mmol/L): Rickets: 2.13 (0.2) Controls: 2.4 (0.1), p < 0.001

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Majid Molla et al., 2000 Kuwait	Children with rickets and controls Rickets: 14.5 (5.2) mo (range 9 mo–8 y) Controls: 15.2 (6.3) mo n = 103 (+ 102 controls) 96.1% from mothers with hijab use	NA	Rickets	25OHD, mean (SD) (nmol/L): Significantly lower in children with rickets Rickets: 26.5 (15.5) Controls: 83.5 (74.75), $p < 0.0001$ Ca, mean (SD) (mmol/L): Rickets: 2.24 (0.28) Controls: 2.45 (0.15), $p < 0.0001$
Oginni et al., 1996 Nigeria	Children with active rickets and healthy controls Children with rickets age range: 1–5 y n = 26 (+ 90 controls) Rickets: 50% female Controls: 61% female Nigerian	NA	Rickets	25OHD, mean (SD) (range) (nmol/L): Significantly lower in rickets group Rickets: 36 (28), range 7–147 Controls: 69 (22), range 32–140, $p < 0.0002$ Ca (albumin-corrected), mean (SD) (mmol/L): Rickets: 2.06 (0.23) Controls: 2.35 (0.14), $p < 0.001$
Thacher et al., 2000 Nigeria	Active rickets and controls Rickets: median (25th and 75th percentile) age: 46 (34–63) mo Controls: 42 (25–70) mo n = 123 (+ 123 controls) 49.6% female Christian/Islam: Rickets: 82/41 Controls: 57/66	NA	Rickets	25OHD median (25th and 75th percentile) (nmol/L): Rickets: 32 (22, 40); < 30: 37% Controls: 50 (42, 62), $p < 0.0001$ Ca, mean (SD) (mmol/L): Rickets: 1.93 (0.22) Controls: 2.24 (0.15), $p < 0.0001$

continued

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TABLE 4-13 Continued

Reference; Country	Population Description	Intervention; Duration	Bone Health Outcomes	Results
Thacher, 1997 Nigeria	Children with active rickets (median duration of 14 mo) and healthy controls with normal weight Rickets: 3.16 (1.53) y Controls 3.14 (1.51) y n = 37 (+ 37 controls) 47% female All Nigerian	NA	Rickets	25(OH)D Rickets: levels > LLN in 16/28 (57%); 2/28 (7%) had values < 12.5 nmol/L Controls: ND Ca, mean (SD) (mmol/L): Rickets: 2.09 (0.30) Controls: 2.08 (0.31), NS 55% of rickets and 51% of controls were hypocalcemic (< 2.1)

NOTE: h = hours; IG = intervention group; IM = intramuscular; iPTH = intact parathyroid hormone; IQR = interquartile range; IU = International Units; LLN = lower limit of normal reference range; mo = month(s); NA = not applicable; ND = not determined; NR = not reported; NS = not significant; PTH = parathyroid hormone; SD = standard deviation; vit = vitamin; wk = week(s); y = year(s).

[a]This table has been truncated for the purposes of this chapter, but it can be found in its entirety in Appendix C.

SOURCE: Modified from Cranney et al. (2007).

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and Skillman, 1971). The increased intestinal calcium absorption causes a net positive calcium balance in the mother early in pregnancy (Heaney and Skillman, 1971). However, in the third trimester, the rapid maternal-fetal calcium transfer results in a maternal calcium balance that is zero or perhaps slightly negative by the end of pregnancy.

There is controversy about the mobilization of calcium from maternal bone during pregnancy and its contribution to fetal calcium needs. A possible loss of BMC has been seen longitudinally using the modern technique of dual-energy X-ray absorptiometry (DXA), but measurements were done 1 to 18 months prior to pregnancy and 1 to 6 weeks postpartum (i.e., not during pregnancy) making it uncertain whether the measured calcium loss had truly occurred during pregnancy (Kovacs and Kronenberg, 1997; Kovacs and Fuleihan Gel, 2006). Further, the effect of pregnancy on bone mineral content may depend on the site examined, with decreases reported for trabecular bone (Black et al., 2000; Naylor et al., 2000; More et al., 2001; Kaur et al., 2003; Ulrich et al., 2003; Akesson et al., 2004; Pearson et al., 2004), but not cortical bone (Naylor et al., 2000; Pearson et al., 2004). Two studies (Kaur et al., 2003; Olausson et al., 2008) used contemporaneous non-pregnant and non-lactating age-matched controls to compare, to the extent feasible, the effects of pregnancy and age on BMD. Kaur et al. (2003) found no significant difference in BMD before and after pregnancy. Olausson et al. (2008) found a significant 1 to 4 percent decrease in whole-body, spine, and total hip BMC before and 2 weeks after pregnancy, whereas controls had an increase in whole-body BMC and a smaller (0.5 to 1 percent) decrease in BMD at the spine and hip. These skeletal changes were unrelated to calcium intake in either group. Collectively, the evidence tends to suggest that mineral mobilization is variable during pregnancy and may contribute, to some extent, to fetal calcium needs.

Relatively few studies have examined the effect of calcium supplementation on either fetal or maternal outcomes. In a placebo-controlled double-blind randomized trial conducted in the United States, Koo et al. (1999) demonstrated that calcium supplementation during pregnancy may benefit the offspring's bone health, but only in those infants whose mothers had very low calcium intake (600 mg/day), based on a post-hoc subgroup analysis. In contrast to this possible benefit to the offspring, calcium supplementation during pregnancy of Gambian women with low calcium intakes resulted surprisingly in greater decreases in BMC and BMD and related biochemical evidence, consistent with higher bone mineral mobilization during lactation (Jarjou et al., 2010).

Maternal serum calcium levels fall during pregnancy (Pedersen et al., 1984) as a consequence of plasma volume expansion and reduced albumin concentration; lower calcium levels do not imply calcium deficiency. The

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ionized calcium (i.e., the physiologically important fraction of calcium) and the albumin-corrected serum calcium levels do not change during pregnancy (Seely et al., 1997). Pregnant women consuming "moderate" calcium (800 to 1,000 mg/day) (Gertner et al., 1986; Allen et al., 1991) to "high" calcium (1,950 mg/day) (Cross et al., 1995) are often hypercalciuric as a result of increased intestinal calcium absorption (i.e., absorptive hypercalciuria); as such, pregnancy itself is a risk factor for kidney stones. Urinary calcium excretion increases as early as the 12th week of gestation and averages 300 ± 61 mg/24 hours in the third trimester with hypercalciuric levels not uncommon (Pedersen et al., 1984; Gertner et al., 1986; Allen et al., 1991; Cross et al., 1995; Seely et al., 1997). While urinary calcium excretion goes up in normal pregnancy, it decreases in women who are developing preeclampsia. The risk of preeclampsia can be reduced with supplemental calcium when the dietary calcium intake is very low; however, there appears to be no effect when dietary calcium intake is adequate (Hofmeyr et al., 2006; Villar et al., 2006; Hiller et al., 2007; Kumar et al., 2009).

In the adolescent, whose skeleton is still growing, pregnancy could theoretically reduce peak bone mass and increase the long-term risk of osteoporosis. Most cross–sectional studies that have compared the BMD in teens early postpartum with that in never-pregnant teens have suggested that there is no reason to be concerned about BMD or bone mass after adolescent pregnancy (Kovacs and Kronenberg, 1997). A few smaller observational studies have reported that lower adolescent age at first pregnancy is associated with lower BMD in the adult (Sowers et al., 1985, 1992; Fox et al., 1993). In contrast, an analysis of NHANES III data on BMD by DXA for 819 women ages 20 to 25 years found that women pregnant as adolescents had the same BMD as women pregnant as adults and as nulliparous women (Chantry et al., 2004). This study's population is diverse and representative of the general U.S. population and thus reassures that teen pregnancy does not reduce BMD in most women. An additional study (O'Brien et al., 2003) found that fractional calcium absorption doubles during adolescent pregnancy (as it does in adults) and during the first 2 months postpartum. Mean BMD of previously pregnant—but not lactating—adolescents was above the expected BMD for age in this study, also suggesting that no loss of BMD had occurred during pregnancy. These data indicate that adolescent women meet the calcium demands of pregnancy by increasing intestinal calcium absorption while preserving maternal bone mass.

Pregnancy: Vitamin D

Maternal outcomes Total calcitriol levels double early in pregnancy and remain at this increased level until delivery (Bikle et al., 1984; Cross et al., 1995; Ardawi et al., 1997; O'Brien et al., 2006; Papapetrou, 2010).

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This is related to a concomitant increase in plasma vitamin D binding protein (DBP) (Bikle et al., 1984; Ardawi et al., 1997). Free calcitriol levels do not increase until the third trimester (Bikle et al., 1984; Specker, 2004; Kovacs, 2008). The main source of calcitriol is from the maternal renal 1α-hydroxylase, with little contribution from the placenta even though it expresses 1α-hydroxylase, based on the case report of a pregnant anephric woman whose low levels of calcitriol increased less than 15 percent by the beginning of the third trimester (Turner et al., 1988). Despite the increased synthesis of calcitriol during pregnancy and the passage of 25OHD across the placenta to the fetus, maternal serum 25OHD levels are relatively unaffected by pregnancy (Hillman et al., 1978; Brooke et al., 1980; Cross et al., 1995; Ardawi et al., 1997; Morley et al., 2006; Papapetrou, 2010), although one report noted a significant decline by the third trimester in Saudi women (Ardawi et al., 1997). Even when baseline serum 25OHD level was in the severely deficient range (mean 20.1 ± 1.9 nmol/L), the serum levels did not change significantly by the end of pregnancy (Brooke et al., 1980).

The increase in maternal intestinal calcium absorption has been positively associated with the increase in maternal serum calcitriol levels in observational studies in humans (Cross et al., 1995; Ritchie et al., 1998). Certain results from studies in animal models are relevant to understanding the changes in vitamin D physiology that occur during human pregnancy. Intestinal calcium absorption is markedly up-regulated in pregnant vitamin D–deficient rats and in mice lacking the VDR (*Vdr*-null mice) to the same high rate achieved in pregnant vitamin D–replete rats and wild-type mice, respectively (Halloran and DeLuca, 1980a; Brommage et al., 1990; Fudge and Kovacs, 2010). This suggests that factors other than vitamin D (e.g., estrogen, placental lactogen, and prolactin) stimulate intestinal calcium absorption during pregnancy.

Very few clinical trials of vitamin D supplementation during pregnancy have been conducted. The work of Wagner et al. (2010a, b), reported currently in abstract form, has focused on high doses of vitamin D (4,000 versus 2,000 and 400 IU/day) in intervention trials in which the focus was on non-skeletal outcomes. A final report from these studies is expected soon. To date, the available intervention studies have shown little effect of vitamin D supplementation on maternal, fetal, or neonatal outcomes, although it would be expected that higher serum 25OHD levels in the newborn should protect against neonatal hypocalcemia (Specker, 2004; Kovacs, 2008). In a study of Asian women with initially low 25OHD levels (mean of 20 nmol/L) at baseline, daily supplementation with 1,000 IU of vitamin D per day did not affect cord blood calcium level or the newborns' crown–heel length, forearm length, triceps skinfold thickness, or head circumference, but it did reduce the fontanelle area by 32.7 percent (Brooke et al.,

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1980). The achieved serum 25OHD level in the vitamin D–supplemented group was 168 nmol/L compared with 10 nmol/L in the control group, raising the question as to whether the actual supplemented dose was considerably higher than the intended dose of 1,000 IU/day. In another trial, 1,000 IU of vitamin D per day was administered during the last trimester of pregnancy, compared with controls with no supplementation (Mallet et al., 1986). Supplementation resulted in higher maternal and cord blood 25OHD levels but had no effect on maternal, cord blood, or neonatal calcium levels or anthropometric parameters in the infants. In a study of vitamin D–deficient Asian women given a single dose of 800,000 IU of vitamin D in the third trimester, cord blood calcium level increased slightly, but there was no other benefit compared with women who received either no supplement or a daily dose of 1,200 IU of vitamin D (Marya et al., 1981, 1988). One small randomized but not blinded intervention trial found that maternal supplementation with 800 IU of vitamin D per day increased both maternal serum and cord blood 25OHD levels significantly, but did not affect gestational age at delivery, compared with unsupplemented controls (Yu et al., 2009). Observational studies have reported an uneventful clinical course of pregnancy in women with abnormalities of the VDR (vitamin D–dependent rickets type I [VDDR I] or VDDR II) when normo-calcemia is maintained (Malloy et al., 1997; St-Arnaud et al., 1997).

Fetal outcomes Animal models (e.g., mice, rats, guinea pigs, and sheep) have contributed to elucidating aspects of the physiology of fetal vitamin D nutriture that cannot be studied during human pregnancy. Studies in genetically altered animal models have provided information about the requirements for vitamin D and VDR signaling during pregnancy. For this reason, reference to such studies is included extensively in this component of the literature review.

There is evidence that 25OHD crosses the placenta relatively freely based on animal studies (Haddad et al., 1971; Noff and Edelstein, 1978) and studies of human perfused placenta (Ron et al., 1984). In contrast, calcitriol transfers across the human perfused placenta (Ron et al., 1984), but not the rat placenta (Noff and Edelstein, 1978). Overall, however, the net transfer from the human mother to fetus appears to be low. By term, cord blood 25OHD levels are typically 75 to 90 percent of maternal 25OHD levels (Seino et al., 1982; Kovacs, 2008), whereas cord blood calcitriol levels are low compared to maternal levels. As already highlighted in previous discussions regarding calcium, fetal serum calcium, ionized calcium, and phosphorus levels are raised above the maternal values, whereas PTH and calcitriol levels are lower. The higher calcium and phosphorus levels in the fetus suppress the 1α-hydroxylase in the placenta and fetal kidneys, and likely explain the low circulating calcitriol levels in normal fetuses.

Despite widespread expression of the VDR in the early embryo and

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later in many different fetal tissues, evidence suggests that vitamin D, calcitriol, and VDR are not required for skeletal development or mineralization prior to birth, as demonstrated in experimental animal studies and human observations.

Experimental animal studies have examined vitamin D deficiency as well as the genetic absence of the VDR (*Vdr*-null mice). In *Vdr*-null fetuses, the rate of placental calcium transfer rates and the expression of the calcium transient receptor potential cation channel, vanilloid family member 6 (TRPV6), increase compared with normal littermates (Kovacs, 2005). Further, in multiple animal models fetuses and neonates have been shown to have normal calcium homeostasis—i.e., normal blood calcium, phosphorus, PTH, and skeletal mineral content—under conditions of severe vitamin D deficiency as shown in: rats (Halloran and DeLuca, 1979, 1980b, 1981; Halloran et al., 1979; Miller et al., 1983); pigs with a null mutation of the 1α-hydroxylase (Lachenmaier-Currle and Harmeyer, 1989); 1α-hydroxylase-null mice (Dardenne et al., 2001; Panda et al., 2001); and *Vdr*-null mice (Li et al., 1997, 1998; Kovacs et al., 2005; Fudge and Kovacs, 2010). In contrast to the fetus, the mothers in these models exhibit severe hypocalcemia, hypophosphatemia, and osteomalacia.

Further, serum calcium levels and skeletal mineral content remain normal for the first 2 to 3 weeks after birth in vitamin D–deficient rats and *Vdr*-null mice (Kovacs et al., 2005). After weaning, the deficient and *Vdr*-null animals develop progressive hypocalcemia, hypophosphatemia, and histomorphometric evidence of rickets, not seen in normal or heterozygous littermates. This situation parallels the maturation of calcium absorption in the intestine, which changes from a non-saturable, passive process in the newborn to an active, saturable, calcitriol-dependent process in the rat (Ghishan et al., 1980, 1984; Halloran and DeLuca, 1980c). It is also consistent with observational evidence from human studies, as discussed below. It should be noted that the guinea pig stands in contrast to other animal models. Vitamin D deficiency in pregnant guinea pigs reduces fetal whole-body BMC, but not BMD (Rummens et al., 2002; Finch et al., 2010). It also appears to reduce guinea pig weight at birth (Rummens et al., 2002; Finch et al., 2010).

The information gleaned from these studies highlights the importance of calcitriol in the regulation of intestinal calcium absorption and the facilitation of skeletal mineralization in the weaned young, adolescent, and adult, but not in the fetus or early neonate. Collectively, the physiological data from several different animal models indicate that fetal calcium homeostasis and skeletal development/mineralization are regulated independently of vitamin D, calcitriol and its receptor. Heterozygous and null fetuses of *Vdr*-null mothers were, however, smaller and weighed less despite

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maintaining normal blood calcium levels and normal mineral content for their proportionately smaller skeletons (Kovacs et al., 2005).

Evidence from humans is mixed on whether the movement of calcium into the fetal body requires calcitriol (Specker, 2004). Both RCTs and observational evidence suggest that calcium transfer and fetal skeletal outcomes are not affected by vitamin D deficiency (Kovacs, 2008). Brooke et al. (1980) reported on an RCT of 126 women in which babies born of placebo-treated mothers had a mean serum 25OHD level of 10 nmol/L and there were no radiographic signs of rickets. Delvin et al. (1986), reporting on an RCT, found that maternal vitamin D supplementation had no effect on cord blood calcium level but resulted in higher 25OHD levels in both the maternal and cord blood. An observational study found no relationship between maternal 25OHD level and whole-body BMC and BMD and a positive relationship of gestational age and birth weight (Akcakus et al., 2006). Another observational study (Congdon et al., 1983) reported that maternal vitamin D supplementation did not affect neonatal BMC for offspring from Asian women with very low serum 25OHD levels.

In human babies lacking the 1α-hydroxylase (VDDR I) or lacking the VDR (VDDR II or hereditary vitamin D–resistant rickets) normal skeletons and blood calcium at birth have been reported (Silver et al., 1985; Takeda et al., 1997; Teotia and Teotia, 1997; Kitanaka et al., 1998; Bouillon et al., 2006). Regarding rickets, observational studies of babies born of severely vitamin D–deficient mothers generally show normal skeletal mineral content with no radiological evidence of rickets at birth (Maxwell and Miles, 1925), followed by the development of hypocalcemia or rickets only in postnatal weeks to months (Pereira and Zucker, 1986; Campbell and Fleischman, 1988; Specker, 1994; Beck-Nielsen et al., 2009). Although some isolated reports have indicated the presence of congenital rickets at birth, the diagnosis was actually made within the first or second week (Begum et al., 1968; Ford et al., 1973; Moncrieff and Fadahunsi, 1974; Sann et al., 1976; Park et al., 1987; Teotia et al., 1995). Radiographic findings have reported rickets present at day 15 but not day 2 (Sann et al., 1976). In many cases, the cause was not isolated vitamin D deficiency, but malnutrition, malabsorption (e.g., celiac disease, pancreatic insufficiency), or very low maternal intakes of both calcium and vitamin D (Begum et al., 1968; Teotia et al., 1995; Innes et al., 2002). In a study in China, neonatal rickets was not found, even though 57 percent of the infants had low cord blood 25OHD levels (Specker et al., 1992).

Overall, data are not consistent regarding skeletal development, in that several observational studies have found an adverse effect of low maternal vitamin D on fetal skeletal outcomes. An ecological study reported a positive association of imputed UVB exposure during the last trimester with some neonatal bone outcomes (Sayers and Tobias, 2009). Another

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study in Korean infants born in winter reported seasonal low neonatal total BMC and maternal 25OHD levels (Namgung et al., 1998). Lower levels of maternal and neonatal serum 25OHD were found for infants with craniotabes (softening of the skull bones along suture lines) compared with those without (Reif et al., 1988). Another study reported only a small non-significant reduced knee–heel length at birth in neonates whose mothers had a serum 25OHD level below 28 nmol/L (Morley et al., 2006). Recently, Mahon et al. (2010) identified an inverse relationship of femur metaphyseal cross–sectional area and splaying index with maternal 25OHD levels at 34 weeks, using high-resolution three-dimensional ultrasound at the 19th and 34th weeks of gestation. Viljakainen et al. (2010), based on a study of 124 mothers and their infants, reported impaired fetal neonatal tibia BMC and cross–sectional area, but not BMD, when maternal 25OHD levels were below the median of 42.6 nmol/L. It is noted that Mahon et al. (2010) concluded that the higher cross–sectional area was evidence of prenatal rachitic deformity, while Viljakainen et al. (2010) considered the higher cross–sectional area to predict higher bone mass in childhood. It is unclear whether the investigators in these various studies examined multiple skeletal measurements in multiple long bones, and these differences may explain some of the differences in the reports.

Regarding so-called developmental programming, recent associational studies have suggested possible adverse programming (including skeletal and selected immunological outcomes) in offspring of mothers with low maternal serum 25OHD levels (Arden et al., 2002; Cooper et al., 2005; Javaid et al., 2006; Miyake et al., 2009; Nwaru et al., 2010) as well as high maternal serum 25OHD levels (Gale et al., 2008). However, a recent observational study reported no association of maternal 25OHD levels with autoimmunity or type 1 diabetes (Marjamaki et al., 2010). Skeletal parameters at birth and nine months were normal but BMC determined later in childhood were reported to be higher in offspring of women with higher serum 25OHD levels during pregnancy compared with those with the lowest serum 25OHD levels (Javaid et al., 2006). The interpretation of these associational studies may be confounded by factors that are associated with maternal serum 25OHD level during pregnancy and affect fetal growth, such as increased maternal weight, lower socioeconomic status, and poorer nutrition. These factors may also be conferred on the offspring during childhood development, complicating the ability to establish a causal relationship.

Overall, the human and experimental animal data indicate that the development and mineralization of the fetal skeleton, as well as fetal blood calcium and phosphorus levels, are generally normal despite extremes of severe vitamin D deficiency, absence of calcitriol, and absence of the VDR. In contrast, the data confirm that vitamin D deficiency that is present at

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birth and left uncorrected will more readily lead to neonatal hypocalcemia and the postnatal development of rickets.

Lactation: Calcium Key physiological changes in the female adolescent or adult occur to meet the calcium demands of lactation that are higher than those in pregnancy, but the adaptations differ from those that occur during pregnancy (Kovacs and Kronenberg, 1997, 2008; Kalkwarf, 1999; Prentice, 2003). Maternal bone resorption is markedly up-regulated (Specker et al., 1994; Kalkwarf et al., 1997), and it appears that most of the calcium present in milk derives from the maternal skeleton. This bone resorption is driven by low estradiol and high plasma PTH-related protein (PTHrP) levels (and possibly other factors), which act through osteoblasts to up-regulate osteoclast number and activity. Maternal BMD can decline 10 to 45 percent during 2 to 6 months of exclusive breastfeeding, but it normally returns to baseline over the succeeding 6 to 12 months post-weaning (Kalkwarf, 1999).

The effect of dietary intake of calcium on the skeletal resorption that occurs during lactation has been examined through randomized trials and in observational studies comparing North American and Gambian women. The consistent finding is that calcium intakes ranging from very low (< 500 mg/day) to supplemented well above normal (1.0 to 2.5 g/day) have no effect on the degree of skeletal demineralization that occurs during lactation, but calcium supplementation does increase urinary calcium excretion (Cross et al., 1995; Fairweather-Tait et al., 1995; Prentice et al., 1995; Kalkwarf et al., 1997; Laskey et al., 1998; Polatti et al., 1999).

The effect of calcium intake on skeletal recovery after weaning has not been rigorously studied. In one RCT that enrolled 95 lactating women prior to weaning, use of a 1 g/day calcium supplement resulted in a 5.9 percent increase in lumbar spine BMD compared with a 4.4 percent increase in women who took a placebo, as well as a 2.5 percent increase in non-lactating women compared with a 1.6 percent increase in women who took a placebo (Kalkwarf et al., 1997). These studies suggest that a higher calcium intake during post-weaning recovery might be beneficial for ensuring restoration of skeletal mineral content; conversely, a low calcium intake during post-weaning might be expected to impair skeletal recovery. However, skeletal recovery was complete in Gambian women with habitually very low calcium intakes. Moreover, the large associational studies mentioned above found no effect (and some found a protective effect) of a history of lactation or the number of months that a mother recalled breastfeeding her child on BMD, osteoporosis, or fracture risk later in life (Sowers, 1996; Kovacs and Kronenberg, 1997). Thus, in the long term, a history of lactation does not increase the risk of low BMD or osteoporosis.

The efficiency of intestinal calcium absorption, which is up-regulated

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during pregnancy, decreases to the non-pregnant level in the puerperium, and remains at that level during lactation (Kent et al., 1991; Specker et al., 1994; O'Brien et al., 2006), then increases slightly during post-weaning compared with the level in non-pregnant or lactating women (Kalkwarf et al., 1996). Urinary calcium excretion also decreases (Allen et al., 1991; O'Brien et al., 2006) and may reach the lower end of the normal range, especially in women with low calcium intakes (Specker et al., 1994). This effect presumably is due to the influence of PTHrP, which stimulates renal calcium reabsorption.

Breast milk calcium content is homeostatically regulated and unaffected by maternal calcium intake. The evidence includes randomized trials in which supplemental calcium from 1 g/day (Kalkwarf et al., 1997) to 1.5 g/day in Gambian women whose habitual calcium intake was low (Jarjou et al., 2006) showed no effect on breast milk calcium content. These results are consistent with the notion that the calcium content of milk derives from resorption of the maternal skeleton and local regulation within mammary tissue. At least one study has confirmed that the breast milk output predicts the decline in maternal BMD during lactation, whereas calcium intake, breast milk calcium concentration, and VDR genotype have no effect (Laskey et al., 1998).

In lactating women, the albumin-corrected serum calcium as well as the ionized calcium levels are normal or slightly increased (Hillman et al., 1981; Specker et al., 1991). The mean ionized calcium level of exclusively lactating women is higher than that of normal controls (Dobnig et al., 1995; Kovacs and Chik, 1995). Also, mothers nursing twins have significantly higher total calcium levels compared with mothers nursing singletons (Greer et al., 1984).

These physiological responses appear to be similar for lactating adolescents. In fact, the largest and most reassuring data set from NHANES III (described previously), which obtained BMD using the DXA method in 819 women ages 20 to 25 years (Chantry et al., 2004) indicates that young women who had breast-fed as adolescents have higher BMD than those who had not breast-fed, even after controlling for obstetrical variables. This indicates that the normal loss of BMD during lactation and recovery afterward occur in adolescent women and may even lead to a higher BMD post-weaning.

Lactation: Vitamin D Breast milk is not normally a significant source of vitamin D for the infant. Because vitamin D (calciferol) is usually present in the circulation only for short intervals after meals, typically very little passes into breast milk. As discussed below, preliminary data may suggest that levels of vitamin D and 25OHD in breast milk can be increased by high

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levels of vitamin D supplementation. Neither 25OHD nor calcitriol passes readily into breast milk.

With respect to the effects of vitamin D supplementation on serum levels of 25OHD in the infant, several studies have examined supplementation of infants with 300 or 400 IU of vitamin D per day, which raised levels above 75 nmol/L (Hollis and Wagner, 2004; Basile et al., 2006; Wagner et al., 2006); however, administering supplements of 300 to 2,000 IU/day to the lactating mother did not increase serum levels of the infant (Greer et al., 1982; Rothberg et al., 1982; Ala-Houhala, 1985; Ala-Houhala et al., 1988; Greer and Marshall, 1989; Hollis and Wagner, 2004). However, very high doses of vitamin D (4,000 to 6,400 IU/day) given to the mother have been reported to raise infant serum 25OHD levels (Hollis and Wagner, 2004; Wagner et al., 2006). As described by the authors, the work was a pilot study and involved 19 subjects. Specifically, when 4,000 IU of vitamin D per day was given to the mothers, the mean serum 25OHD level of the infants exceeded 75 nmol/L; with a dose of 6,400 IU/day, the serum 25OHD level of all infants exceeded this value. However, the functional impact of raising infants' serum 25OHD levels above 75 nmol/L by increasing maternal dietary intake to such high levels is not clear, and the small sample size of this pilot study (n = 19) precluded conclusions about safety. One RCT found no benefit in raising infants' serum 25OHD level above 50 nmol/L relative to measures of weight, length, and skeletal mineral content (Chan et al., 1982). Other work with the administration of high dosages of vitamin D to the mother has not specifically reported any functional health outcome to the breast-fed infant other than increased serum 25OHD levels; the breast milk calcium content is unaffected (Hollis and Wagner, 2004; Wagner et al., 2006). The administration of 400 IU/day to the infant remains the American Academy of Pediatrics' recommendation (Wagner and Greer, 2008).

Serum 25OHD levels do not appear to change significantly during lactation compared with non-lactating states, although this has been assessed in only two small studies (Kent et al., 1990; Sowers et al., 1998). Because 25OHD does not pass readily into milk, it is not lost to the mother via this route. One study (Cross et al., 1995) reported an increase in maternal serum 25OHD level post-weaning, and although calcitriol levels increased in two studies (Cross et al., 1995; Kalkwarf et al., 1997), they did not in another (Specker et al., 1991). Studies have generally shown that providing vitamin D to lactating mothers increased their serum 25OHD levels, but otherwise had no significant effect on maternal outcome parameters (Cancela et al., 1986; Okonofua et al., 1987; Takeuchi et al., 1989; Kent et al., 1990; Alfaham et al., 1995; Sowers et al., 1998) and in clinical trials (Rothberg et al., 1982; Ala-Houhala, 1985; Ala-Houhala et al., 1988; Kalkwarf et al., 1996; Hollis and Wagner, 2004; Basile et al., 2006; Wagner et al., 2006; Saadi et al., 2007).

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An observational study in Gambian women consuming a low-calcium diet reported no relationship between maternal 25OHD levels and breast milk calcium (Prentice et al., 1997). However, many studies measured no outcome other than the achieved serum 25OHD level in mothers and neonates and were not powered to examine outcomes such as hypocalcemia or clinical rickets (Rothberg et al., 1982; Ala-Houhala, 1985; Hollis and Wagner, 2004; Basile et al., 2006; Wagner et al., 2006).

Maternal skeleton recovers BMC after lactation ceases, but no RCTs have tested whether vitamin D sufficiency affects the speed and net recovery of maternal skeletal mineral content after weaning. An observational study in Saudi women found no relationship of serum 25OHD level with BMD at the lumbar spine, femoral neck, Ward's triangle, or trochanter, as well as no difference in BMD at these sites in women with or without severe hypovitaminosis D (Ghannam et al., 1999). None of the intervention studies that examined the use of vitamin D supplementation during lactation enrolled sufficient adolescent women to permit conclusions to be drawn about the effect of the intervention.

As noted above, animal models are of interest; in the case of vitamin D, rodent models have predominated. Unlike the situation in humans, calcitriol levels in lactating rodents remain elevated, but increase further in response to a low-calcium diet or larger litter size (Lobaugh et al., 1990, 1992). This may indicate a compensatory mechanism that increases intestinal calcium absorption even further when extra demands are placed on the mother during lactation. However, studies in vitamin D–deficient rats and *Vdr*-null mice have indicated that sufficiency of vitamin D, or responsiveness to calcitriol is not required for lactation. Vitamin D–deficient rats and *Vdr*-null mice lactated normally and resorbed the expected proportion of bone (Halloran and DeLuca, 1980b; Miller et al., 1982; Fudge and Kovacs, 2010), although one study in vitamin D–deficient rats found that more skeletal mineral content was lost than normal (Marie et al., 1986). Intestinal calcium absorption was twice the control level in lactating vitamin D–deficient rats, confirming that vitamin D is not required for the intestinal adaptation to take place (Halloran and DeLuca, 1980a; Boass et al., 1981).

In rodents, the skeleton is substantially resorbed during lactation, and this is followed in the post-lactation period by an interval of up-regulated bone formation, which effectively restores BMD to a normal level within 10 to 14 days. Two studies of vitamin D–deficient rats reported at least partial recovery of skeletal mineral content after lactation, with the final value exceeding the pre-pregnancy value in one study (Halloran and DeLuca, 1980b; Miller et al., 1982). Likewise, in *Vdr*-null mice, BMC after weaning also exceeded the pre-pregnancy level (Fudge et al., 2006). Thus, these animal studies suggest that calcitriol may not be required for the skeleton to recover its normal mineral content after lactation is completed.

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In both *Vdr*-null and 1α-hydroxylase-null mice, the provision of a high-calcium, high-phosphorus, lactose-enriched diet, initiated in the neonates prior to weaning, prevented the development of rickets in the adult (Li et al., 1998; Amling et al., 1999; Van Cromphaut et al., 2001; Hoenderop et al., 2002; Dardenne et al., 2003; Rowling et al., 2007). Similar outcomes have been reported for children with VDDR-I or VDDRI-II in which rickets is mitigated with high levels of oral calcium or intermittent intravenous infusions of calcium (Balsan et al., 1986; Hochberg et al., 1992; Kitanaka et al., 1998). These results indicate that the main role of calcitriol is to stimulate active intestinal calcium absorption rather than to directly affect skeletal development. Moreover, it suggests that the role of calcitriol can be bypassed if the calcium content of the experimental diet is suitably manipulated.

Other Measures of Interest Related to Bone Health: PTH

PTH is potentially of interest as an indicator of bone health because vitamin D intake can lower serum PTH levels (Malabanan et al., 1998) and because elevated serum PTH levels have been recognized as a risk factor for osteoporosis (Hodsman et al., 2002). The role of PTH in the calcium–vitamin D homeostatic system is highlighted in Chapter 2. A critical question is what levels of PTH are harmful to bone, as only a small amount of PTH is needed to maintain a normal level of serum 25OHD.

Measures that have been explored are the levels of serum 25OHD at which PTH levels rise as well as the level of serum 25OHD at which PTH levels no longer decline (Aloia et al., 2006a; Durazo-Arvizu et al., 2010). However, because serum PTH levels increase with age, it is not clear what level of PTH should be regarded as normal (Dawson-Hughes et al., 1997a; Vieth et al., 2003) or whether the relationship is meaningful for all age groups (Abrams et al., 2005). These studies have led some to suggest that a serum 25OHD level of 75 nmol/L is consistent with the PTH plateau point (Malabanan et al., 1998) and hence demarcates sufficiency and insufficiency for vitamin D. However, a review of the literature does not show widespread agreement on a plateau consistent with a serum 25OHD level of 75 nmol/L. In most cases, serum PTH level reaches a plateau at different levels of serum 25OHD varying between 37.5 and 125.0 nmol/L. Box 4-7 summarizes the study outcomes.

Race/ethnicity may be a factor in determining the relationship between serum 25OHD and PTH levels, although the measures used have focused on calcitriol rather than serum 25OHD concentrations. African American and dark-skinned populations have lower serum 25OHD and calcitriol levels compared with white populations (Bell, 1995, 1997). A study of more than 500 healthy women ages 20 to 80 years found that PTH

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BOX 4-7
Studies Demonstrating PTH Plateaus at Various Serum 25OHD Levels

Serum 25OHD < 30 nmol/L:
- Ooms et al. (1995a)

Serum 25OHD < 50 nmol/L:
- Malabanan et al. (1998)
- Levis et al. (2005)
- Steingrimsdottir et al. (2005)
- Aloia et al. (2006a)

Serum 25OHD < 75 nmol/L:
- Vieth et al. (2003)
- Holick et al. (2005)
- Durazo-Arvizu et al. (2010)

Serum 25OHD ~ 88 nmol/L:
- Kinyamu et al. (1998)

Serum 25OHD 100–125 nmol/L:
- Krall et al. (1989)
- Dawson-Hughes et al. (1997a)

No plateau:
- Bates et al. (2003)
- Benjamin et al. (2009)

No relationship:
- Rucker et al. (2002)

and calcitriol levels were higher in black than in white women and that the black women had lower bone turnover rates compared with white women (Aloia et al., 1996b). Some evidence, however, suggests that PTH levels are similar in both populations (Benjamin et al., 2009).

As reviewed by Prentice et al. (2008), an inverse relationship between PTH and serum 25OHD concentrations has been reported in many cross–sectional and intervention studies in elderly people (Krall et al., 1989; Ooms et al., 1995a; Chapuy et al., 1997; Bates et al., 2003), post menopausal women (Krall et al., 1989; Lappe et al., 2006), and young persons (Guillemant et al., 1999). Some studies suggest that the plasma PTH concentration reaches a plateau as the 25OHD concentration increases

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(Chapuy et al., 1997; Lappe et al., 2006), whereas others describe an exponential inverse relationship (linear when the data are expressed in logarithms) throughout the physiological range of 25OHD concentrations (Bates et al., 2003; Vieth et al., 2003). The reasons for these discrepancies are unclear, but they could reflect differences among the populations studied and statistical methods used (Prentice, 2008).

Further, the plasma PTH concentration varies widely within and among individuals at any given concentration of 25OHD (Chapuy et al., 1997; Bates et al., 2003), because the plasma PTH concentration depends upon many factors other than vitamin D, such as stage of life, ethnic background, intakes of dietary calcium (Steingrimsdottir et al., 2005) and phosphorus, time of day, kidney function (Ooms et al., 1995a), physical activity level, and drug use (Slovik et al., 1981; van der Wiel et al., 1991; Vieth et al., 2003; Fraser et al., 2004; Patel et al., 2007; Prentice, 2008). In addition, the choice of assay method is important because an assay could detect both PTH fragments and intact molecules (van der Wiel et al., 1991).

Most of the studies supporting the use of serum PTH level as either a biomarker of exposure or a biomarker of biological effect have been conducted among older white persons living in Europe and the United States. However, the available studies in other age groups and in people from different geographic locations and ethnic backgrounds do not provide evidence that PTH measures can be universally applied relative to information about vitamin D intakes and effects. Studies in Africa and China, for example, have reported that plasma PTH measures are elevated in populations with low calcium intake, even when vitamin D nutriture is good, and the inverse correlations between plasma PTH measures and bone health outcomes such as BMD and fracture risk observed in Western countries are not found (Yan et al., 2003; Aspray et al., 2005). Also, PTH measures increase during puberty (Abrams et al., 2005; Tylavsky et al., 2005), a period of skeletal growth. Therefore, although the potential for PTH as a useful indicator of bone health is acknowledged, it is not a useful indicator for DRI development at this point in time.

Integration of Evidence for the Potential Indicator of Bone Health

To be useful for judging bone health outcomes as potential indicators for DRI development, the available evidence must be considered in the context of its relevance to bone accretion, bone maintenance, and bone loss. The committee therefore arranged the data consistent with these physiological states. Although the AHRQ analyses were useful overall for this purpose, the committee recognized that there were useful studies published after the completion of the AHRQ-Tufts as well as several relevant studies that did not meet the inclusion criteria stipulated by the

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AHRQ analyses. These are included and identified in the discussions below. The following sections integrate data on bone accretion, maintenance, and loss and refer to the DRI life stage groups as appropriate. Initially, the utility of serum 25OHD level for the purposes of DRI development is discussed, as is the relationship between calcium absorption and serum 25OHD concentrations.

Utility of serum 25OHD level for examining bone health outcomes Although serum 25OHD is indicative of vitamin D exposure (see Chapter 3), the seemingly logical next step of using serum 25OHD level to explore the levels at which vitamin D effects a health outcome requires caution. There are a number of studies of good quality that report serum 25OHD concentrations in relation to health outcomes such as fracture incidence—and which have been described in the AHRQ analyses—but such associations are not necessarily causal or predictive, and not all of the variables associated with serum 25OHD levels are reported. In short, these associations are not yet an adequate basis for validating serum 25OHD concentrations as a "biomarker of effect" (see Chapter 1) for either an intermediate health endpoint (e.g., blood pressure) or disease (e.g., rickets or osteoporosis).

Others have also concluded that the usefulness of serum 25OHD level as an indicator of functional outcomes has not been demonstrated in many cases, with the possible exception of elderly people (Brannon et al., 2008). Further, Brannon et al. (2008) pointed out that the value of the measure appears to be most useful at the extremes of the range for detecting deficiency and toxicity, but may be less useful in the middle range and subject to confounders. Additionally, it has not been ruled out that vitamin D may act to produce health outcomes in a manner that is separate from circulating 25OHD levels and thereby function in a pathway different from that known for 25OHD. Currently there is no evidence confirming pathways not involving 25OHD, although their existence is plausible.

Nonetheless, serum 25OHD concentration is a useful measure for several reasons, not the least of which is that 25OHD has a long half-life in the circulation and its concentration is not under tight homeostatic control. It generally demonstrates a direct relationship with "exposure" or "supply" (i.e., dietary intake and cutaneous synthesis), although, as discussed in Chapter 3, the relationship is known to vary with factors ranging from body adiposity to aging and is also known to be curvilinear, with decreased response as intakes increase. Despite these caveats, serum 25OHD concentration is a useful biomarker of the supply of vitamin D available to target tissues in most situations (Prentice et al., 2008). It therefore is relevant as a stand-in for overall vitamin D nutriture, although distinguishing between

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concentrations due to intake and those due to sun exposure is not possible for most studies.

The utility of serum 25OHD level as a biomarker of effect is less certain. Prentice et al. (2008) pointed out that the adequacy of the vitamin D supply in meeting functional requirements depends upon many factors, including the uptake of 25OHD by target cells, the rate of conversion of calcitriol and its delivery to target tissues, the expression and affinity of the VDR in target tissues, the responsiveness of cells to the activated VDR, and the efficiency of induced metabolic pathways.

Nonetheless, despite these uncertainties, serum 25OHD levels can be regarded as a useful tool in considering vitamin D requirements; in fact, such measures are virtually the only tool available at this time. As pointed out by AHRQ-Tufts, when a non-validated intermediate outcome must be considered, the implicit assumption is that it would have the properties of a validated surrogate outcome, and this assumption should be made explicit and the uncertainties identified. This is a reasonable approach and allows the appropriate inclusion of consideration of serum 25OHD concentrations for the purposes of specifying the potential indicator of bone health.

Relationship between calcium absorption and serum 25OHD level Ensuring desirable rates of calcium uptake from the intestinal lumen into the body—calcium absorption—is an important aspect of bone health. Because vitamin D is instrumental in calcium absorption, the relationship between vitamin D and calcium absorption is relevant to an indicator for bone health. The literature in this area focuses on fractional calcium absorption (i.e., fraction of a given dose of calcium that is absorbed) and its association with serum 25OHD level.

Although calcitriol has been shown to stimulate intestinal calcium absorption directly and calcitriol levels correlate with absorption, the understanding of the current relationship between 25OHD level and calcium absorption requires examination. Widely quoted as evidence of the threshold for maximal calcium absorption at serum 25OHD levels above 75 nmol/L is an analysis of results from three separate studies (Barger-Lux and Heaney, 2002; Bischoff et al., 2003; Heaney et al., 2003) as put forward by Heaney (2005). Less widely understood is the nature of the evidence from each of these studies and, thus, the limitations of this graphic analysis. In only one of these studies (Barger-Lux and Heaney, 2002) was calcium absorption directly measured using a single calcium isotope method; the difference between the lower (approximately 75 nmol/L) and higher (approximately 125 nmol/L) serum 25OHD levels was non-significant. Although the single-isotope method is considered less accurate than the dual-isotope method for measuring calcium absorption, this method is viewed as appropriate. The two values from the Barger-Lux and Heaney

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(2002) study are the most reliable of the values in this analysis. Two additional values taken from Heaney et al. (2003) in this graphic analysis (at approximately 50 and 85 nmol/L) are not direct measures of calcium absorption, but instead are indirect pharmacokinetic measures based on the plasma calcium response to a 500 mg oral calcium load (Heaney et al., 2003). Thus, the committee found these values limited in their usefulness in this analysis. The remaining 25OHD level of a calcium absorption of 0.15 at serum 25OHD 29 nmol/L was taken from Bischoff et al. (2003). It does not represent either a direct or indirect measurement of calcium absorption, but was derived from measured urinary calcium excretion using subjects that did not reduce serum PTH while on calcium supplements (Heaney, 2005; personal communication, R. P. Heaney, Creighton University, Omaha, NE, August 25, 2009). This approach is not generally acceptable, and the committee could not consider the value to be valid. In conclusion, the portion of this analysis showing a rise in calcium absorption with an increase in 25OHD level from approximately 28 nmol/L to 80 or 90 nmol/L is unreliable, because the two values showing this rise either are not based on directly measured calcium absorption or are based on an unreliable method for estimating calcium absorption as discussed by Aloia et al. (2010) and as described below. The remaining two values, although reliable, are insufficient to determine the relationship of 25OHD level to calcium absorption, if any exists.

The gold standard for assessing fractional calcium absorption is to administer two calcium isotopes (one orally, one intravenously) under conditions in which blood and/or urine can be collected and assayed for both isotopes. Alternatively, calcium absorption can be assessed using a single isotope test, although results may be less precise. As discussed below, the data from studies published after the Heaney (2005) paper either do not show increased calcium absorption with higher levels of 25OHD or show only a very slight increase in calcium absorption as serum 25OHD level rises.

With respect to children, Abrams et al. (2009) performed dual-label calcium absorption studies in 251 children ranging from 4.9 to 16.7 years of age and found no effect of higher serum 25OHD level on fractional calcium absorption. In fact, children with 25OHD levels of 28 to 50 nmol/L had higher fractional calcium absorption than did children with 25OHD levels of 50 to 80 or greater than 80 nmol/L. Data from a 2008 study in girls (Weaver et al., 2008) indicated that serum 25OHD level did not predict net calcium absorption and retention. A study conducted in Nigeria (Thacher et al., 2009) demonstrated that in children with rickets, increases in serum 25OHD level did not coincide with increased fractional calcium absorption.

With respect to adults, there are a number of single-isotope studies of

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interest. Need et al. (2008) studied fractional calcium absorption in 319 men (66 ± 10 years) with serum 25OHD levels less than 40 nmol/L. Fractional calcium absorption was 0.36 in men with the lowest quartile serum 25OHD levels (< 10 nmol/L) and rose significantly to 0.56 in the second quartile (11 to 20 nmol/L). No further change in fractional calcium absorption occurred with 25OHD levels of 21 to 30 or 31 to 40 nmol/L (Need et al., 2008). Kinyamu et al. (1998) performed a cross–sectional study of 376 healthy women (71 ± 4 years) and compared calcium absorption in those who took vitamin D supplements with that in non-supplemented women. Serum 25OHD level was significantly higher in women who took vitamin D (87.9 ± 28.2 nmol/L vs. 73.6 ± 23.0 nmol/L), whereas fractional calcium absorption did not differ between the two groups (Kinyamu et al., 1998). In addition, Devine et al. (2002) used a single isotope of calcium in a study of 120 older women and plotted a linear relationship between intestinal calcium absorption and serum 25OHD level. Intestinal calcium absorption rose from 35 percent at a mean serum 25OHD level of 15 nmol/L to 50 percent at 150 nmol/L. However, there were few data points at any serum 25OHD level, and an alternative fit to the data suggested an increase to 50 nmol/L and a plateau thereafter.

Hansen et al. (2008) studied 18 postmenopausal women before and after 15 days of supplementation with 50,000 IU of vitamin D_2 daily. Serum 25OHD level rose markedly from 55 ± 10 nmol/L to 160 ± 53 nmol/L ($p < 0.001$), while fractional calcium absorption changed only modestly from 24 ± 7 percent at baseline to 27 ± 6 percent after vitamin D repletion ($p < 0.04$), indicating that the large rise in serum 25OHD levels was statistically significant, as was the small rise in intestinal calcium absorption. The 3 percent absolute increase in fractional calcium absorption was considered by the authors to be a minor increment given the large increase in serum 25OHD level (Hansen et al., 2008).

In a randomized controlled study, postmenopausal women with a mean baseline serum 25OHD level of 44 nmol/L receiving 1,000 mg of calcium citrate daily were randomized to daily placebo or 1,000 IU of vitamin D_2 (Zhu et al., 2008a). Using a single-isotope method, this research group found a 36 nmol/L rise in serum 25OHD level and no increase in calcium absorption. This outcome is consistent with findings from their longer-term study (over 5 years), also demonstrating no rise in absorption (Zhu et al., 2008a). In both placebo and vitamin D groups, the calcium absorption decreased compared with baseline, most likely as a result of greater calcium intake (1 g calcium supplementation in both treatment arms). Also, Francis et al. (1996) found that 500 to 1,000 IU of vitamin D_2 did not increase calcium absorption in elderly women. In a randomized double-blind controlled pilot trial in women (mean age = 57 years; dual-isotope method) with a baseline serum 25OHD level of 52 nmol/L, it was found that 400 IU

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of vitamin D per day raised serum 25OHD level (by 14 nmol/L) and did not significantly increase calcium absorption compared with placebo.[4]

In a recent study, Aloia et al. (2010) performed a single-isotope assay of intestinal calcium absorption in 492 white and black women ages 20 to 80 years. They tested whether serum 25OHD or calcitriol level predicted the rate of intestinal calcium absorption in a multivariate model that included age, menopausal status, calcium intake, and other factors. The serum 25OHD levels ranged from 30 to 150 nmol/L, and were 51.62 ± 33.67 nmol/L overall or 32.87 ± 21.20 nmol/L in blacks and 67.73 ± 34.11 nmol/L in whites. Whereas calcitriol level was an important predictor of intestinal calcium absorption in the final model, 25OHD level had no effect. The authors concluded that serum 25OHD level is not an indicator of intestinal calcium absorption efficiency by itself, but 25OHD does interact at low levels with calcitriol to predict calcium absorption.

Overall, the data are mixed, but most studies show no increase in intestinal calcium absorption across a broad range of serum 25OHD levels. The single-isotope study by Need et al. (2008) indicates no increase in fractional calcium absorption above 20 nmol/L. The single-isotope studies by Heaney et al. (2003), Kinyamu et al. (1998), and Aloia et al. (2010) indicate no change in fractional calcium absorption across higher ranges of 25OHD levels—specifically, from 60 to 154 nmol/L in Heaney et al. (2003), from 50 to 116 nmol/L in Kinyamu et al. (1998), and from 30 to 150 nmol/L in Aloia et al. (2010). Others (Francis et al., 1996; Patel et al., 2001; Zhu et al., 2008a, b) demonstrate no effect on absorption of increasing the serum 25OHD concentrations by 14 to 36 nmol/L, whereas the Hansen et al. (2008) study indicates a 3 percent increase in absorption after raising the serum 25OHD level from 55 to 160 nmol/L in the short-term (15 days).

The data currently suggest that fractional calcium absorption reaches a maximum between 30 and 50 nmol/L in both children and adults. A value of 50 nmol/L allows for some uncertainty in the data and a buffer against seasonal and dietary variations in calciferol intake that, in turn, cause fluctuations in serum 25OHD levels.

Bone accretion Bone accretion resulting in bone growth and skeletal development occurs during the younger life stages. Measures of the amount of calcium needed to achieve normal bone accretion as well as the levels of vitamin D that support accretion are, therefore, relevant considerations. The topics of pregnancy and lactation among adolescent girls, who are still accruing bone tissue, are discussed in other sections below jointly with pregnancy and lactation among women.

[4]Personal communication, S. Shapses, Rutgers University, New Brunswick, NJ, April 10, 2010.

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Calcium retention levels Total body calcium at birth in healthy, full-term infants is approximately 30 g (Givens and Macy, 1933; Widdowson et al., 1951). Based on bone mineral accretion derived as a function of change in body weight, total body calcium increases to approximately 80 g by 1 year of age (Leitch and Aitken, 1959). This suggests an average accretion rate of approximately 140 mg calcium per day during the first year of life. This greatly exceeds the earlier accretion rate estimates, derived from cadaveric sources, of approximately 30 to 35 mg/day and 50 to 55 mg/day for infants through 4 months of age and 4 through 12 months of age, respectively (Fomon and Nelson, 1993; Koo and Tsang, 1997). Yet another mean accretion rate of approximately 80 mg/day during the first year of life has been derived using metacarpal morphometry data (Garn, 1972; Weaver, 1994). Resolution of these different values for usual accretion rate is not currently possible, but assessment of these data and the balance data suggests that a mean accretion rate of about 100 mg/day overall during the first year of life may serve as a reasonable approximation for primarily breast-fed infants (Abrams, 2010).

Information about bone accretion in young children is limited given the impracticalities associated with studies of young subjects. Lynch et al. (2007), using an isotope-based method, evaluated the relationship between calcium intake and balance in healthy children 1 to 4 years of age. They reported mean calcium retention of 161 mg/day with a mean calcium intake of 551 mg/day, reflecting a positive calcium balance. Linear and non-linear modeling indicated that calcium intakes of 470 mg/day yielded a calcium retention of 140 mg/day, consistent with the growth needs of this population (Lynch et al., 2007).

For slightly older children in the 7- to 8-year age range, the work by Abrams et al. (1999) has also demonstrated that the average calcium accretion rate is 140 mg/day[5] calcium. A small increase is seen in late pre-puberty (Leitch and Aitken, 1959; Ellis et al., 1996), yielding a bone calcium accretion rate ranging from 140 to 160 mg/day across this age group, within which a small percentage will be pre-pubertal. Based on modeling, a curvilinear dose–response relationship between calcium intake and retention was made evident as shown in Figure 4-8.

A recent publication from Wu et al. (2010) that focused on Chinese American boys and girls 11 to 15 years of age reported calcium retention to be 1,100 mg/day in boys and 970 mg/day in girls, but these estimates were based on intakes to achieve maximal calcium retention as opposed to average calcium retention, the value needed to determine an EAR. A recent study of white children in Canada (Vatanparast et al., 2010) has provided bone calcium accretion levels for children and adolescents between the

[5]The 140 mg/day value is a modeled value as described in the study (Abrams et al., 1999).

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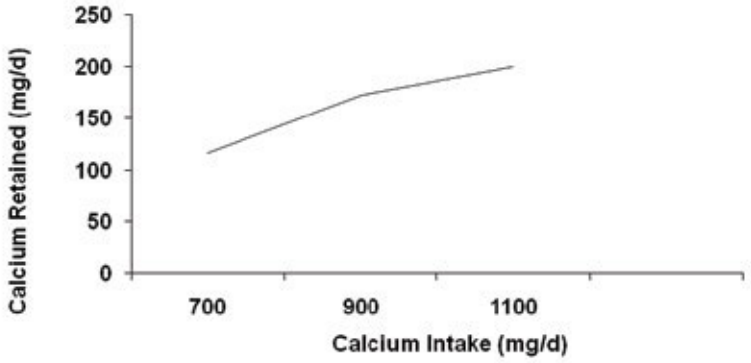


FIGURE 4-8 Dose–response relationship between calcium intake and retention.
SOURCES: Abrams et al. (1999); Ames et al. (1999).

ages of 9 and 18 years as shown in Table 4-14. The differences between girls and boys and between the 9- to 13-year and the 14- to 18-year age groups are small, but statistically significant. The data provide a basis for estimating intake levels needed for this age group relative to bone accretion.

Although it would be expected that bone maintenance is characteristic of young adults overall, there is some evidence of a small accretion of bone mass for persons in their 20s. The magnitude of this reported accretion varies. Specifically, Recker et al. (1992) followed 156 college-age women for 5 years and reported an increase of 12.4 percent per decade (about 1.24 percent per year) in whole-body BMC, but there were smaller increases in clinically relevant sites such as the forearm (4.8 percent per decade or 0.48 percent per year) and the lumbar spine (5.9 percent per decade or 0.59 percent per year). Further, the rate of increase declined each year for this group. The variance was also large in this study, which may be due to the method selected to assess BMC. Barger-Lux et al. (2005) more recently reported an accretion rate of 0.28 percent per year for women in the 20-

TABLE 4-14 Mean Bone Calcium Accretion for Three Age Groupings of Girls and Boys

	Mean Bone Calcium Accretion (mg/day)	
Age (years)	Girls	Boys
9–13	151	141
14–18	92	210
9–18	121	175

SOURCE: Vatanparast et al. (2010).

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to 30-year age range. This lower reported accretion rate is equivalent to a calcium accretion rate of only 6 mg/day. In addition to reporting only very small bone accretion for ages 19 through 30 years, Barger-Lux et al. (2005) also noted the possibility that there was no further effect on bone accretion above calcium intake levels of approximately 800 mg/day.

In short, bone accretion may continue during this early stage of adulthood, but at very low, almost indiscernible, levels. Interpretation of the data is further complicated by evidence from the Canadian Multicentre Osteoporosis Study (Berger et al., 2010), which demonstrates that attainment of peak bone mass depends upon which site is measured; peak bone mass is achieved by age 18 at some sites, but by age 25 or so at others. This newer population-based study is much larger than earlier studies, for example, Recker et al. (1992), and relies on the newer technique of DXA to estimate BMC. Tuck and Datta (2007) reported that maximal bone mass is attained in the second decade of life followed by a period of consolidation lasting 5 years, such that maximal levels are achieved in the early to mid 20s. Interestingly, peak trabecular bone mass is achieved earlier, at 15 to 18 years of age, is maintained for several years, and then begins to decline in young adulthood (Riggs et al., 2008).

Bone mineral content/bone mineral density: Calcium Measures of BMC and BMD in addition to calcium retention levels are also of interest as a measure of bone accretion. In a cross–sectional evaluation in 136 boys and men and 130 girls and women, including children beginning at the age of 4 years as well as adults through the age of 27 years, BMD of total body, lumbar spine, and femoral neck increased significantly with age until 17.5 years in boys and 15.8 years in girls (Lu et al., 1994). However, care must be taken in interpreting calcium intakes—specifically, calcium supplementation that results in total intakes above 1,500 mg/day for these groups—relative to BMC or BMD measures. Studies have suggested that increasing intakes of calcium in girls above their habitual intake of about 900 mg/day is associated with positive effects on bone mineral accretion and, in turn, BMD (Johnston et al., 1992; Lloyd et al., 1993; Chan et al., 1995). However, there is evidence that the bone mass gained through calcium or milk supplementation during childhood and adolescence is not retained post-intervention, suggesting that there is no benefit to intakes above that needed to ensure normal bone accretion (Fehily et al., 1992; Lee et al., 1996; Slemenda et al., 1997). A study conducted by Matkovic et al. (2004) evaluated BMD measures among female white adolescents 15 to 18 years of age in the United States, and reported that there was a positive influence of calcium supplementation and dairy products on BMD of the hip and forearm. The background level of calcium intake was approximately 833 mg/day, whereas the supplemented subjects had total calcium intakes of 1,586 mg/day. The Matkovic et al. (2004) study, however, did not follow

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the subjects after the intervention ceased in order to determine whether the bone mass was retained. Overall, it would appear that levels of calcium intake consistent with levels established as supportive of bone accretion are associated with a normal, healthy increase in BMD. However, calcium intake levels above those consistent with established bone accretion rates appear to offer no meaningful benefit.

Bone mineral content/bone mineral density: Vitamin D Regarding vitamin D nutriture and very young children, virtually no data are available to link vitamin D intake or serum 25OHD level to bone accretion measures. However, for older children and adolescents, as described above, there was *fair evidence* from the AHRQ-Ottawa analyses of an association between 25OHD levels and baseline BMD and change in BMD or BMC indexes based on observational data. However, the results from the RCTs, as described above, did not confirm a consistent benefit on BMD or BMC across skeletal sites and age groups. Reasons for these differences may be due to the difficulty in controlling confounding variables for bone mass in observational studies.

Rickets Although consideration of rickets provides only a starting point for considering nutrient reference values, AHRQ-Ottawa, as described above, analyzed serum 25OHD concentrations in the context of the onset of rickets in children up to 5 years of age. It identified serum concentrations below 27.5 nmol/L as consistently associated with rickets. However, many of the relevant studies were from developing countries where dietary calcium intake is low; therefore, for these studies the onset of rickets was associated with higher levels of serum 25OHD, likely due to low calcium intakes. Specker et al. (1992) concluded that serum 25OHD concentrations of below 27 to 30 nmol/L place the infant at an increased risk for developing rickets, although they indicated that the measure is not diagnostic of the disease. It is worth noting that there is very limited evidence of rickets due to calcium deficiency in the face of vitamin D sufficiency (Abrams, 2002). The minimum calcium intake needed to prevent calcium-deficiency rickets has not been precisely identified, and the available studies (all outside North America) reflect varying levels at which calcium-deficiency rickets occurred. Levels of intake between 200 and 300 mg of calcium per day in infants and small children have been associated with risk for rickets in these cases (Abrams, 2002).

Calcium absorption and serum 25OHD As described above, life stages that experience bone accretion demonstrate a maximal calcium absorption associated with serum 25OHD levels of at least 30 nmol/L and closer to 40 to 50 nmol/L. Fractional calcium absorption does not appear to increase with serum 25OHD concentrations above 50 nmol/L. In addition, rickets in populations that are not calcium deficient does not occur until serum 25OHD levels drop below 30 nmol/L.

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Summary of evidence for bone accretion In summary, average calcium retention (100 to 140 mg/day) during periods of bone accretion provide critical evidence to support the development of DRIs for calcium for these life stages using the factorial method as outlined in the 1997 DRI report (IOM, 1997), based on the average calcium retention, the specific age period, fractional calcium absorption rate, urinary calcium losses, and other small calcium losses. Data of good quality have been made available in the past 10 to 15 years for ages 1 through 18 for average bone mineral calcium accretion or retention; these can be used to determine the EAR and Recommended Dietary Allowance (RDA) for calcium for these age groups. BMC is of less utility for developing the DRIs for calcium, as noted above, but intakes of calcium that support average calcium accretion are also associated with normal healthy BMC/BMD.

Neither rickets nor calcium absorption is informative for establishing DRIs for calcium. During bone accretion, low serum 25OHD levels (< 30 nmol/L) are associated with increased risk of rickets when calcium intakes are not limiting. Further, fractional calcium absorption may be impaired at low serum 25OHD levels (< 30 nmol/L) and does not appear to be enhanced further above serum 25OHD above 50 nmol/L. Although the AHRQ-Ottawa report found fair evidence of an association between serum 25OHD levels and BMC/BMD based on observational data, results from the RCTs did not confirm a consistent benefit on BMC/BMD across skeletal sites and age groups.

Bone maintenance Whereas bone accretion ceases in early adulthood, bone continues to be remodeled throughout life. The goal—bone maintenance—is to provide adequate levels of calcium and vitamin D to support the process and maintain healthy bone and bone density. In turn, maintaining neutral calcium balance is the measure of interest—positive balance no longer occurs and negative calcium balance is to be avoided. In addition to highlighting the five key indicators relevant to bone maintenance, this section addresses pregnancy and lactation within the context of relevant indicators for DRI development.

Neutral calcium balance An important body of evidence is contributed by a recent comprehensive analysis of metabolic studies, as reported by Hunt and Johnson (2007). Their work not only offers solutions for some of the confounding associated with the interpretation of data from calcium balance studies, as discussed in Chapter 2, but also it provides new information on the levels of calcium associated with neutral calcium balance.

Participants in the Hunt and Johnson (2007) study included 73 women 20 to 75 years of age (average 47 years) and 82 men 19 to 64 years of age (average 28 years). The analysis included 19 feeding studies conducted at one site in a metabolic unit under carefully controlled conditions. Balance

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data from the final 6 to 12 days of each dietary period were analyzed. For these studies, only healthy individuals participated, calcium intakes below and near the presumed required amounts were included, and adequate dietary adaptation was ensured by examining only dietary periods greater than or equal to 18 days. The statistical model used by Hunt and Johnson (2007) predicted neutral calcium balance at calcium intakes of 741 mg/day for healthy adults, regardless of age or gender. The upper limit of the 95 percent prediction interval around this estimate was 1,035 mg/day. Given the subjects, the outcomes are most readily applicable for adults up to the age of 50 years. These authors concluded that their data indicated tight control of calcium homeostasis in the range of typical calcium intakes and far above the point at which calcium balance is neutral (i.e., 741 mg/day). Moreover, they indicated that calcium balance is highly resistant to changes in calcium intake across a broad range of intakes—specifically, 414 to 1,740 mg/day, the approximate 25th and 99th percentiles from their studies.

Bone mineral density: Calcium In the case of female subjects, there are observational studies relating calcium intake to bone mass in premenopausal women, but virtually all are confounded by the absence of data on vitamin D (either intake or serum 25OHD concentrations) and factors such as physical activity and hormonal status. In addition, there are only two randomized trials of calcium supplementation and bone mass in women (and none in men) from the fourth to the sixth decade of life, despite the relative importance of this period for the maintenance of skeletal health. Thus, overall, little information specifically for BMD and calcium is available, and there is no evidence that levels above that needed for neutral calcium balance are beneficial. Needless to say there are no fracture studies, in part because of the relative rarity of osteoporotic fractures in this age group. However, BMD is considered predictive of future fracture risk.

One recent observational study of 300 premenopausal Greek women demonstrated that those who had calcium intakes above 800 mg per day and were physically active had higher ultrasound bone mass measurements than those with lower calcium intakes, regardless of physical activity level (Dionyssiotis et al., 2010). Furthermore, a 10-year observational study of 133 premenopausal Finnish women demonstrated that those with high calcium intake had less trochanteric BMC loss than those with lower intake (Uusi-Rasi et al., 2008). A recent observational study from Bischoff-Ferrari et al. (2009b) examined NHANES data and calcium intake against the incidence of hip BMD and serum 25OHD level among individuals without previous fractures across a wide age range. These authors found that among premenopausal women, a higher calcium intake was associated with greater BMD only for those women with a serum 25OHD level below 50 nmol/L. No such association was found for men. The methodologies do not indicate whether the authors applied the prescribed weighting factors

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for NHANES data, which if not carried out could significantly impact the nature of the results.

Other observational data provide only marginal evidence to suggest that calcium intakes can have an impact on bone mass in men. One observational study of nearly 2,400 young Swedish men (mean age 18.4 years) suggested that physical activity level but not calcium intake was related to calcaneal BMD (Pettersson et al., 2010). Similarly, in a study of 131 men ages 20 to 75 years, calcium intake had no relationship to lumbar or femoral BMD at any age (Atalar et al., 2009). In the Amsterdam Growth and Health Longitudinal Study of 225 men 27 to 36 years of age during a 10-year period, calcium intake was not related to lumbar BMD. In contrast, in a study of 300 Greek men ages 18 to 30 years, only calcium intakes below 400 mg per day were associated with the lower BMD (Kyriazopoulous et al., 2006).

As mentioned, randomized trial data are few and underpowered. In one very small (n = 37) randomized trial of women 30 to 42 years of age, those who increased their dietary calcium intake by an average of 600 mg/day for 3 years exhibited no vertebral bone loss compared with the women with no calcium supplementation, who lost an average of 1 percent of their spine BMD per year (Baran et al., 1990). In another small randomized study of 300 women between 45 and 55 years of age who were considered "perimenopausal," Elders et al. (1991) demonstrated that supplemental calcium at 1,000 mg/day over 2 years prevented a relatively small degree of bone loss in the spine compared with placebo-treated controls.

Bone mineral density: Vitamin D Regarding vitamin D and BMD measures, the AHRQ analyses incorporated largely studies that administered vitamin D in combination with calcium. Further, regarding the relationship between serum 25OHD levels and BMD measures for persons likely to be experiencing bone maintenance, very few studies for persons between the ages of 18 and 20 years were located. Regarding the intake of vitamin D with and without calcium supplementation, again most studies focused on postmenopausal women. In any case, bone density is known to vary among adults with age, gender, and race/ethnicity (Looker et al., 2009).

For studies of vitamin D nutriture and BMD, observational data are available. For example, Bischoff-Ferrari et al. (2009b) examined a cohort of men and women from NHANES III with average age of 47 years (20 to 69 years) and found that for both genders, there was a stepwise increase in BMD for higher serum 25OHD concentrations, even among individuals less than 50 years of age. The analysis is reported on the basis of cutoff point, and overall distributions were not provided; further it is not clear that the NHANES III sampling weights were applied. Van Dijk et al. (2009) studied vitamin D intake and BMD in 320 Dutch men and women at 36 years of age and found that vitamin D intake was positively associated with BMD

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at all sites in men but not in women. AHRQ-Tufts reported inconsistent outcomes for the measures of BMD relative to serum 25OHD. In any case, observational data are best used when causality has been demonstrated by RCTs, which does not appear to be the case for BMD and serum 25OHD.

One recent RCT that focused on vitamin D measures included persons between the ages of 18 and 64 years, the period at which bone maintenance is paramount. Andersen et al. (2008) analyzed 89 women and 83 men separately; subjects were Pakistani immigrants living in Copenhagen, Denmark. The men and women were assigned to receive either a daily dose of 400 or 800 IU of vitamin D_3 or placebo for 1 year. For women, the mean baseline dietary calcium intake was 495 mg/day, and mean serum 25OHD concentration was 12 nmol/L. For men, the mean baseline dietary calcium intake was 548 mg/day, and the mean serum 25OHD concentration was 21 nmol/L. At the end of the study, there was no significant difference in lumbar spine BMD changes regardless of the dose in both women or men.

Not unexpectedly, osteoporotic fractures are not a factor during the younger years of adulthood, a life stage not characterized by bone loss. In young adult women, stress fractures and overuse injuries in Navy recruits were examined in relation to calcium and vitamin D intake (Lappe et al., 2008). Supplementation with these two nutrients (2,000 mg of calcium per day and 800 IU of vitamin D per day) reduced the incidence of stress fractures. However, the generalizability of this study to the normal population is questionable.

Osteomalacia Recent data on osteomalacia are illuminating. A study conducted by Priemel et al. (2010) provides useful information on serum 25OHD levels and osteomalacia. Postmortem bone biopsies and measurement of serum 25OHD levels were performed in 675 individuals between 20 and 100 years of age. Subjects had been residing in Germany and died for reasons not related to cancer, metabolic disorders, or bone diseases. The mean age of the persons biopsied was 58.7 years for the 401 men, and 68.3 years for the 274 women. The authors noted that unlike PTH or calcium, serum 25OHD level has been found to be stable in various experiments for at least 10 days postmortem; one question is the extent to which serum 25OHD levels at one point in time (death) correlate with levels during adulthood. This is the largest study to date examining vitamin D (in the form of serum 25OHD) and undermineralization of bone as reflected by pathological accumulation of osteoid.

The Priemel et al. (2010) group defined a mineralization defect as a value of greater than or equal to 2 percent for the ratio of osteoid volume (i.e., bone matrix that is not mineralized) to total bone volume, referred to as OV/BV. The authors pointed out that, based on their findings, no subject experienced the defect at serum 25OHD levels of 75 nmol/L. That is, 100 percent of the population could be considered "covered" by a se-

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rum 25OHD concentration of 75 nmol/L. However, this conclusion from Priemel et al. (2010) over-states the levels of 25OHD in serum consistent with population coverage akin to an RDA. The question for DRI development is not whether a maximal level provides benefit, but at what level can the vast majority of the population (97.5 percent) expect benefit.

The committee, therefore, examined the data provided in Panel D of Figure 4 (osteoid volume versus 25OHD scatterplot) from Priemel et al. (2010) in detail. Determination of the number of cases with serum 25OHD levels above 50 nmol/L and above 40 nmol/L was of interest. The number of data points above 50 nmol/L was counted by inspection of the data. At a serum 25OHD level of 50 nmol/L, there were seven data points reflecting persons who failed to achieve the prescribed bone mineralization (OV/BV > 2 percent). This suggested that a serum 25OHD level of 50 nmol/L met the needs of 99 percent of the persons in the study (that is, only 7 of 675 surpassed the measure). In fact, the analysis suggested that 97.5 percent of the population met the measure at a serum 25OHD level of approximately 45 nmol/L; however, as it could not be precisely calculated from the graphic, 50 nmol/L was selected to err on the side of caution. Thus, more than 97.5 percent of the cohort was protected from the defect (OV/BV of ≥ 2 percent) at a serum 25OHD concentration of 50 nmol/L. Further, it is noteworthy that a majority of subjects for whom serum 25OHD levels were below 40 nmol/L actually achieved adequate bone mineralization (OV/BV < 2 percent) as measured by this study. In fact, even at levels lower than 25 nmol/L more than half of the subjects were below the threshold defect measure. Premortem calcium intakes were not available, and this remains a limitation of this study. It is apparent that calcium intake is an important variable in bone mineralization. Calcium intake in children can prevent rickets even in the face of low serum 25OHD levels or in the genetic conditions of absent calcitriol (VDDR I) and absent VDR (VDDR II). From this unique data set of Priemel et al. (2010) it is likely that higher calcium intake in adults can have a positive impact on the skeleton even in the face of lower vitamin D levels. In this regard, the observational data from Bischoff-Ferrari et al. (2009b) using NHANES II, is also noted. In short, the indication is that higher calcium intakes can compensate for lower intestinal calcium absorption as a result of low serum 25OHD levels. Conversely, higher serum 25OHD levels cannot compensate for inadequate calcium intake.

Earlier observational studies from the UK (Leeds, Cardiff) and the United States (New York) histologically examined the hips of first-time hip fracture patients and found that 30 to 40 percent had proven osteomalacia in the fractured hip (Jenkins et al., 1973; Aaron et al., 1974; Sokoloff, 1978; Doppelt, 1984). Additional studies have found serum 25OHD levels to be significantly lower, PTH levels higher, and biochemical or histo-

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logical evidence of osteomalacia more likely in patients with hip fracture than those without hip fracture (Hoikka et al., 1982; Lips et al., 1982; von Knorring et al., 1982; Wilton et al., 1987; Diamond et al., 1998; LeBoff et al., 1999). Osteomalacia was also seen on bone biopsy in about 4 to 5 percent of general medical and geriatric patients who had not suffered a fracture (Anderson et al., 1966; Stacey and Daly, 1989). Of 111 women with postmenopausal vertebral compression fractures attributed to osteoporosis, 8 percent had evidence of osteomalacia (Avioli, 1978). Overall, these data suggest that the contribution of osteomalacia to fragility may be more significant than previously realized: 30 to 40 percent of hip fractures may be due to frank osteomalacia not osteoporosis; the remaining 60 to 70 percent of hip fractures may represent a spectrum that includes earlier stages of osteomalacia/demineralization due to inadequate calcium/vitamin D as well as osteoporosis. These data may also explain why vitamin D supplementation was found to effectively prevent hip fractures in an elderly population (Chapuy et al., 1992): it could be healing various degrees of underlying osteomalacia in the hip.

Calcium absorption and serum 25OHD As described earlier, studies of serum 25OHD concentrations and calcium absorption in adults (mostly in postmenopausal women and older men) have suggested that adequate calcium absorption occurs in the range of 30 to 50 nmol/L serum 25OHD for most persons. Fractional calcium absorption generally does not appear to increase with serum 25OHD concentration levels above 50 nmol/L. In addition, osteomalacia as explored in one study is not found to be meaningfully present until levels of serum 25OHD are at least below 30 nmol/L.

POTENTIAL INDICATORS FOR PREGNANCY: CALCIUM For the majority of women, pregnancy comes at a period of life when the mother's body is normally experiencing bone maintenance. Key physiological changes during pregnancy, mediated by hormonal action, assure delivery of adequate calcium to meet the needs of the fetus, as discussed earlier (e.g., Kovacs and Kronenberg, 1997; Prentice, 2003; and Kovacs, 2008). These key changes also affect the utility of the bone health indicators detailed above for assessing dietary calcium needs. Potential indicators for calcium requirements during pregnancy are discussed below.

- *Calcium absorption* Absorption efficiency doubles during pregnancy in adults (Heaney and Skillman, 1971; Kent et al., 1991) and adolescents (O'Brien et al., 2003). Calcium absorption is, thus, informative in the DRI development for pregnancy.
- *Calcium balance* Pregnant women are in positive calcium balance early in pregnancy as indicated by the measures of hypercalciuria and direct measurement (Heaney and Skillman, 1971). However,

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the utility of calcium balance in DRI development in pregnancy is complex, because the positive calcium balance achieved early in pregnancy is reduced to a neutral calcium balance or a slightly negative calcium balance by term.

- *Maternal BMD/fetal BMC/maternal fracture risk* Neither AHRQ-Ottawa nor AHRQ-Tufts addressed calcium and bone health in pregnancy. Bone turnover is modestly increased from as early as the first trimester, and the analysis concludes there is inconsistent evidence that BMD may decrease between prepartum and postpartum measurements, as discussed above.

  Olausson et al. (2008) reported 1 to 4 percent decreases in whole-body, spine, and total hip BMC and BMD from before pregnancy to 2 weeks postpartum compared with a nonpregnant, nonlactating group, but calcium intake was not related to this skeletal change. Thus, it is conceivable that some calcium provided to the fetus derives from the maternal skeleton during pregnancy. Calcium supplementation among Gambian women with low calcium intakes (355 mg/day) during pregnancy resulted in significantly lower maternal hip BMC and BMD and greater loss of bone mineral in the lumbar spine and distal radius compared with that found in the placebo group (Jarjou et al., 2010). The rate of increase in whole-body BMC is also slower in the breast-fed offspring of calcium-supplemented women during the first year (Jarjou et al., 2006). These two RCTs suggest no benefit to the fetus and possibly an adverse effect on the mother and infant, at least in the short term, of calcium supplementation during pregnancy. Further, the majority of epidemiological and prospective studies report that parity is associated with a neutral or even a protective effect relative to maternal BMD or fracture risk later in life (Sowers, 1996; Kovacs and Kronenberg, 1997; O'Brien et al., 2003; Chantry et al., 2004).

  Thus, additional calcium intake during pregnancy does not appear necessary for maternal or fetal bone health. Similarly, pregnant adolescents, who are in an active period of bone accretion, do not have impaired BMD or increased fracture risk as reported in observational and large cohort studies (Sowers et al., 1985, 1992; Fox et al., 1993; Sowers, 1996; Kovacs and Kronenberg, 1997; Chantry et al., 2004). Thus, maternal and fetal BMD/BMC and maternal fracture risk have utility as an indicator for DRI development for pregnant adults and adolescents.
- *Hypercalciuria* Most pregnant women are hypercalciuric with typical intakes of calcium (Gertner et al., 1986; Dahlman et al., 1994; Cross et al., 1995; Seely et al., 1997). This suggests that increased intakes of calcium could aggravate hypercalciuria as well as the

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inherent risk of kidney stones associated with pregnancy. Thus, hypercalciuria may be of some utility in DRI development, in that it indicates that dietary intake of calcium is more than adequate.

In sum, although no studies have directly explored levels of calcium intake sufficient for pregnant women, indirect measures suggest that the maternal calcium requirement is not increased over the non-pregnant state because of the physiological changes in calcium absorption and possibly, to some extent, bone turnover during pregnancy. The majority of epidemiological and long-term prospective studies that have examined the effect of parity on BMD, risk of osteoporosis, and incidence of fracture have found that parity is associated with a neutral or even a protective effect relative to these outcomes (Sowers, 1996; Kovacs and Kronenberg, 1997). In short, pregnancy does not impair long-term BMD or skeletal health of the mother.

POTENTIAL INDICATORS FOR PREGNANCY: VITAMIN D Key physiologic changes that occur in pregnancy to assure delivery of adequate calcium to meet fetal needs are relevant for DRI development. Potential indicators for vitamin D requirements during pregnancy are described below.

- *Calcium absorption* Although the efficiency of calcium absorption doubles in pregnancy, evidence from studies in the *Vdr*-null mouse shows that this up-regulation occurs independently of vitamin D or calcitriol (Van Cromphaut et al., 2001; Fudge and Kovacs, 2010). Mechanistic evidence is not available from humans; indeed, if such data were available they would still be difficult to interpret because of the known concomitant physiological adaptations. Thus, this measure is not useful for an integrated bone health indicator for vitamin D in pregnancy.
- *Maternal, fetal, and childhood BMC/BMD* Regarding biological plausibility, fetal calcium homeostasis, skeletal development, and bone mineralization appear independent of vitamin D, the VDR, and calcitriol, based on animal models, and human genetic mutations, as discussed above. Regarding AHRQ-Ottawa, this analysis identified three cohort studies and found insufficient evidence on the association of serum 25OHD levels with maternal BMD during pregnancy. No additional studies were identified addressing vitamin D and maternal BMD. One RCT (Delvin et al., 1986) found no effect of vitamin D supplementation on fetal calcium homeostasis. One observational study (Akcakus et al., 2006) reported no relationship between maternal 25OHD level and fetal BMC or BMD. A number of observational studies found normal fetal skeletal devel-

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opment and mineral content (Maxwell and Miles, 1925; Congdon et al., 1983) and no radiological evidence of rickets at birth (Pereira and Zucker, 1986; Campbell and Fleischman, 1988; Specker et al., 1992; Specker, 1994; Beck-Nielsen et al., 2009) in severe vitamin D deficiency, or even in the absence of 1α-hydroxylase or the VDR (Silver et al., 1985; Takeda et al., 1997; Teotia and Teotia, 1997; Kitanaka et al., 1998; Bouillon et al., 2006). In contrast, four associational studies reported lower maternal serum 25OHD levels associated with craniotabes (Reif et al., 1988), lower tibia BMC and cross–sectional area, maternal serum 25OHD level below 42.6 nmol/L (Viljakainen et al., 2010), and higher fetal femur metaphyseal cross–sectional area and splaying (Mahon et al., 2010).

Regarding the developmental programming of later skeletal health in older offspring, one observational study, using 33 percent of the initial infants in a cohort, reported an association of lower whole-body and lumbar spine BMC and areal BMD at age 9 years in children whose mothers had low serum 25OHD levels late in gestation, even though no skeletal parameters differed at birth or nine months of age (Javaid et al., 2006). In offspring of mothers whose serum 25OHD levels late in gestation were less than 27.5 nmol/L or between 27.5 and 50.0 nmol/L, whole-body BMC was reduced compared with those whose mothers had serum 25OHD levels above 50.0 nmol/L. The definition of developmental programming as an indicator per se is questionable; in any case, the evidence for developmental programming of offspring skeletal health outcomes is insufficient to permit the committee to draw any conclusions, but it may be considered within the larger context of fetal skeletal BMD.

Although the congruence of the limited RCT data and majority of the observational data in humans suggests that fetal skeletal outcomes are not adversely affected by maternal vitamin D intake or serum 25OHD concentrations, fetal BMD and related skeletal outcomes may still be of some utility for DRI development. Little evidence could be identified for maternal BMD, making it unclear as to this measure's utility for DRI development.

- *Neonatal rickets* The AHRQ-Ottawa report included neonatal rickets infants 0 to 6 months of age and young children 1 to 6 years of age and found fair evidence for an association between low serum 25OHD levels and rickets identified as early as 2 months, but inconsistent evidence about the threshold level of 25OHD in serum above which rickets does not occur. AHRQ-Tufts identified no additional studies. Generally, the available observational studies do not report the development of vitamin D–deficiency rickets

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until weeks or months after birth (Begum et al., 1968; Ford et al., 1973; Moncrieff and Fadahunsi, 1974; Sann et al., 1976; Pereira and Zucker, 1986; Park et al., 1987; Campbell and Fleischman, 1988; Specker, 1994; Teotia et al., 1995; Beck-Nielsen et al., 2009). Thus, neonatal rickets is of limited utility in the development of DRIs for pregnancy.

- *Maternal and cord blood 25OHD levels* Regarding pregnancy outcomes, maternal and cord blood 25OHD levels may be of interest. AHRQ-Ottawa reported inconsistent evidence on changes in serum 25OHD levels during pregnancy, with two studies reporting no change and one study reporting a decline. In a few other studies, maternal serum 25OHD levels have responded to supplemental vitamin D (Marya et al., 1981, 1988; Mallet et al., 1986; Yu et al., 2009). In observational studies, babies born of vitamin D–deficient mothers have the lowest serum 25OHD levels and are at higher risk for complications sooner after birth than are babies born of vitamin D–replete mothers. Maternal serum 25OHD levels were stable and largely unaffected by pregnancy (Hillman et al., 1978; Brooke et al., 1980; Ardawi et al., 1997; Morley et al., 2006), even when the baseline serum 25OHD level was very low (20.1 ± 1.9 nmol/L) (Brooke et al., 1980).

Overall, fetal BMC and related skeletal outcomes are informative for DRI development for pregnancy.

Potential indicators for lactation: Calcium The key physiological changes to meet the calcium demands of lactation occur through increased bone resorption, and most of the calcium in human milk comes from the maternal skeleton (Kalkwarf, 1999; Prentice, 2003; Kovacs, 2005; Kovacs and Kronenberg, 2008). Thus, lactation is a period of transient bone mineral loss and not, per se, a period of bone maintenance, although BMD is restored post-weaning (Kalkwarf, 1999). However, lactation is included in the category of bone maintenance in order to discuss pregnancy and lactation contiguously, and because bone mineral is restored in the immediate period post-lactation. Potential indicators related to calcium requirements during lactation are outlined below.

- *Maternal BMD* The need to provide calcium to the infant—a need that is two to three times greater than the daily amount needed for fetal development during pregnancy—is met by the maternal adaptation of increased bone resorption (Specker et al., 1994; Kalkwarf et al., 1997), resulting in a 5 to 10 percent decline in BMD during the first 6 months of exclusive breastfeeding

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(Kalkwarf et al., 1997). Neither of the AHRQ analyses addressed calcium and BMD during lactation. Both RCTs and observational studies indicate that increased dietary calcium intake does not suppress maternal bone resorption during lactation (Cross et al., 1995; Fairweather-Tait et al., 1995; Prentice et al., 1995; Kalkwarf et al., 1997; Laskey et al., 1998; Polatti et al., 1999) nor does it alter the calcium content of human milk (Kalkwarf et al., 1997; Jarjou et al., 2006). Further, the calcium content of human milk does not predict maternal BMD decline, but breast milk volume does (Laskey et al., 1998), although milk calcium content is known to vary within and between feeds, complicating interpretation. During the post-lactation period (6 to 12 months), maternal bone mineral is deposited; in turn, maternal BMD is restored to pre-lactation levels without any consistent evidence of a need for higher calcium intake compared with non-pregnant women (Sowers, 1996; Kovacs and Kronenberg, 1997; Kalkwarf, 1999). Two RCTs found no effect of calcium supplementation post-weaning (Cross et al., 1995; Prentice et al., 1995), although one RCT found a slightly greater (1.5 percent) increase in BMD in calcium-supplemented women post-weaning (Kalkwarf et al., 1997). Adolescents, like adults, resorb bone during lactation and recover fully afterward, with no evidence that lactation impairs achievement of peak bone mass (Chantry et al., 2004). Maternal BMD is therefore informative for DRI development.

- *Calcium balance* Although calcium balance is negative during lactation owing to the enhanced bone resorption discussed above, mothers are restored to a positive balance and net accretion of bone mineral immediately upon cessation of lactation, followed by BMD restoration. Notably, urinary calcium excretion decreases during lactation. Thus, during lactation, higher calcium intakes will be less well tolerated and may not be needed, because higher calcium intake does not suppress bone loss. Calcium balance in lactation can be informative for DRI development.

Overall, available evidence indicates that the maternal calcium requirement is not increased during lactation, and it may also not be increased during the post-weaning interval in which the skeleton recovers to its pre-pregnancy baseline BMC.

POTENTIAL INDICATORS FOR LACTATION: VITAMIN D As noted above, lactation is a period of transient bone loss, but it is discussed here in order to consider pregnancy and lactation contiguously and because BMD is restored in

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the post-lactation period. Potential indicators for vitamin D requirements during lactation are discussed below.

- *Maternal BMD* AHRQ-Ottawa found good evidence from one cohort study that there is no association between serum 25OHD level and maternal BMD during lactation. No studies have examined what level of maternal vitamin D intake is required for the maternal skeleton to recover lost mineral content after lactation, although one observational study (Ghannam et al., 1999) in Saudi women found no relationship between maternal serum 25OHD levels (including levels consistent with hypovitaminosis D) and lumbar or femoral neck BMD. There is no evidence that lactating adolescents require any more vitamin D or higher serum 25OHD levels than non-lactating adolescents. Thus, maternal BMD is of limited use in DRI development for lactation.
- *Maternal and infant serum 25OHD levels* Regarding lactation, maternal and infant serum 25OHD levels are of limited use given the present lack of consistent data. AHRQ-Tufts identified only one RCT, which it graded C (i.e., the report from Wagner et al., 2006), that found no effect of maternal supplemental vitamin D (6,400 IU) during lactation on infants' weight or length. Eight other RCTs (Rothberg et al., 1982; Ala-Houhala, 1985; Ala-Houhala et al., 1988; Kalkwarf et al., 1996; Hollis and Wagner, 2004; Basile et al., 2006; Wagner et al., 2006; Saadi et al., 2007) suggest that maternal vitamin D supplementation increases maternal serum 25OHD levels but does not affect neonatal serum 25OHD levels unless the maternal intake of vitamin D is extremely high, in the range of 4,000 to 6,400 IU/day (Hollis and Wagner, 2004; Wagner et al., 2006). With respect to observational studies, maternal serum 25OHD levels are not affected by lactation (Kent et al., 1990; Sowers et al., 1998), although one study found an increase post-weaning (Cross et al., 1997). Observational studies (Cancela et al., 1986; Okonofua et al., 1987; Takeuchi et al., 1989; Kent et al., 1990; Alfaham et al. 1995; Sowers et al., 1998) also show little impact of maternal serum 25OHD levels. Thus, maternal and fetal serum 25OHD concentrations have limited utility for DRI development.

Summary of evidence for bone maintenance During bone maintenance, calcium intakes that maintain a neutral calcium balance have been recently elucidated in an important 2007 study (Hunt and Johnson, 2007) and are informative for the development of an EAR as well as an RDA. The relationship of calcium intake to BMD is more difficult to discern given the limited, and often contradictory observational data and relatively few and

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small RCTs. There is little evidence that levels of calcium intake above that needed for neutral calcium balance are consistent with an improvement in BMD. Of note, the pregnancy-induced increase in fractional calcium absorption allows the needs of pregnancy to be met without an increase in calcium intake above normal requirements. Although it does result in bone resorption, lactation does not increase the risk of reduced BMD or osteoporosis.

Osteomalacia, as explored in one recent study, is not found to be meaningfully present until serum 25OHD levels are at or below at least 30 nmol/L and is rarely present when serum 25OHD levels are above 50 nmol/L, suggesting the possibility of a population distribution. Further, fractional calcium absorption is not additionally enhanced when serum 25OHD levels are above 50 nmol/L. Both osteomalacia and fractional calcium absorption are, thus, informative for the development of DRIs for vitamin D in periods of bone maintenance.

Finally, calcium and vitamin D requirements are not increased during pregnancy or lactation. Nor does vitamin D supplementation alter the development of the fetal, infant, or maternal skeletal health outcomes.

Bone loss A sustained bone loss is associated with the normal aging process and with menopause, as discussed in Chapter 2. The older adult loses bone at an estimated 1 percent per year (Sowers et al., 2010), although the rate of loss varies. The loss is abrupt for women at menopause and is quite rapid until approximately the sixth or seventh year after the onset of menopause. For men, bone loss begins later in life and generally declines steadily over time. Although neutral calcium balance is desired, the realities focus on reducing bone loss and mitigating the degree of negative calcium balance to the extent possible.

Calcium balance Bone loss is reflected by negative calcium balance, and ideally the degree of negative calcium balance would be reduced to the extent possible. Therefore, a reasonable starting point for considering the nutrient intake levels that may be relevant during the life stages associated with bone loss is information on calcium balance. However, relatively few data are available. The study conducted by Hunt and Johnson (2007), described previously, included a few older men up to the age of 64 years and some older women up to the age of 75 years. Specifically, information provided by the study authors[6] indicated that there were 2 men and 34 women between 51 and 70 years of age and 4 women more than 70 years of age. The Hunt and Johnson (2007) analysis suggested that, overall, per-

[6]Specific age breakdown for subjects in Hunt and Johnson (2007): ages 19 to 50 years (35 women, 80 men); ages 51 to 70 years (34 women, 2 men); ages > 70 years (4 women, 0 men). Personal communication, L. Johnson, June 30, 2010.

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sons of any age in the study achieved neutral calcium balance at calcium intakes of 741 mg/day.

Although these data may be relevant for the younger aging male, the Hunt and Johnson (2007) analysis may not be adequate for considering specific issues of bone loss due to aging among men; there were only two men in the age range of 51 to 70 years and no men over the age of 70 years in the analysis. Further, it is uncertain what proportion of women in the Hunt and Johnson (2007) study were menopausal, although approximately half were over the age of 50 years.

Heaney et al. (1977), in examining 130 Catholic nuns as part of a longitudinal study, reported that neutral calcium balance during the perimenopausal state for these women (between the ages of 35 and 50 years) was achieved at 1,240 mg/day. This intake is notably higher than that reported by Hunt and Johnson (2007). In a second study of the same group of women (n = 168), Heaney et al. (1978) reported that perimenopausal and estrogen-treated women reached neutral calcium balance with calcium intakes of 990 mg/day, whereas untreated postmenopausal women required 1,504 mg of calcium per day for neutral calcium balance. This suggests, in contrast to the findings of Hunt and Johnson (2007), that menopausal state may be relevant to considerations of calcium requirements. In any case, because the indicator of interest is bone health, other measures, such as bone density and fracture risk are also considered.

Bone mineral density and fracture risk: Calcium Fracture risk occurs in the later years of life and can be useful as an indicator of bone health, but fractures are less common in persons less than 70 years of age. Therefore, as an indicator, it is not particularly revealing as far as the effects of nutrient intake in slowing the bone loss of early menopause, when many women are in their 50s. It is also of questionable relevance to men less than 70 years of age who generally have yet to experience the full impact of bone loss due to aging. However, BMD measures are predictive of future fractures and can serve as a relevant indicator to ensure bone health to the extent possible during the onset of menopause and during the early aging process.

Regarding BMD measures and calcium intake among younger menopausal women, the AHRQ analyses are not specifically helpful in that the analyses used primarily studies that supplemented participants with both vitamin D and calcium, and neither AHRQ analysis addressed calcium alone relative to bone health. One report reviewed by AHRQ, which used a combination of calcium and vitamin D supplements, should be noted, especially given the large size of the cohort. The study (Jackson et al., 2006), stemming from the WHI, randomly assigned more than 36,000 postmenopausal women between the ages of 50 and 79 years (mean = 62 years) to a placebo or 1,000 mg of calcium with a supplement of 400 IU of vitamin D_3. Fractures were ascertained during a period of about 7 years, and

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BMD was measured for some of the subjects. On average, the background intake of these women provided a relatively high intake of calcium (average 1,150 mg/day), compared with that typically reported for the general population. With the addition of the supplement given as part of the study protocol, calcium intakes approached 2,150 mg/day. Overall, following the intervention, the authors found a small, but significant, improvement in hip BMD; however, the study did not demonstrate a reduction in hip fracture. This appears to be consistent with the understanding that fracture risk is less prevalent under the age of 70 years, particularly among persons 50 to 60 years of age. On the basis of age stratification, women 50 to 59 years of age showed a hazard ratio for hip fracture of 2.17, whereas the HR for women 60 to 69 years of age was 0.74. It is notable that the vitamin D supplementation was relatively low, thereby enhancing the ability to consider the effects of calcium per se. Under these conditions, there is the suggestion that calcium intakes of 2,150 mg/day increased BMD slightly compared with intakes of 1,150 mg/day (placebo with background diet). However, the calcium–vitamin D treatment was associated with an increased risk of kidney stones.

Several studies (Table 4-15) are noted in the context of examining the effect of calcium on BMD at times when menopause occurs or is on-going. As shown in the table, the data suggest mixed results. None measured the nature of the dose–response relationship. Some indicate benefit at lower levels of calcium intake, whereas others show no effect at higher levels of intake. The benefits vary by bone site, but not consistently; and lifestyle factors, such as exercise, appear to be related to outcome. However, the meta-analysis of Shea et al. (2002), which examined calcium supplementation with minimal vitamin D intake, suggested a relatively small, but consistent, effect of calcium supplementation on BMD in postmenopausal women, many of whom were less than 70 years of age. The authors reported that the inference that calcium increases bone density for this group was strengthened by the consistency of the findings across four sites of measurement, but pointed out that loss to follow-up and unexplained heterogeneity confounded the conclusions. In a study of free-living menopausal women that measured total body calcium (by neutral activation analysis), a retardation of bone loss in the femoral neck in early menopause was reported with a calcium intake of 1,700 mg/day (Aloia et al., 1994). However, the study protocol combined the calcium supplementation with 400 IU of vitamin D per day. In contrast, in some studies focused on reducing bone loss in menopausal women using various treatments including increased calcium intake, it was found that the retardation of bone loss with calcium intake was not equivalent to that associated with hormone replacement therapy, but also that it appeared to have minimal effect on retarding that compo-

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TABLE 4-15 Intervention Studies of Interest: Calcium Supplementation (without Vitamin D) and Bone Mineral Density Among Menopausal Women < 71 Years of Age

Reference; Study Type; Country	Population Description	Calcium Intake and BMD Measures
Dawson-Hughes et al., 1990 RCT United States	Healthy, postmenopausal women Average age = 58 years *n* = 301	500 mg with background diet (intakes grouped as < 400 mg or 400–650 mg) of 274 ± 80 mg/day and 513 ± 71 mg/day (early postmenopausal); 283 ± 89 mg/day and 530 ± 95 mg/day (late postmenopausal) If postmenopausal for 6+ years, maintenance of BMD at hip and radius, but loss at spine Bone loss from spine not affected by calcium supplementation if menopausal for 5 or fewer years
Reid et al., 1993 RCT New Zealand	Healthy, postmenopausal women (≥ 3 years postmenopause) Average age = 58 years *n* = 122	1,000 mg supplement with background diet of 750 ± 260 mg/day at 2 years (mean) Loss of total body BMD reduced by 43%
Prince et al., 1995 RCT Australia	Healthy, postmenopausal women (> 10 years postmenopause) Average age = 62 years *n* = 168	1,000 mg supplement with background diet of 822 ± 286 mg/day (Ca group) and 919 ± 411 mg/day (Ca + exercise group) (means) Cessation of bone loss at the intertrochanteric and trochanteric hip site; reduced bone loss of the tibias (ultradistal); no significant bone loss at the spine site in any group Exercise with calcium supplementation resulted in less bone loss at the femoral neck site compared with calcium supplementation alone
Riggs et al., 1998 RCT United States	Healthy, postmenopausal women Average age = 66 years *n* = 177	1,600 mg with background diet of 711 ± 276 mg/day Small retardation of rate of bone loss (total body BMD, lumbar spine, proximal femur), but significant difference

NOTE: BMD = bone mineral density; RCT = randomized controlled trials.

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nent of bone loss that was due to estrogen withdrawal (Riis et al., 1987; Dawson-Hughes et al., 1990).

Although the meta-analysis of Tang et al. (2007) concluded that in addition to fracture risk reduction, calcium supplementation was associated with a larger reduction in the rate of bone loss when the supplemented dose was 1,200 mg/day, the analysis included both men and women, many of whom were over the age of 70 years. Further, the authors noted that the regimen was most effective for persons who were quite elderly, lived in institutions, had low body weight, and had low calcium intakes at the time of the study. Such persons are likely different from the younger menopausal women undergoing the rapid bone loss associated with the early stages of menopause.

Taken as a whole, the evidence suggests some benefit for BMD/bone loss related to calcium intake, but the minimum level of intake that is effective is difficult to ascertain, because dose–response relationships were not examined and there were many confounding variables. Most studies added a large supplemental dose to existing background calcium intakes of approximately 700 to 800 mg/day. Therefore, the benefit has been studied at calcium intakes ranging from about 750 mg/day to 1,700 to 1,800 mg/day.

Bone loss becomes more characteristic of both genders as age increases, and the risk of osteoporotic fracture becomes more common, along with decreased bone density. Although most evidence for fractures focuses on women, fracture rate for men has also been studied and is of concern. Overall, the question is whether and at what levels calcium intake can mitigate or reduce fracture risk in older persons.

Relative to calcium intake alone, the meta-analysis offered by Tang et al. (2007) and discussed previously, provides some information. While all subjects were over 50 years of age, many were in the age range of 70 years and above. The results suggested benefit for BMD and fracture risk reduction relative to calcium in combination with vitamin D *or* calcium alone. However, the number of calcium-alone studies was small, and the vitamin D status of those in the trials was not always evident. The authors' conclusion that a calcium intake of 1,200 mg/day was effective in demonstrating this benefit must be considered in light of the fact that most studies did not provide supplementation at lower levels, such as 1,000 mg/day.

Many of the same studies that were relevant at the time of the 1997 DRI review (IOM, 1997) remain relevant today, such as Chevalley et al. (1994) and Recker et al. (1996). The available studies in 1997 suggested that there was a favorable effect of calcium on reduction in fracture rate, but there were insufficient data to allow estimation of the magnitude of the impact of calcium intake on fracture rates. The Bischoff-Ferrari et al. (2007) meta-analysis of RCTs with calcium basically came to similar conclusions. In that paper, a summary of prospective cohort studies of calcium alone suggested

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TABLE 4-16 Intervention Studies of Interest: Calcium Supplementation (without Vitamin D) and Fracture Risk and/or BMD in Persons > 70 Years of Age

Reference; Country	Subjects	Calcium Intake and Fracture Risk and/or BMD
Peacock et al., 2000 United States	Independent, mobile older men and women *n* = 316 women *n* = 122 men Average age = 75 years	750 mg/day Background diet of 670 ± 325 mg/day (men) and 564 ± 294 mg/day (women) [Note: Study protocol included a group receiving $25OHD_3$] For hip, calcium supplement recipients had similar BMD compared with placebo (at the spine, both placebo and calcium supplementation increased BMD during the study)
Grant et al., 2005 UK	Older men and women with previous fracture; 85% women *n* = 5,292 Average age = 78 years	1,000 mg/day Background diet not reported [Note: Study protocol included a group receiving vitamin D_3 and a group receiving combination of calcium and vitamin D_3] Incidence of new fractures (26% were of the hip) did not differ significantly between participants allocated calcium and those who were not Compliance with tablets containing calcium was significantly low
Prince et al., 2006 Australia	Healthy, older women over age of 70 years *n* = 1,460 Average age = 75 years	1,200 mg/day Background diet of 897 mg/day (placebo, compliant with regimen), 915 mg/day (calcium, compliant with regimen), 950 mg/day (placebo, noncompliant with regimen), 903 mg/day (calcium, noncompliant with regimen) Supplementation overall did not significantly reduce fracture risk, but subanalysis on the basis of compliance showed significantly reduced fracture incidence with calcium supplementation Calcium recipients had improved quantitative ultrasonography findings of the heel, femoral neck, and whole-body DXA

NOTE: BMD = bone mineral density; DXA = dual-energy X-ray absorptiometry; UK = United Kingdom.

no effect on non-vertebral fracture risk. However, in a pooled meta-analysis of five RCTs of calcium alone, the authors found that risk reduction was 8 percent (HR = 0.92 [95% CI: 0.81-1.05]) for non-vertebral fractures. This is consistent with the committee's conclusion that calcium supplementation alone has a modest benefit for skeletal health, both in terms of increased

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BMD and a suggestion of non-vertebral fracture risk reduction. Peacock et al. (2000) more recently reported no effect of calcium compared with placebo relative to hip BMD (see Table 4-16).

In summary, considering calcium alone, intakes at or above 1,200 mg/day, whether with supplements or diet, are not associated with a reduced fracture risk, although calcium supplementation can prevent bone loss from both the hip and spine in both young and older postmenopausal women. In contrast, there is evidence from several meta-analyses to suggest that sufficient calcium (≥ 1,200 mg/day) with vitamin D supplementation (800 IU/day) reduces fracture risk, particularly hip, in those over age 70 years and those institutionalized (Tang et al., 2007; Avenell et al., 2009b).

Bone mineral density and fracture risk: Vitamin D The vast majority of the studies that consider bone health and the issues of bone loss, BMD, and fracture risk contain protocols that administered a combination of vitamin D with calcium. These are well described in the AHRQ analyses, which focused on postmenopausal women and older men. AHRQ-Tufts concluded that there is *good evidence* that vitamin D_3 plus calcium supplementation resulted in small increases in BMD of the spine, total body, femoral neck, and total hip. Based on included trials, it was less certain whether vitamin D_3 supplementation alone has a significant effect on BMD. Two of the three relevant new RCTs identified by AHRQ-Tufts showed a significant increase in hip or total BMD in postmenopausal women, supplemented with vitamin D_3 or vitamin D_2 (300 or 1,000 IU/day, respectively) plus calcium (1,200 mg/day), compared with placebo. Only one of these three trials did not combine calcium supplementation with vitamin D supplementation. AHRQ-Ottawa concluded that supplementation with vitamin D (most studies used vitamin D_3) plus calcium was effective in reducing fractures in institutionalized older populations, although the benefit in community-dwelling individuals was inconsistent. AHRQ-Tufts did not identify any new RCTs examining the combined effect of vitamin D plus calcium supplementation on fractures in postmenopausal women and older men. For vitamin D alone, the evidence was specified as inconsistent for a relationship with reduction in fracture risk. Three new RCTs identified by AHRQ-Tufts (Bunout et al., 2006; Burleigh et al., 2007; Lyons et al., 2007) did not show significant effects of either vitamin D_2 or vitamin D_3 (daily doses ranged from 400 to 822 IU) in reducing the risk of total fractures.

Avenell et al. (2009b) performed a meta-analysis comparing the effects of vitamin D alone with those of vitamin D plus calcium relative to fracture risk. In nine trials encompassing nearly 25,000 participants, vitamin D supplementation alone had no effect on risk reduction for hip, vertebral, or any fracture. In contrast, calcium plus vitamin D (typical intake range of 400 to 800 IU/day, but up to a high of 2,286 IU/day, as well as bolus doses

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on a weekly basis) suggested a 16 percent risk reduction for hip fractures, particularly among institutionalized elders.

The meta-analysis conducted by Tang et al. (2007) did not consider the effect of vitamin D independently, but is nonetheless of interest. These authors analyzed 17 trials that used calcium or calcium in combination with vitamin D supplementation and reported fracture as an outcome, concluding that a supplementation of 800 IU of vitamin D per day or greater in combination with a calcium intake of at least 1,200 mg per day is more effective for fracture risk reduction than supplementation with less than 800 IU of vitamin D per day with the same level of calcium supplementation.

Another meta-analysis with fewer studies (Bischoff-Ferrari et al., 2009c) examined the prevention of non-vertebral fractures with vitamin D supplementation alone. These authors concluded that non-vertebral fracture rate is reduced with vitamin D supplementation in a dose-dependent manner. The analysis, however, has some limitations. First, it did not take into account baseline vitamin D intake, which could have been as high as 250 to 300 IU/day, as was noted in a cohort study of older women (Jackson et al., 2006). Second, their approach to defining a dose–response relationship included a sensitivity analysis, based on analysis of a subgroup of women identified as having been the most compliant in taking their supplement. Finally, the regression line that produced a 75 nmol/L threshold serum 25OHD level at which fractures were prevented used an x-axis with irregularly spaced intervals of serum 25OHD level from 50 to 80 nmol/L. With this confounding as a limitation on the utility of the data, the Bischoff-Ferrari et al. (2009c) analysis may support the possibility that vitamin D intakes of approximately 400 IU/day provide some level of benefit relative to fracture risk reduction.

Two recent RCTs are now available that were not considered in the AHRQ analyses. Sanders et al. (2010) treated nearly 2,300 women 70 years of age or older with either placebo or 500,000 IU of vitamin D once yearly for 3 years. The mean serum 25OHD level in the treated group at baseline was 49 nmol/L and rose at 1 month to 120 nmol/L. Remarkably, the risk of any fracture was 25 percent higher in the treated group than in the placebo group, primarily during the first 3 months of treatment. Salovaara et al. (2010) performed a recent 3-year randomized trial of 3,432 free-living Finnish postmenopausal women ages 65 to 71 years, testing the effects of 1,000 mg of calcium per day plus 800 IU of vitamin D per day on incident fractures. Baseline average calcium intake was the same for treatment and control groups, approximately 950 mg/day likewise, serum 25OHD levels were 50 nmol/L for each. After 3 years, the serum 25OHD level in the treated group was 75 nmol/L compared with 55 nmol/L in controls. There was no statistically significant effect of the combination of calcium and vitamin D on incident fractures at any site, although as with other studies,

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there was a trend in overall fracture risk reduction for the treated group (adjusted HR = 0.83; 95% CI: 0.61–1.12).

Osteomalacia The data from Priemel et al. (2010) as examined by the committee have been discussed above in the section on bone maintenance. Given that this study included persons from 20 to 100 years of age, with a majority between 60 and 100 years of age, the information from the study is relevant to considerations of bone loss. As determined by the committee, nearly all persons were free of the measure of osteomalacia used in the study when serum 25OHD levels were above 50 nmol/L; a significant increase in the number of people displaying the mineralization defect was not observed until the serum 25OHD level had decreased below 30 nmol/L. A number of subjects continued to achieve adequate bone mineralization even at very low levels of 25OHD.

Calcium absorption and serum 25OHD level As described above, studies of serum 25OHD concentrations and calcium absorption in adults (most studies used postmenopausal women and older men) have suggested that ample calcium absorption occurs in the serum 25OHD concentration range of 30 to 50 nmol/L for most persons. Fractional calcium absorption generally does not appear to increase with serum 25OHD concentration above 50 nmol/L. In addition, osteomalacia as explored in one study, is not found to be meaningfully present until serum 25OHD levels are at least below 30 nmol/L 25OHD.

Integration of evidence for bone accretion, maintenance, and loss

Calcium The indicator of bone health for calcium depends on the stage of bone health: accretion, maintenance, or loss. For the accretion stage, average bone calcium accretion/retention is informative when combined with a factorial approach (IOM, 1997) to develop an EAR and calculate an RDA. During bone maintenance, neutral calcium balance maintains bone health. For the bone loss stage, integrating BMD with neutral calcium balance may provide additional information for women in the early menopausal period, as discussed above. For younger men entering the same life stage, neutral calcium balance maintains bone health. In later menopause and with aging, fracture risk integrated with the limited information on BMD is informative. Of special note is the pregnancy-induced increase in fractional calcium absorption that precludes an increased calcium requirement during pregnancy. The period of transient but notable bone mineral loss during lactation is not affected by calcium intake and is remedied within a short period post-lactation without increased calcium intake.

Vitamin D Specifying the indicator for vitamin D and bone health across the key stages of bone accretion, bone maintenance, and bone loss presents a challenge because of the limitations of the data and the desirable features of an indicator of effect. Serum 25OHD concentrations are

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often reported for a range of outcomes of interest, making this indicator of "exposure" useful, even though it is not a validated intermediate indicator of effect. Potentially further complicating the specification of an indicator is the public health interest in developing a reference value that addresses bone health beyond the impact of classic vitamin D deficiency, such as rickets. Of note is that existing evidence does not suggest a unique role for vitamin D during pregnancy or lactation beyond that which it plays during non-pregnant and non-lactating states.

As the committee considered the limitations and variability of the evidence across the stages of bone accretion, bone maintenance, and bone loss, a strong congruence of several indicators of bone health—no one of which was sufficiently informative to serve as a basis for a reference value—emerged in relation to serum 25OHD levels and, thus, vitamin D exposure. Integrating these indicators—BMC/BMD, fractional calcium absorption, rickets, osteomalacia, and fracture risk—revealed, as can be seen in the conceptual model in Figure 4-9, an increase in risk of rickets or osteomalacia, impaired fractional calcium absorption, and fractures in older persons when serum 25OHD levels were low, and no apparent benefit for these

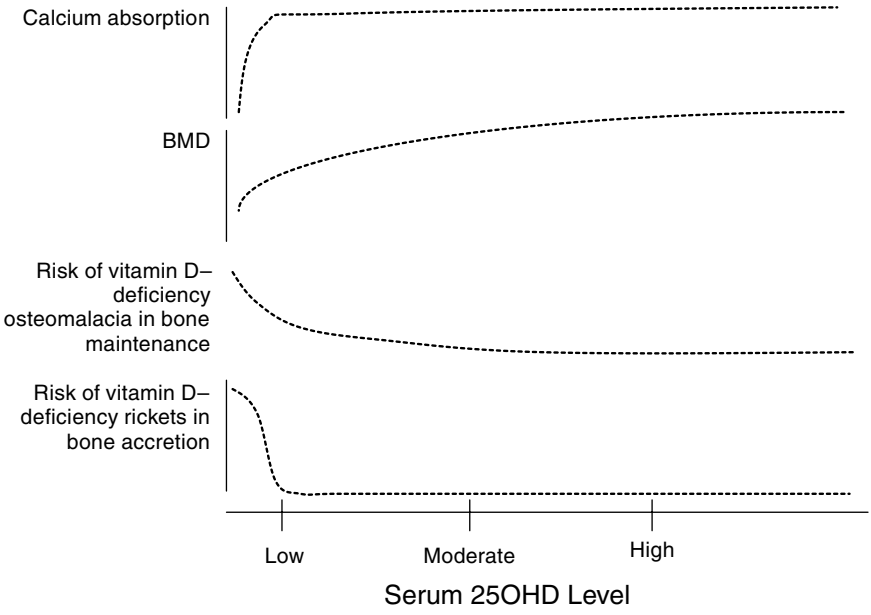


FIGURE 4-9 Conceptualization of integrated bone health outcomes and vitamin D exposure.

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measures when serum 25OHD levels were higher. At moderate levels of serum 25OHD, risk was variable, depending on the specific measure. Collectively, however, the integration of these indicators, if used for DRI development, would support the development of an EAR within this moderate range in which risk for one or more of these bone health indicators may be increased in approximately 50 percent of the healthy population, but reduced in the remaining 50 percent of the population. Illustrated, then, in the companion Figure 4-10 is the consistency of this integrated conceptual model with the classical requirement distribution, or, more specifically, with a marker of exposure. As this is a conceptual model, specific values are not assigned in this figure for "low," "moderate," and "high."

CONSIDERATIONS RELATED TO AFRICAN AMERICAN ANCESTRY

As a result of their greater skin pigmentation, African Americans as well as other dark-skinned groups have lower serum 25OHD concentrations throughout life—as discussed in the section below. However, despite lower serum 25OHD concentrations, African Americans have a superior "calcium economy" compared with whites in North America and have less risk for osteoporosis and fracture (Cohn et al., 1977; Anderson and Pollitzer, 1994; Bell et al., 1995; Aloia et al., 1996b, 1999, 2000; Finkelstein et al., 2002; Barrett-Connor et al., 2005; Cauley et al., 2005a; Tracy et al., 2006). Racial/ethnic differences have been sought to explain the paradox

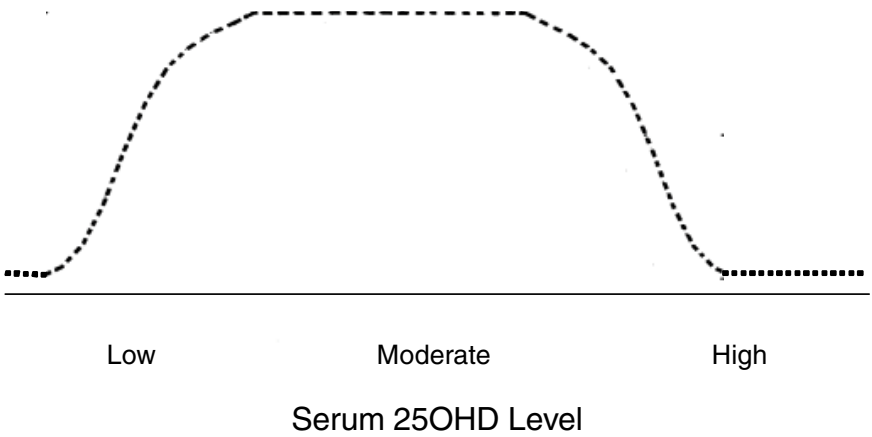


FIGURE 4-10 Theoretical distribution of serum 25OHD level in healthy populations based on integrated bone health outcomes.

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of a decreased incidence of osteoporosis in the presence of lower serum 25OHD levels (Aloia, 2008).

Initially, there is an important caution in considering available data. For many comparative studies, the derivation of the assignment of "race" is not clear. Detailed ancestry is not included in most studies, and socioeconomic characteristics of the ethnic groups are not described. Usually, no genetic data are collected. When the approach has been to consider social and behavioral variables in relation to a single ethnic group, there have been studies suggesting that there is considerable variability in the black ethnic group (Melton et al., 2002; Nelson et al., 2004; Thandrayen et al., 2009). For instance, spinal BMD is lower in recent Sudanese immigrants than in African Americans or whites (Gong et al., 2006). Thus, our interpretation of studies considering bone health in Americans of African heritage must be approached with caution. Moreover, the search for genomic explanations for bone mass variability has thus far not been rewarding (Fleet et al., 1995; Harris et al., 1997; Zmuda et al., 1997, 1999, 2003; Koller et al., 2000; Nelson et al., 2000; Peacock et al., 2002; Gong and Haynatzki, 2003; Edderkaoui et al., 2007; Shaffer et al., 2007; Wang et al., 2007; Engelman et al., 2008; Foroud et al., 2008; Eisman, 2010).

In any case, numerous studies have demonstrated that bone mass is higher in African Americans throughout the life cycle (Cohn et al., 1977; Li et al., 1989; Luckey et al., 1989; Bell et al., 1991; Gilsanz et al., 1991;

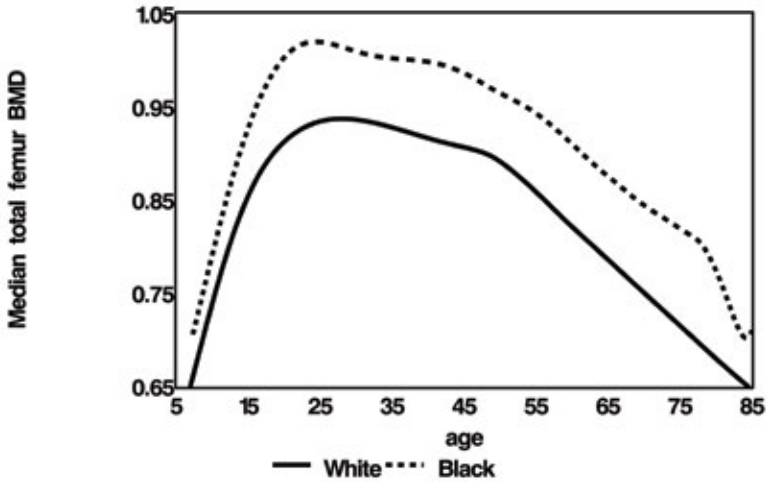


FIGURE 4-11 Bone density of the femur in the black and white population.
SOURCE: Aloia, 2008. Reprinted with permission from the *American Journal of Clinical Nutrition* (2008; volume 88: 545S-550S), American Society for Nutrition.

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Kleerekoper et al., 1994; Nelson et al., 1995, 1997; Aloia et al., 1996a, 1997). The evidence is illustrated in Figure 4-11, using data from one study (Kalkwarf et al., 2007). The advantage in bone mass is associated with one-half the prevalence of osteoporosis and one-half the fracture risk of whites (Barrett-Connor et al., 2005). Longitudinal BMD studies demonstrate that this skeletal advantage for African Americans is present before 6 years of age and increases during adolescence, a stage when the skeleton accrues 50 percent of its peak bone mass (Li et al., 1989; Gilsanz et al., 1991; Cromer et al., 2004; Kalkwarf et al., 2007). The skeletal advantage developed during adolescence is maintained throughout adult life, with African Americans having the same pattern of bone loss as whites in each life stage but at a slower rate (Meier et al., 1992; Bryant et al., 2003; Cauley et al., 2005b; Looker et al., 2009).

However, the Study of Osteoporotic Fractures has also demonstrated that for any given bone density value the risk for fracture is less in African Americans, indicating that bone mass is not the only protective factor against fracture (Cauley et al., 2005a). Other possible factors are lower bone turnover, the micro-architecture of bone, bone geometry, body composition, and heredity (Weinstein and Bell, 1988; Schnitzler et al., 1990; Faulkner et al., 1993; Cummings et al., 1994; Han et al., 1996; Wang et al., 1997; Nelson et al., 2000; Gundberg et al., 2002; Hanlon et al., 2002; Faulkner et al., 2005; Schnitzler and Mesquita, 2006; Travison et al., 2008). Bone biopsies in African Americans show an advantageous architecture with more osteocytes and a lower bone formation rate (Parfitt et al., 1997; Parisien et al., 1997; Qiu et al., 2006).

African American girls have higher calcium absorption efficiency, presumably because of their higher calcitriol levels, and lower urinary calcium excretion compared with white girls (Abrams et al., 1995; Bryant et al., 2003; Harkness and Cromer, 2005; Braun et al., 2007; Weaver et al., 2008) (Figure 4-12). There is no threshold for calcium retention at calcium intakes up to 2,000 mg/day, leading to the conclusion that calcium requirements should be the same in the two races.

African American adults retain superior renal calcium conservation and generally have higher serum PTH and calcitriol levels and lower urinary calcium excretion (Bell et al., 1985; Meier et al., 1991; Dawson-Hughes et al., 1993; Kleerekoper et al., 1994; Harris et al., 2000; Aloia et al., 2006a, b; Cosman et al., 2007). Skeletal resistance to PTH is also present in adult African Americans, demonstrated by lower bone turnover despite elevated PTH levels and by resistance of bone resorption to PTH infusion (Aloia et al., 1996a; Cosman et al., 1997; Han et al., 1997, 1999).

Older African Americans—similar to older persons in other population groups in the United States and Canada—develop secondary hyperparathyroidism and accelerated bone turnover and bone loss, but it is

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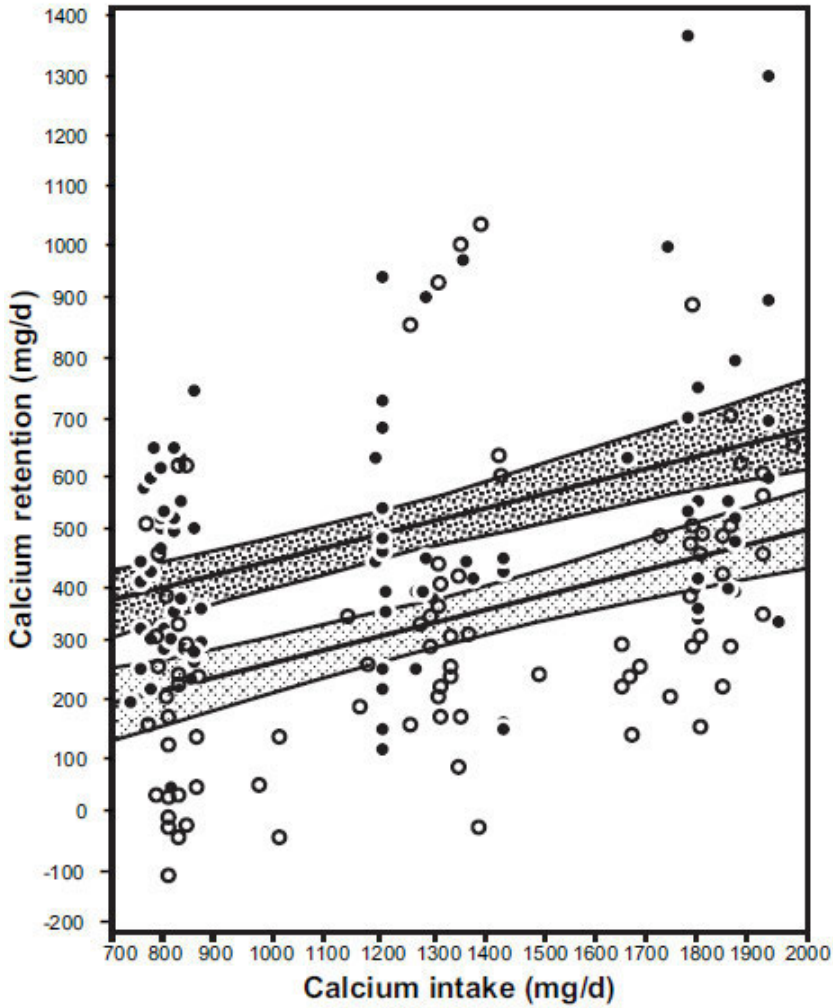


FIGURE 4-12 Mean calcium retention and 95% CIs for regression lines across different calcium intakes, by race. The darker shading represents African American girls (●, 84 observations in 55 girls), and the lighter shading represents white girls (○, 98 observations in 66 girls).

SOURCE: Braun et al., 2007. Reprinted with permission from the *American Journal of Clinical Nutrition* (2007, volume 85, pages 1657-63), American Society for Nutrition.

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unknown if this is attenuated by increasing calcium or vitamin D intake (S. S. Harris et al., 2001; Cauley et al., 2005b; Tracy et al., 2005). There is limited information on the effect of calcium and vitamin D supplements on bone mass or fracture in older subjects, because African Americans have usually not been included in clinical trials in meaningful numbers. A 3-year randomized, double-blind, placebo-controlled vitamin D_3 intervention in postmenopausal black women showed no difference in rate of bone loss between treatment and control groups (Aloia et al., 2005). There was also no relationship between serum 25OHD and rates of bone loss. The WHI did include African American subjects, who took part in a calcium plus vitamin D trial. Hip fracture risk was not reduced by the intervention (Jackson et al., 2006). Changes in bone density in this trial were adjusted for race, but separate analyses by race for the positive outcome on BMD of the hip were not provided. However, a more recent meeting presentation using data from the WHI Observational Study (Cauley et al., 2009) has revealed the concerning finding that fracture risk was directly related to serum 25OHD level in the African American subgroup.

Thus, although the available, emerging evidence would suggest that there is perhaps a lower requirement for calcium and vitamin D among African Americans relative to ensuring bone health, at least compared with whites, there is a notable lack of high-quality and convincing evidence to act on this possibility or to set different requirements for persons of African American ancestry. See Chapter 6 for discussions related to race/ethnicity and estimation of the Tolerable Upper Intake Levels (ULs) for vitamin D.

SELECTION OF INDICATORS

As described in Chapter 1, following the examination of the relevance and quality of the data for the potential indicators of interest, the next step in the DRI development process is to select the indicator or indicators to be used for estimating average requirements or EARs, in this case for calcium and vitamin D. Overall, the selection of indicators is evidence-based; indicators for levels of dietary adequacy are selected based on the strength and quality of the evidence and their demonstrated public health significance, taking into consideration sources of uncertainty in the evidence.

The indicator of bone health is selected as to form the basis of the DRIs for calcium and vitamin D for all life stage groups. With the exception of measures related to bone health, the potential indicators examined are currently not associated with evidence that could be judged either compelling or sufficient in terms of cause and effect, nor informative regarding dose–response relationships for the purposes of determining nutrient requirements. Cancer/neoplasms, cardiovascular disease and hypertension, diabetes and metabolic syndrome, falls and physical performance, im-

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mune functioning and autoimmune disorder, infections, neuropsychological functioning, and preeclampsia could not be causally linked reliably or consistently with relevant outcomes as a function of calcium or vitamin D intake. Although the conclusions at this time do not preclude the possibility that future studies may specify the existence of such relationships, they are currently best described as hypotheses of emerging interest, and the conflicting nature of the available evidence means that it cannot be used to establish a positive impact on health outcomes with any level of confidence.

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5

Dietary Reference Intakes for Adequacy: Calcium and Vitamin D

OVERVIEW

Bone health has been selected as the indicator to serve as the basis of the Dietary Reference Intakes (DRIs) for calcium and vitamin D. The review that underpins this conclusion has been described in Chapter 4, the component of this report addressing the hazard identification step of risk assessment and specifying the selected indicator. The next step in the risk assessment approach for DRI development—the hazard characterization component of risk assessment—is contained in this chapter. The dose–response relationship between the nutrient intake and bone health is examined and dietary reference values for adequacy are specified. In the case of DRIs for calcium and vitamin D, such values take the form of Estimated Average Requirements (EARs) and Recommended Dietary Allowances (RDAs) or, alternatively, Adequate Intakes (AIs). The discussions related to the Tolerable Upper Intake Level (UL), which is also a DRI value, are contained in Chapter 6.

Currently available data on bone health outcomes—when considered as an integrated body of evidence—can be used to derive EARs and RDAs for calcium and vitamin D for all life stages except infants. Bone health measures associated with bone accretion, bone maintenance, and bone loss are relevant to different DRI life stages, and thus the indicator of bone health has been reflected by different bone health measures depending upon the life stage. With respect to infants 0 to 12 months of age, for whom data were very sparse, an AI can be specified for each nutrient based on the available evidence concerning levels of intake observed to be adequate.

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The DRIs for calcium and vitamin D established in 1997 (IOM, 1997) also relied on bone health as the indicator in setting reference values for adequacy. However, the 1997 report established an AI for all life stage groups; no EARs or RDAs were specified. Newer data plus an integration of data have allowed the estimation of EARs and RDAs for all life stages except infants. Quantitative comparisons between AIs and EARs and RDAs are not appropriate.

In 1997, AIs were established for calcium in lieu of EARs and RDAs as a result of uncertainties associated with balance studies, lack of concordance between observational and experimental data, and lack of longitudinal data to verify the relationship between calcium intake, calcium retention and bone loss (IOM, 1997). In the past 10 years, newer evidence on skeletal health has emerged from a combination of large-scale randomized trials and calcium balance studies as described in Chapter 4. Further, there are now data relative to a number of life stage groups, and these help to avoid reliance on extrapolating or scaling data from one life stage to another unstudied life stage.

In the case of vitamin D, the 1997 report concluded that there were inadequate data available for EARs and RDAs as a result of uncertainties about sun exposure, the vitamin D content of the diet, and vitamin D stores (IOM, 1997). In the intervening years data have emerged that allow a requirement distribution to be simulated for vitamin D, which, in turn, has been found to be concordant with other available data. This analysis unexpectedly indicated that the dose–response relationship regarding median requirements is not significantly affected by age. Further, several newer studies can be used to elucidate the contributions made by sun exposure and to help separate total intake contributions from contributions stemming from cutaneous synthesis. Strides have been made in estimating the vitamin D content of foods as well as the amounts of vitamin D consumed by the U.S. and Canadian populations.

Despite new data since the earlier Institute of Medicine (IOM) report (IOM, 1997), there remain a number of uncertainties that have caused challenges in estimating DRI values for calcium and vitamin D. Notable among these is the absence of intervention trials that study dose–response relationships for the nutrients. Rather, most of the evidence is derived from a single dose that is often relatively high. Further, some studies fail to specify information about the background diet and hence the total level of intake is lacking. When this is the case, the mean population requirement may be below the dose used in the study, but cannot be further specified. In addition, there is the common practice of designing studies to examine calcium and vitamin D in combination, thereby precluding the ability to discern the effects of each nutrient alone, which is of interest when establishing a reference value for a nutrient.

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As discussed in Chapter 4, there are very limited data to suggest that there may be some biological differences in the way in which different ethnic/racial groups respond to calcium and vitamin D, most notably among those of African American ancestry. The extent to which such observations may affect requirements for the nutrients is unknown at this time. Although it is important to take into account biological differences where they may exist among, for example, African Americans, Hispanics, and those of Asian descent, the available data are too limited to permit the committee to assess whether separate, quantitative reference values for such groups are required. The DRIs established in this report are based on the current understanding of the biological needs for calcium and vitamin D across the North American population. Other factors may come into play in terms of ensuring adequate intakes of these nutrients—for example, lactose intolerance or food choices—but as far as is known these factors do not affect the basic biological need for these nutrients. Rather, they are discussed in Chapter 8 as issues relevant to the application of the DRIs by dietary practitioners.

Described in this chapter is the committee's decision-making regarding the dose–response relationships for calcium and bone health, and for vitamin D and bone health. From these conclusions, DRI values for adequacy are specified. A significant underlying assumption made by the committee is that the DRIs for calcium are predicated on intakes that meet requirements for vitamin D and that the DRIs for vitamin D rest on the assumption of intakes that meet requirements for calcium. In other words, the requirement for one nutrient assumes that the need for the other nutrient is being met. This is an essential assumption, for three reasons:

1. Given that reference values are intended to act in concert for the purposes of planning diets, health policy makers would be working to meet all nutritional needs; therefore it would be inappropriate to establish requirements for such purposes on the basis that one or more related nutrients would be consumed by the population in inadequate amounts.
2. An inadequacy in one of the nutrients could cause changes in the efficient handling of or physiological response to the other nutrient that might not otherwise be present. For example, in vitamin D–deficient states with minimal calcium intake, absorption of calcium from the gut cannot be enhanced. The compensatory metabolic response to this scenario is the accelerated conversion of 25-hydroxyvitamin D (25OHD) to its active form (calcitriol) through an increase in parathyroid hormone (PTH) levels. Such perturbations confound the estimation of the true requirement under neutral circumstances.

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3. No amount of vitamin D is able to compensate for inadequate total calcium intake; thus, setting a realistic DRI value for vitamin D requires that calcium is available in the diet in adequate amounts.

However, the committee has also commented on the consequences for one nutrient when the other is inadequate, in order to be transparent regarding the science underpinning the determination of reference values for these two nutrients.

CALCIUM: DIETARY REFERENCE INTAKES FOR ADEQUACY

The EARs, RDAs, and AIs for calcium are shown in Table 5-1 by life stage group. The studies used to estimate these values have been included in the review of potential indicators contained in Chapter 4. Therefore, in the discussions below, the relevant data are highlighted but not specifically critiqued again.

Infants 0 to 12 Months of Age

Infants 0 to 6 Months of Age	AI 200 mg/day Calcium
Infants 6 to 12 Months of Age	AI 260 mg/day Calcium

Data are not sufficient to establish an EAR for infants 0 to 6 and 7 to 12 months of age, and therefore AIs have been developed based on the available evidence. An AI value is not intended to signify an average requirement, but instead reflects an intake level based on approximations or estimates of nutrient intakes that are assumed to be adequate. Whether and how much the AI values for infants could be lowered and still meet the physiological needs for human milk-fed infants are unknown because mechanisms for adaptation to lower intakes of calcium are not well described for the infant population, and experimental data with overall relevance to estimating average requirements are extremely limited.

Calcium requirements for infants are presumed to be met by human milk (IOM, 1997). There are no functional criteria for calcium status that reflect response to calcium intake in infants (IOM, 1997). Rather, human milk is recognized as the optimal source of nourishment for infants (IOM, 1991; Gartner et al., 2005). There are no reports of any full-term, vitamin D–replete infants developing calcium deficiency when exclusively fed human milk (Mimouni et al., 1993; Abrams, 2006). Therefore, AIs for calcium

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TABLE 5-1 Calcium Dietary Reference Intakes (DRIs) for Adequacy (amount/day)

Life Stage Group	AI	EAR	RDA
Infants			
0 to 6 mo	200 mg	—	—
6 to 12 mo	260 mg	—	—
Children			
1–3 y	—	500 mg	700 mg
4–8 y	—	800 mg	1,000 mg
Males			
9–13 y	—	1,100 mg	1,300 mg
14–18 y	—	1,100 mg	1,300 mg
19–30 y	—	800 mg	1,000 mg
31–50 y	—	800 mg	1,000 mg
51–70 y	—	800 mg	1,000 mg
> 70 y	—	1,000 mg	1,200 mg
Females			
9–13 y	—	1,100 mg	1,300 mg
14–18 y	—	1,100 mg	1,300 mg
19–30 y	—	800 mg	1,000 mg
31–50 y	—	800 mg	1,000 mg
51–70 y	—	1,000 mg	1,200 mg
> 70 y	—	1,000 mg	1,200 mg
Pregnancy			
14–18 y	—	1,100 mg	1,300 mg
19–30 y	—	800 mg	1,000 mg
31–50 y	—	800 mg	1,000 mg
Lactation			
14–18 y	—	1,100 mg	1,300 mg
19–30 y	—	800 mg	1,000 mg
31–50 y	—	800 mg	1,000 mg

NOTE: AI = Adequate Intake; EAR = Estimated Average Requirement; RDA = Recommended Dietary Allowance.

for infants are based on mean intake data from infants fed human milk as the principal fluid during the first year of life and on the studies that have determined the mean calcium content of breast milk. Additionally, information on calcium absorption and calcium accretion is taken into account.

With respect to estimating AIs for calcium for infants, studies reviewed previously in this report have provided the following information:

- Based on infant weighing studies, a reasonable average amount of breast milk consumed is 780 mL/day. The average level of calcium within a liter of breast milk is 259 mg (± 59 mg). It is therefore estimated that the intake of calcium for infants fed exclusively human milk is 202 mg/day. This number is rounded to 200 mg/day.

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- Calcium absorption for this age group ranges somewhat above and below 60 percent depending upon the total amount of calcium consumed. For development of the AI, a 60 percent calcium absorption rate was assumed.
- The usual accretion rate for calcium in infants can be estimated using the approximation of 100 mg/day overall during the first year of life, with the recognition that the available literature contains reports of varying rates above and below that level.

Infants 0 to 6 Months of Age

Using the estimates described above for the calcium content of breast milk and the amount of milk consumed per day, the AI for calcium for infants 0 to 6 months of age is 200 mg/day, a value reflective of the calcium provided to exclusively breast-fed infants. The expected net retention of calcium from human milk assuming 60 percent absorption would be 120 mg/day, which is in excess of the values predicted from calcium accretion based on cadaver and metacarpal analysis. An AI of 200 mg/day is expected, therefore, to result in retention of sufficient amounts of calcium to meet growth needs.

Further, for infants in the first 4 months of life, balance studies suggest that 40 to 70 percent of the daily calcium intake is retained by the human milk-fed infant (Widdowson, 1965; Fomon and Nelson, 1993). In balance studies using human milk–fed infants, the mean calcium intake was 327 mg/day, and calcium retention was 172 mg/day on average (Fomon and Nelson, 1993). If infants consume calcium at the AI daily, they would achieve similar or greater calcium retention even if the efficiency of absorption was at the lower observed value of 30 percent. Thus, the AI should meet most infants' needs.

The AI established here of 200 mg/day is similar to the AI of 210 mg/day derived by the 1997 report (IOM, 1997). The difference is extremely small—only 10 mg/day—and likely within measurement error; however, the new AI reflects the current best estimate for calcium levels obtained exclusively from human milk

Infants 6 to 12 Months of Age

Estimation of the AI for infants 6 to 12 months of age takes into account the additional intake of calcium from food. From 6 to 12 months of age, the intake of solid foods becomes more significant, and calcium intakes may increase substantially from these sources. Only extremely limited data are available for typical calcium intakes from foods by older milk-fed infants, and mean calcium intake from solid foods has been approxi-

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mated as 140 mg/day for formula-fed infants (personal communication, Dr. Steven Abrams, February 22, 2010).

For the purpose of developing an AI for this age group, it is assumed that infants who are fed human milk have intakes of solid food similar to those of formula-fed infants of the same age (Specker et al., 1997). Based on data from Dewey et al. (1984), mean human milk intake during the second 6 months of life would be 600 mL/day. Thus, calcium intake from human milk with a calcium concentration of about 200 mg/L during this age span (Atkinson et al., 1995) would be approximately 120 mg/day. Adding the estimated intake from food (140 mg/day) to the estimated intake from human milk (120 mg/day) gives a total intake of 260 mg/day. Again, this AI is slightly and probably insignificantly less than the 1997 AI (IOM, 1997) but is the current best estimate.

Children and Adolescents 1 Through 18 Years of Age

Children 1 Through 3 Years of Age	
	EAR 500 mg/day Calcium
	RDA 700 mg/day Calcium
Children 4 Through 8 Years of Age	
	EAR 800 mg/day Calcium
	RDA 1,000 mg/day Calcium
Children 9 Through 13 Years of Age Adolescents 14 Through 18 Years of Age	
	EAR 1,100 mg/day Calcium
	RDA 1,300 mg/day Calcium

For these life stage groups, the focus is the level of calcium intake consistent with bone accretion and positive calcium balance. Studies conducted primarily between 1999 and 2009 (see Table 5-2) provide a basis for estimating EARs and calculating RDAs. In contrast to earlier reference value deliberations for which there were virtually no available studies focused on children and adolescents, this committee benefited from several recent studies that used children as subjects.

The approach used for children was to determine average calcium accretion through bone measures such as DXA and average calcium retention as estimated by calcium balance studies (i.e., positive balance). Next, the factorial method (IOM, 1997) was used with these two data sets to estimate the intake needed to achieve the bone accretion. Average bone calcium accretion is used rather than peak calcium accretion because the committee judged this value to be more consistent with meeting the needs

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TABLE 5-2 Calcium Intake Estimated to Achieve Average Bone Calcium Accretion for Children and Adolescents Using the Factorial Method

Study Author, Year	Age/ Gender	Average Calcium Accretion (mg/day)	Urinary Losses (mg/day)	Endogenous Fecal Calcium Losses (mg/day)	Sweat Losses (mg/day)	Total Needed (mg/day)	Absorption (percent)	Estimated Total Intake (Adjusted for Absorption)
Lynch et al., 2007	1–3 Male/Female	142	34	40	—	216	45.6	474
Abrams et al., 1999; Ames et al., 1999	4–8 Male/Female	140–160	40	50	—	240	30.0	800
Vatanparast et al., 2010	9–13 Female	151	106	112	55	424	38.0	1,116
	9–13 Male	141	127	108	55	465	38.0	1,224
	14–18 Female	92	106	112	55	365	38.0	961
	14–18 Male	210	127	105	55	500	38.0	1,316
	9–18 Female	121	106	112	55	394	38.0	1,037
	9–18 Male	175	127	108	55	465	38.0	1,224

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of 50 percent of this population, and hence an EAR (rather than an AI). Moreover, as discussed in Chapter 2, peak calcium accretion with higher total calcium intakes is likely transitory and, thus, not consistent with DRI development.

The application of the factorial method using average bone calcium accretion allows an estimate of the calcium intake required to support bone accretion and net calcium retention, as shown in Table 5-2. The approach is described below, specifically for each life stage for children and adolescents.

Children 1 Through 3 Years of Age

The data are very limited for children 1 through 3 years of age given the challenges in studying young children. However, a report by Lynch et al. (2007) provides relevant data. Linear and non-linear modeling in this study suggested a target average calcium retention level of 142 mg/day, consistent with the growth needs of this life stage group. Through the factorial method, a calcium intake of 474 mg/day is estimated to meet this need (see Table 5-2). Given that these data are derived from mean estimates and are assumed to be normally distributed, the mean value is very likely the median value. An estimated EAR is, therefore, established as 500 mg of calcium per day, rounded from 474 mg/day.

An assumption specified by Lynch et al. (2007) is that an additional 30 percent calcium retention would meet the needs of 97.5 percent of this age group. This was calculated as 180 mg/day and is based on calcium absorptive efficiency for young children, and it is judged reasonable. This results in an estimated RDA for calcium of 700 mg/day calcium, with rounding.

Clearly, there are uncertainties when reliance is placed on a single study. The ability to study calcium requirements in a controlled study, however, does offer the ability to estimate an average requirement rather than an AI. The study is of high quality, and the reference values specified are in line with those specified for younger and older children.

Children 4 Through 8 Years of Age

The work of Abrams et al. (1999) and Ames et al. (1999) has indicated that, like that for younger children, an average calcium retention level of approximately 140 mg/day is consistent with the needs of bone accretion. However, there is evidence of a small increase during pre-puberty, yielding a calcium retention range of approximately 140 to 160 mg/day to allow for bone accretion across this age group of which a portion will be pre-pubertal. Using the factorial method (see Table 5-2) and from the non-linear dose–response relationship identified by the work of Ames

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et al.(1999) and Abrams et al. (1999), a calcium intake of 800 mg/day could be expected to achieve the levels of calcium needed for bone accretion. Again, the assumption that another approximately 30 percent is needed to cover about 97.5 percent of the population—through derivation as mean estimates and the assumption of normal distribution—results in a calculated and rounded RDA value for calcium of 1,000 mg/day.

Again, as with younger children, there are relatively few studies available and most have small sample sizes. While the studies included some ethnic/racial diversity, they focused on girls. These limitations cannot be remedied at this time. However, the data are sufficiently robust to support an estimation of an average requirement of 800 mg/day calcium.

Children 9 Through 13 Years of Age and Adolescents 14 Through 18 Years of Age

As reviewed in Chapter 4, data from a recent study (Vatanparast et al., 2010) have provided bone calcium accretion levels for children and adolescents ranging from 92 to 210 mg/day. Average bone calcium accretion was included in the factorial method, and the intake levels can be estimated as shown in Table 5-2.

While the committee was aware of data suggesting that calcium retention may vary by gender among children, these differences between girls and boys and between the 9- to 13- and 14- to 18-year age groups are relatively small quantitatively, and the limited nature of the data do not allow further specification of these differences to the extent they are real. Given the application of DRI values in real world settings such as school meal planning, recommending that boys receive a small amount more calcium than girls is not practicable, but it is also not warranted given the limited nature of the data suggesting this possibility. Additionally, there is wide variability in the onset of puberty and the pubertal growth spurt, and it is reasonable to conclude that increases in calcium intake may be needed early in puberty at times when children may be only 9 or 10 years old. Thus, for reference values for both boys and girls in the 9- to 13- and 14- to 18-year life stages, the differences in calcium intake to achieve mean bone calcium accretion as elucidated by Vatanparast et al. (2010) have been interpolated between 9- to 18-year old girls (1,037) and boys (1,224). This interpolation yields an estimated mean need for calcium for boys and girls of 1,100 mg/day with rounding, a value approximately at the midpoint between the two groups. Again, assuming a normal distribution, this estimate to achieve a mean calcium accretion represents the median and, thus, an EAR. The EAR is therefore set at 1,100 mg for both boys and girls for both life stages encompassed by the 9 through 18 year age range. In order to cover 97.5 percent of the population, an estimated RDA value for calcium of 1,300 mg/day is established.

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The uncertainties surrounding the reference value stem from reliance on primarily a single study. Although carefully carried out, the study included only white children. These newer data, however, provide the opportunity to identify an average requirement.

Adults 19 Through 50 Years of Age

Adults 19 Through 30 Years of Age Adults 31 Through 50 Years of Age	EAR 800 mg/day Calcium RDA 1,000 mg/day Calcium

While there is evidence of minor bone accretion into early adulthood, the levels required to achieve this accretion—which appears to be site dependent—are very low. The goal, therefore, is intakes of calcium that promote bone maintenance and neutral calcium balance.

The report from Hunt and Johnson (2007) provides virtually the only evidence for these life stage groups. Based on a series of controlled calcium balance studies, they have established a calcium intake level of 741 mg/day to maintain neutral calcium balance. They further provide the 95 percent prediction interval around the level required for neutral calcium balance.

Other available measures that relate to bone maintenance include bone mineral density (BMD), but studies that measured bone mass concomitant to calcium intake are highly confounded by failures to control for other variables that impact bone mass and to specify a dose–response relationship. There is no evidence that intakes of calcium higher than those specified by Hunt and Johnson (2007) offer benefit for bone health in the context of bone maintenance for adults 19 to 50 years of age. Osteoporotic fracture is not a relevant measure for this life stage, therefore extrapolating from the more prevalent data focused on older adults is not appropriate, nor is extrapolating from the data for younger persons for whom the concern is bone accretion.

Therefore, the Hunt and Johnson (2007) data, which reflect the outcomes of a series of metabolic studies, provide a reasonable basis for an EAR for calcium of 800 mg/day calcium. That is, the observed value of 741 mg/day is rounded, but rounded up to 800 mg/day given the uncertainty. The upper limit of the 95 percent prediction interval around this estimate (1,035 mg/day) is appropriate as the basis for an RDA for calcium and rounded to 1,000 mg/day. As is the case with younger life stage groups, there is now the 2007 Hunt and Johnson study on the topic of calcium and bone health, which has allowed the estimation of an average requirement.

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However, the data are still very sparse, and the DRI for this age group relies on one study, albeit a well-controlled and carefully analyzed study.

Adults 51 Years of Age and Older

Men 51 Through 70 Years of Age	
	EAR 800 mg/day Calcium
	RDA 1,000 mg/day Calcium
Women 51 Through 70 Years of Age	
	EAR 1,000 mg/day Calcium
	RDA 1,200 mg/day Calcium
Adults >70 Years of Age	
	EAR 1,000 mg/day Calcium
	RDA 1,200 mg/day Calcium

Men and Women 51 Through 70 Years of Age

The natural process of bone loss begins to manifest itself in the latter stages of adulthood. It begins earlier for women than for men as a result of the onset of menopause, which usually occurs when women reach 50 to 55 years of age. By the time both men and women have reached 70 or more years of age, each are experiencing bone loss. However, women—who have been undergoing the loss longer—are more at risk for adverse consequences. It is important to underscore that the goal of calcium intake during these life stages is to lessen the degree of bone loss; calcium intake at any level is not known to prevent bone loss.

Although calcium absorption (active calcium transport) has been reported to decrease with age (Avioli et al., 1965; Bullamore et al., 1970; Alevizaki et al., 1973; Gallagher et al., 1979; Tsai et al., 1984), it is challenging to consider higher calcium intake as a remedy given that calcium intake must be extremely high to have an effect on calcium uptake via passive absorption (i.e., paracellular transport, see Chapter 2).

The relative lack of data pertaining to bone changes in men as they age has received comment (Orwoll et al., 1990). It has been pointed out that cross–sectional data suggest that, overall, the rate of bone loss in men is substantially slower than that in women, and men have a lower incidence of fractures (Khosla et al., 2008); perhaps this accounts for the lack of research focused on this group. The limited available trials and observation studies (e.g., Osteoporotic Fractures in Men [MrOS] study) concerning bone health focus on men older than the 5 through 70 year age range (usu-

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ally > 65 years) and typically include vitamin D administration. Likewise, organizations such as the National Osteoporosis Foundation have issued guidelines that do not stipulate BMD testing for men until the age of 70 years (NOF, 2008), whereas they recommend BMD testing at an earlier age for women. Given this context, the data from Hunt and Johnson (2007) with respect to neutral calcium balance among adults can provide some information for specifying requirements among men between the ages of 51 and 70 years. Although there were only two men over the age of 50 years in the Hunt and Johnson (2007) study, the absence of evidence that significant changes occur in skeletal maintenance for men in their 50s and 60s results in the assumption that their needs are akin to those of younger men. Therefore, the calcium EAR and RDA for men 51 to 70 years of age are set at the same levels as for persons 31 to 50 years of age: the EAR for calcium is established as 800 mg/day, and the RDA for calcium is 1,000 mg/day. The newer calcium balance data are used with caution, given its limitations for this purpose.

Women 51 through 70 years of age are considered separately from men. Although it is evident that calcium intake does not prevent bone less during the first few years of menopause, there is the question of whether or to what extent calcium intake can mitigate the loss of bone during and immediately following the onset of menopause. Although about half of the women in the Hunt and Johnson (2007) study were over the age of 50, the authors did not stratify on the basis of menopausal status. Therefore, there are some uncertainties surrounding the use of these newer calcium balance data for the purposes of determining an EAR and RDA for women. However, other information is available that can be useful. Absolute hip fracture rates are lower than for women in this age rang than for women over the age 70 but still greater than for premenopausal women. Moreover, BMD is a reliable predictor for fracture risk later in life and therefore becomes a useful measure for DRI purposes.

The available data for BMD among women 51 through 70 years of age provide mixed results concerning the relationship between BMD and calcium intake in menopausal women. This may be due in part to study protocols—which usually have relied on a single dose of 1,000 mg or more daily—that have failed to clarify background diet or estimate total intake. On balance, there is somewhat more evidence for a benefit of higher calcium intake among women over the age of 60 years, a group that is likely about half of the DRI life stage of women 51 through 70 years of age. Specifically, the meta-analysis conducted by Tang et al. (2007), which included studies in women ranging in mean age from 50 to 85 years, indicated that total calcium intake alone equal to 1,200 mg or more per day had a positive effect on BMD as well as a modest (relative risk [RR] = 0.88; 95%

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confidence interval [CI]:0.83–0.95), but significant, effect on fracture risk reduction. In breaking down the meta-analysis further, there were six studies of more than 1,100 women with a mean age of 60 years who received additional calcium without vitamin D compared with placebo. The average calcium supplementation was 1,100 mg/day in the treated group, and those women had risk reduction for hip fracture and significant increases in both hip and spine BMD.

Further, evidence from the Women's Health Initiative trial (WHI) (Jackson et al., 2006) conducted using 36,282 women ages 50 to 79 years indicated that participants who were randomized to 1,000 mg of calcium plus 400 International Units (IU) of vitamin D per day experienced a small, but significant, improvement in hip bone density and a modest reduction in hip fractures, although the change in hip fracture risk was not statistically significant. A subgroup analysis indicated that women over the age of 60 years also experienced a small but statistically non-significant reduction in hip fracture risk (hazard ratio [HR] = 0.74; 95 percent CI 0.52–1.06) compared with those randomized to placebo. These data are taken into account cautiously for several reasons. The WHI study may be confounded by both hormone replacement therapy considerations as well as the inclusion of vitamin D, although the supplementation level of vitamin D was relatively low. The appropriateness of conducting a subgroup analysis for fracture risk, although interesting, is always considered questionable. Further, the same subgroup analysis revealed that women between the ages of 50 and 60 years experienced a greater hip fracture risk when they were supplemented with calcium and vitamin D. The absolute risk of hip fractures for women 50 to 60 years of age is derived from a small number of fractures per total cohort (i.e., 13 fractures in 6,694 women 50 to 60 years of age). The Tang et al. (2007) meta-analysis is compromised by the inability to study a true dose–response relationship; many studies were grouped at the 1,200 mg/day level of intake and could not be used to reveal the effects at lower levels of intake.

Within the confines of these limitations, there is nonetheless the emerging conclusion that in regard to the relevant indicator for this group, that is, BMD, a somewhat higher intake of calcium than required by men or suggested by the newer calcium balance data is justified for all postmenopausal women within the life stage 51 through 70 years. Not unexpectedly, absolute hip fracture rates are very low in the 50- to 60-year age group (e.g., 0.03 percent per year in WHI), and therefore fracture risk is not a particularly relevant factor, although to the extent that a subgroup analysis can be relied upon, women greater than 60 years of age appear to experience some benefit from calcium intake relevant to fracture risk reduction.

It would appear that the life stage consisting of women 51 through

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70 years of age reflects a diverse set of physiological conditions—notably premenopausal, perimenopausal, and postmenopausal—with respect to the condition of bone health, and cannot be reliably characterized as a homogeneous single group for the purpose of deriving EARs and RDAs for calcium. Some may benefit from increased calcium, and some may not. Further, there is considerable variability in the age of onset of menopause, and so assumptions about the proportion of this age group that may or may not benefit cannot be made. Therefore, to ensure public health protection and to err on the side of caution, preference is given to covering the apparent benefit for BMD with higher intakes of calcium for postmenopausal women within this group. The EAR for women 51 through 70 years is set at 1,000 mg calcium per day. The addition of 200 mg/day to the estimates provided by Hunt and Johnson (2007) gives a reasonable margin of safety for lessening bone loss to the extent that is possible and is reasonably consistent with data from the existing intervention trials. Further, the value of 1,000 mg/day is still within the 95 percent prediction interval offered by Hunt and Johnson (2007) for a value that encompasses a wider range of persons than younger menopausal women. Although this does result in a different DRI for women than for men in the 51 through 70 year age group, the physiological differences and apparent response to increased calcium intake evidenced from randomized trials warrants this difference.

As there is no reason to assume that requirements for this life stage are not normally distributed, the approximate 20 to 30 percent addition to achieve the level needed to cover 97.5 percent of the population results in an estimated RDA of 1,200 mg/day. The level errs in the direction of a lower value given concerns about an upper level of intake (see Chapter 6).

This reference value for women 51 to 70 years of age is notably uncertain and reflects a decision to provide public health protection in the face of inconsistent data. It also identifies menopausal women between the ages of 51 and 70 years as the basis for the reference value, rather than nonmenopausal women, on the assumption that during this life stage many and eventually all will become menopausal. The value cannot be more certain until such time as there is information on calcium balance specifically for women experiencing the early stages of menopause, as well as well-controlled trials that more clearly elucidate dose–response measures for menopausal younger women relative to calcium intake and bone health.

Adults >70 Years of Age

Bone loss and the resulting osteoporotic fractures are the predominant bone health concern for persons >70 years of age. Although measures to ascertain fracture risk are often self-reported and can be challenging to

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verify, fracture risk represents the best measure for bone health for this life stage. One important caution is that the estimation of the effect of fracture risk is greatly complicated by the limited evidence concerning dose–response data relative to calcium intake. Importantly, calcium balance studies to determine the levels of calcium that result in neutral calcium balance are lacking in the literature for persons over the age of 70 years. Hunt and Johnson (2007) were able to incorporate only two women over the age of 70 years.

The analysis of Tang et al. (2007) is limited by the nature of the studies available, in that most studies tested intervention levels at or above 1,200 mg/day and often did not report total calcium intake. Those studies in the Tang et al. (2007) analysis that examined calcium alone, without vitamin D supplementation, were few. The authors' conclusion that 1,200 mg/day was beneficial relative to reduced fracture risk is relevant, but may be compromised by the inability to examine the effectiveness at other levels. In contrast to the Tang et al. (2007) analysis, Peacock et al. (2000), Grant et al. (2005), and Prince et al. (2006), who studied calcium intake alone, were unable to demonstrate benefits for bone health among persons over 70 years of age with supplemental calcium intakes (750 to 1,200 mg/day); however, a compliance sub-analysis conducted by Prince et al. (2006) suggested reduced fracture incidence with calcium supplementation of 1,200 mg/day.

The data available do not clearly elucidate a requirement for calcium and primarily suggest values that may result in covering nearly all of the population group in terms of reduced fracture risk. That is, the available studies were not examining the levels of calcium intake that were effective, but rather were examining whether their administered calcium intake was effective. Further, the benefit of calcium supplementation was evident in the case of sub-analysis on the basis of compliance, which, while informative, are not ideal data sets. In addition, the populations studied varied considerably, many could be considered at high risk (such as institutionalized older persons and persons with low body weight), and the effect of calcium supplementation was usually not taken into account in the context of vitamin D status or existing calcium nutriture.

For this reason, public health protection was considered, and it was determined that a requirement somewhat above that established by calcium balance studies for bone maintenance was appropriate despite the unknowns and the inability to clearly estimate a dose–response for calcium relative to fracture risk. As with those estimates used for postmenopausal women, a 200 mg/day calcium increment was added to the estimated requirements for younger persons, resulting in an EAR value of 1,000 mg of calcium per day. It is assumed that the rapid and notable bone loss ob-

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served for early menopause has ceased, and the bone loss for women in this life stage group is similar to that experienced by men. The estimation of an RDA to cover more than 97.5 percent of the life stage group consistent with normally distributed data results in an RDA of 1,200 mg/day, again in the face of concerns about high levels of intake (see Chapter 6).

Pregnancy and Lactation

Pregnant 14 Through 18 Years of Age	
	EAR 1,100 mg/day Calcium
	RDA 1,300 mg/day Calcium
Pregnant 19 Through 30 Years of Age	
Pregnant 31 Through 50 Years of Age	
	EAR 800 mg/day Calcium
	RDA 1,000 mg/day Calcium
Lactating 14 Through 18 Years of Age	
	EAR 1,100 mg/day Calcium
	RDA 1,300 mg/day Calcium
Lactating 19 Through 30 Years of Age	
Lactating 31 Through 50 Years of Age	
	EAR 800 mg/day Calcium
	RDA 1,000 mg/day Calcium

Pregnancy

The EAR for non-pregnant women and adolescents is appropriate for pregnant women and adolescents based on the randomized controlled trials (RCTs) of calcium supplementation during pregnancy that reveal no evidence that additional calcium intake beyond normal non-pregnant requirements has any benefit to mother or fetus (Koo et al., 1999; Jarjou et al., 2010). Consistent with the RCT data indicating the appropriateness of the non-pregnant EAR and RDA for the pregnant woman is (1) the epidemiologic evidence suggesting that parity is associated with a neutral or even a protective effect relative to maternal BMD or fracture risk (Sowers, 1996; Kovacs and Kronenberg, 1997; O'Brien et al., 2003; Chantry et al., 2004), and (2) the physiologic evidence that maternal calcium needs are met through key changes resulting in a doubling of the intestinal fractional calcium absorption, which compensates for the increased calcium transferred to the fetus (200 to 250 mg/day) and potentially some transient mobilization of maternal bone mineral, particularly in the late third trimester.

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Overall, it appears that pregnant adolescents make the same adaptations as pregnant women, and there is no evidence of adverse effects of pregnancy on BMD measures among adolescents.

The EARs are thus 800 mg/day for pregnant women and 1,100 mg/day for pregnant adolescents. Likewise, the RDA values for non-pregnant women and adolescents are applicable, providing RDAs of 1,000 mg/day and 1,300 mg/day, respectively.

Lactation

The EAR for non-lactating women and adolescents is appropriate for lactating women and adolescents based on (1) the strong evidence of physiologic changes resulting in a transient maternal bone resorption to provide the infant with calcium (Kalkwarf et al., 1997; Specker et al. 1997; Kalkwarf, 1999) and (2) evidence from RCTs and observational studies that increased total calcium intake does not suppress this maternal bone resorption (Cross et al., 1995; Fairweather-Tait et al., 1995; Prentice et al., 1995; Kalkwarf et al., 1997; Laskey et al., 1998; Polatti et al., 1999) or alter the calcium content of human milk (Kalkwarf et al., 1997; Jarjou et al., 2006). Post-lactation maternal bone mineral is restored without consistent evidence that higher calcium intake is required, as based on two RCTs (Cross et al., 1995; Prentice et al., 1995) and several observational studies (Sowers, 1996; Kovacs and Kronenberg 1997; Kalkwarf, 1999).

Adolescents, like adults, resorb bone during lactation and recover fully afterward with no evidence that lactation impairs achievement of peak bone mass (Chantry et al., 2004).

The EARs are thus 800 for lactating women and 1,100 mg/day for lactating adolescents. Likewise, the RDA values for non-lactating women and adolescents are applicable, providing RDAs of 1,000 and 1,300 mg/day, respectively.

VITAMIN D: DIETARY REFERENCE INTAKES FOR ADEQUACY

The EARs, RDAs, and AIs for vitamin D are shown in Table 5-3 by life stage group. The identical EARs across age groups are notable and, as discussed below, reflect the concordance of serum 25OHD levels with the integrated bone health outcomes as well as the lack of an age effect on the simulated dose–response. Studies used to estimate these values have been included in Chapter 4 in the review of potential indicators.

While at the outset the consideration of vitamin D requirements recognizes that humans are physiologically capable of obtaining vitamin D

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TABLE 5-3 Vitamin D Dietary Reference Intakes (DRIs) for Adequacy (amount/day)

Life Stage Group	AI	EAR	RDA
Infants			
0 to 6 mo	400 IU (10 µg)	—	—
6 to 12 mo	400 IU (10 µg)	—	—
Children			
1–3 y	—	400 IU (10 µg)	600 IU (15 µg)
4–8 y	—	400 IU (10 µg)	600 IU (15 µg)
Males			
9–13 y	—	400 IU (10 µg)	600 IU (15 µg)
14–18 y	—	400 IU (10 µg)	600 IU (15 µg)
19–30 y	—	400 IU (10 µg)	600 IU (15 µg)
31–50 y	—	400 IU (10 µg)	600 IU (15 µg)
51–70 y	—	400 IU (10 µg)	600 IU (15 µg)
> 70 y	—	400 IU (10 µg)	800 IU (20 µg)
Females			
9–13 y	—	400 IU (10 µg)	600 IU (15 µg)
14–18 y	—	400 IU (10 µg)	600 IU (15 µg)
19–30 y	—	400 IU (10 µg)	600 IU (15 µg)
31–50 y	—	400 IU (10 µg)	600 IU (15 µg)
51–70 y	—	400 IU (10 µg)	600 IU (15 µg)
> 70 y	—	400 IU (10 µg)	800 IU (20 µg)
Pregnancy			
14–18 y	—	400 IU (10 µg)	600 IU (15 µg)
19–30 y	—	400 IU (10 µg)	600 IU (15 µg)
31–50 y	—	400 IU (10 µg)	600 IU (15 µg)
Lactation			
14–18 y	—	400 IU (10 µg)	600 IU (15 µg)
19–30 y	—	400 IU (10 µg)	600 IU (15 µg)
31–50 y	—	400 IU (10 µg)	600 IU (15 µg)

NOTE: AI = Adequate Intake; EAR = Estimated Average Requirement; IU = International Unit; RDA = Recommended Dietary Allowance.

through exposure to sunlight, the estimation of DRIs for vitamin D immediately requires a plethora of related considerations ranging from factors that affect and alter sun exposure and vitamin D synthesis, to public health recommendations regarding the need to limit sun exposure to avoid cancer risk. Just as importantly, the available data have not sufficiently explored the relationship between total intake of vitamin D per se and health outcomes. In short, a dose–response relationship between vitamin D intake and bone health is lacking. Rather, measures of serum 25OHD levels as a biomarker of exposure (i.e., intake) are more prevalent.

After considering the available evidence, including data published

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after the 2009 analysis by the Agency for Healthcare Research and Quality (Chung et al., 2009), hereafter referred to as AHRQ-Tufts, the committee concluded:

- A dose–response relationship can be simulated based on serum 25OHD measures. That is, serum 25OHD levels can reflect intake, and there are studies that relate bone health outcomes to serum 25OHD levels, as described in Chapter 4.
- Newer data provide the ability to link vitamin D intakes to the change in serum 25OHD level under conditions of minimal sun exposure, thereby reducing the confounding introduced by the effect of sun exposure on serum 25OHD concentrations. These data also provide an approach for estimating dietary reference values related to intakes that will achieve targeted serum 25OHD concentrations, albeit without regard to the contributions from sun exposure.

Generally, association studies that use a biomarker of exposure in relation to health outcomes can present challenges when establishing reference values. Such measures are not necessarily valid or reliable markers, and they can be subject to considerable confounding by a host of variables. In the case of vitamin D, there are certain factors that allow more confidence in using this measure in the estimation of reference values. Specific deficiencies of vitamin D lead to recognized, measurable deficiency states with adverse effects on the indicator of interest, in this case bone health as evidenced by rickets and osteomalacia. The next consideration is whether the biomarker is an accurate reflection of intake. In the case of serum 25OHD concentrations, despite the lack of clarity about the impact of a number of variables on serum 25OHD concentrations, the measure can be reasonably associated with total intake when sunlight exposure is minimal.

On this basis, serum 25OHD concentrations were used to simulate a dose–response relationship for bone health. Next, the available data—notably those obtained under conditions of limited sun exposure—were integrated in order to estimate a total intake that would result in the desired serum 25OHD relative to measures of bone health. This step-wise process for simulating a dose–response relationship for vitamin D considered, first, the relevance to this study of the confounding introduced by 25OHD assay methodologies and related measurement problems, including "assay drift." Next, the data from three bodies of evidence described in Chapter 3—the relationship between calcium absorption and serum 25OHD levels; serum 25OHD levels and bone health in children; and serum 25OHD levels and bone health in older adults—were summarized and used to specify a dose–response curve for serum 25OHD. Interestingly, concordance of

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serum 25OHD levels and bone health for median requirements emerged across all age groups. Finally, the relationship between changes in vitamin D intake and changes in serum 25OHD concentrations was considered.

Simulation of a Dose–Response Relationship for Vitamin D Intake and Bone Health

"Assay Drift" and Implications for Interpretation of Serum 25OHD Data in the Literature

In considering serum 25OHD levels as reported by various studies, the committee was aware of the so-called "assay drift" associated with longitudinal comparison of assay results collected in the National Health and Nutrition Examination Survey (NHANES), as well as the large inter-laboratory variation worldwide (Carter et al., 2010) and the differences in performance characteristics between the various antibody-based and liquid chromatography (LC)-based assays. Although it was reported that a consistent assay bias was recognized within the NHANES data for certain time periods (2000–2006)[1], this assay drift as described in Chapter 3 is small in comparison with the inter-laboratory variation or the methodological differences observed in data from the Vitamin D External Quality Assurance Scheme (DEQAS) (Carter et al., 2010).

Accordingly, for the purposes of this study, a correction of data based on knowledge of assay drift was neither practical nor necessary for the determination of DRI values. The NHANES assay drift applies to certain data analyzed within a known time frame (2004–2006), but at the same time other data using similar methods might have experienced drift that was unknown and therefore could not be accounted for or corrected. Moreover, the dispersion of serum 25OHD levels across the range of vitamin D intakes is very large, as exemplified by data from Millen et al. (2010).

Although methodological issues contribute to uncertainty in comparing data among studies, the differences in serum 25OHD over time due to assay drift are relatively small and thus inconsequential when viewed relative to other sources of biological variation. In essence, assay drift is considered to be a component of the noise within the signal, and one of the contributors to uncertainty. But for DRI purposes it did not require re-evaluation or normalization of data. Regarding NHANES data specifically as they were used by the committee as a basis for the intake assessment (Chapter 7), the ramifications of "assay drift" are more significant

[1]Centers for Disease Control and Prevention (CDC). Available online at http://www.cdc.gov/nchs/data/nhanes/nhanes3/VitaminDanalyticnote.pdf (accessed July 8, 2010).

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for longitudinal comparisons, which were not a component of the intake assessment.

Conclusions Regarding Data for Serum 25OHD and Bone Health

The evidence presented in Chapter 4 allows the following conclusions about serum 25OHD concentrations relative to DRI development:

- *Calcium absorption*
  Given that an identified key role of vitamin D is to enhance calcium absorption, evidence regarding the level of serum 25OHD associated with maximal calcium absorption is relevant to establishing a dose–response relationship for serum 25OHD level and bone health outcomes. As outlined in Chapter 4, for both children and adults there was a trend toward maximal calcium absorption between serum 25OHD levels of 30 and 50 nmol/L, with no clear evidence of further benefit above 50 nmol/L.

- *Rickets*
  In the face of adequate calcium, the risk of rickets increases below a serum 25OHD level of 30 nmol/L and is minimal when serum 25OHD levels range between 30 and 50 nmol/L. Moreover, when calcium intakes are inadequate, vitamin D supplementation to the point of serum 25OHD concentrations up to and beyond 75 nmol/L has no effect.

- *Serum 25OHD level and fracture risk: Randomized clinical trials using adults*
  Because available trials often administered relatively high doses of vitamin D, serum 25OHD concentrations varied considerably. Although some studies suggested that serum 25OHD concentrations of approximately 40 nmol/L are sufficient to meet bone health requirements for most people, findings from other studies suggested that levels of 50 nmol/L and higher were consistent with bone health. Given that causality has been established between changes in serum 25OHD levels and bone health outcomes, information from observational studies can be useful in determining the dose–response relationship.

- *Serum 25OHD level and fracture risk: Observational studies using adults*
  Melhus et al. (2010) found that serum 25OHD levels below 40 nmol/L predicted modestly increased risk of fracture in elderly men, but there was no additional risk reduction above 40 nmol/L,

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suggesting maximum population coverage at 40 nmol/L. In contrast, Ensrud et al. (2009) observed that men with 25OHD levels below 50 nmol/L had greater subsequent rates of femoral bone loss, and there was no additional benefit from serum 25OHD concentrations higher than 50 nmol/L, suggesting maximum population coverage at 50 nmol/L. Still other studies suggested that somewhat higher serum 25OHD concentrations were needed to provide maximum population coverage. For example, Cauley et al. (2008), in a prospective cohort study, reported that serum 25OHD concentrations in the range of 60 to 70 nmol/L were associated with the lowest risk of hip fracture; above this level, risk was reported to increase, but not significantly. Looker and Mussolino (2008), using NHANES data, found that, among individuals with serum 25OHD levels above 60 nmol/L, the risk of hip fracture was reduced by one-third. The van Schoor et al. (2008) study reported that in more than 1,300 community-dwelling men and women ages 65 to 75 years, serum 25OHD levels less than or equal to 30 nmol/L were associated with a greater risk of fracture. Cauley et al. (2010) noted that men in the MrOs cohort with levels of serum 25OHD less than 50 nmol/L experienced a significant increase in hip fracture risk that was attenuated somewhat when considering hip BMD.

- *Osteomalacia from postmortem observational study*
  Data from the work of Priemel et al. (2010) have been used by the committee to support a serum 25OHD level of 50 nmol/L as providing coverage for at least 97.5 percent of the population. The data, however, do not allow specification of serum 25OHD levels above which half of the population is protected from osteomalacia and half is at risk; rather the evidence indicated that even relatively low serum 25OHD levels were not associated with the specified measures of osteomalacia, mostly likely owing to the impact of calcium intake. This is consistent with a number of studies, both from trials and from observational work, indicating that vitamin D alone appears to have little effect on bone health outcomes; it is most effective when coupled with calcium.

The wide variation in the precise relationship of serum 25OHD levels to any specific outcome for bone health is evident in the discussion above and the conclusion of the 2007 AHRQ report (Cranney et al., 2007; hereafter referred to as AHRQ-Ottawa) that a specific threshold serum 25OHD level could not be established for rickets. Nonetheless, the committee found a striking concordance of the data surrounding serum 25OHD lev-

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els across several of the specific outcomes and across age groups, which, in turn, allows an estimation of serum 25OHD concentrations that are consistent with an EAR- and RDA-type reference value when the indicators of bone health are integrated (see Figure 5-1). As shown above, the levels range between 30 and 50 nmol/L, respectively, for the EAR and the RDA. Further, the higher level of 75 nmol/L proposed by some as "optimal" and hence consistent with an RDA-type reference value is not well supported.

The congruence of the data links serum 25OHD levels below 30 nmol/L with the following outcomes: increased risk of rickets, impaired fractional calcium absorption, and decreased bone mineral content (BMC) in children and adolescents; increased risk of osteomalacia and impaired fetal skeletal outcomes; impaired fractional calcium absorption and an increased risk of osteomalacia in young and middle-aged adults; and impaired fractional calcium absorption and fracture risk in older adults. Similarly, for all age groups, there appears to be little causal evidence of additional benefit to any of these indicators of bone health at serum 25OHD levels above 50 nmol/L, suggesting that this level is consistent with an RDA-type reference value in that this value appears to cover the needs of 97.5 percent of the population. For some bone health outcomes, such as BMD

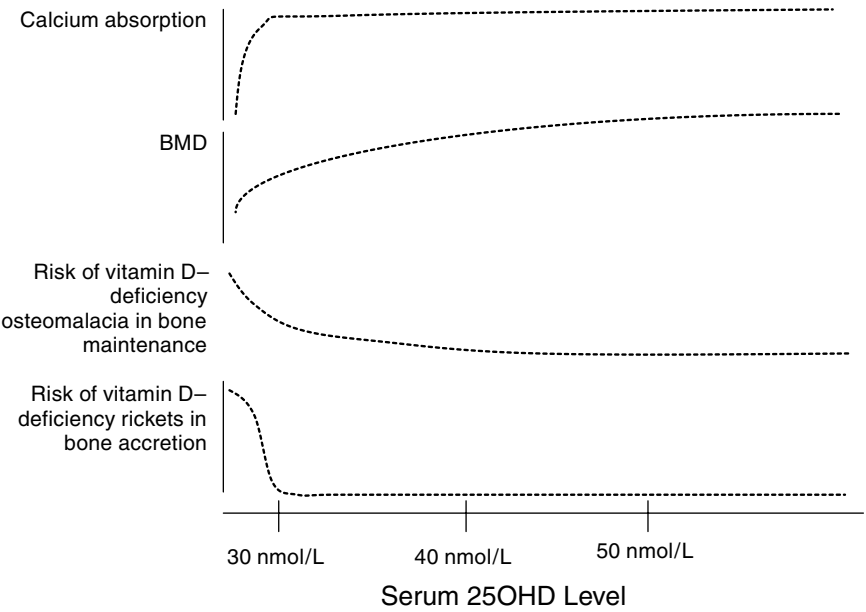


FIGURE 5-1 Conceptualization of integrated bone health outcomes and vitamin D exposure.

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in adults, the results of the available RCT(s) show a negative relationship between serum 25OHD level and outcome, and the available observational studies yield mixed results. In addition, for several of these specific outcomes, the RCTs that show benefit for what is generally a single tested dose of supplemental vitamin D do not allow inference of intermediate levels of 25OHD in serum between the placebo and dose. When evaluating the congruence of the data, the committee, therefore, looked at the lowest effective dose and the achieved serum 25OHD level. Uncertainty does exist for the selected serum 25OHD levels consistent with an EAR- and RDA-type level; this uncertainty stems from the wide range of effects and relationships and the lack of a relevant dose–response relationship.

Overall, when the data are examined for an EAR-type of serum 25OHD concentration—that is, a median type of value, a level above which approximately half the population might meet requirements and below which one-half might not—the data do not specifically provide such information, although this value can be concluded to lie between 30 and 50 nmol/L for all age groups. This is likely due to the unique inter-relationship between calcium and vitamin D. At lower levels of vitamin D, there appears to be a compensation on the part of calcium, and calcium intake can overcome the marginal levels of vitamin D. Calcium appears to be the more critical nutrient in the case of bone health, and therefore has an impact the dose–response relationship. Therefore, calcium or lack thereof may "drive" the need for vitamin D.

In the case of vitamin D—or more precisely serum 25OHD concentrations—the data, especially for adults, do not lend themselves readily to the usual DRI model, which is based on the assumption that data concerning a median intake will be as available or even more prevalent than data concerning coverage for most of the population. The standard model specifies, based on the assumption of a normal distribution for requirements, that the average or median requirement (i.e., the EAR) is used to calculate the RDA. This unanticipated situation is primarily evident for adults for whom it is not possible to estimate the level of 25OHD in serum at which 50 percent of the population is at increased risk of osteomalacia. Rather, in this case, the data allow a better estimation of the serum 25OHD level that likely covers most persons in the population. In children and adolescents, however, and to some extent in adults, the integration of these indicators as shown in Figure 5-1 enables an approximation of a level of serum 25OHD at which the risk of adverse bone health outcomes increases; however, there is uncertainty associated with this value given the limitations of the data at present. Thus, for children and adolescents, a serum 25OHD level of 40 nmol/L from the middle of the range of 30 to 50 nmol/L, at which risk to the population is increasing, was selected to serve as the targeted level for a median dietary requirements. For adults, the evidence that most are covered by a serum 25OHD level of 50 nmol/L is used

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as the starting point, and a value of 40 nmol/L is estimated as the targeted level for a median dietary requirement.

Overall, as shown in Figure 5-1, the data suggest that 50 nmol/L can be set as the serum 25OHD level that coincides with the level that would cover the needs of 97.5 percent of the population. The serum 25OHD level of 40 nmol/L serum 25OHD is consistent with the median requirement. The lower end of the requirement range is consistent with 30 nmol/L, and deficiency symptoms may appear at levels less than 30 nmol/L depending upon a range of factors. What remains is to ascertain the level of vitamin D intake that would achieve these levels of 25OHD in serum.

Integration of Data to Estimate Vitamin D Intakes to Achieve Serum 25OHD Concentrations

As diet is not necessarily the only source of vitamin D for the body, it would be ideal if the relative contribution made by sunlight to the overall serum 25OHD levels could be quantified, thereby clearing the path to better estimate total intakes of the nutrient needed to maintain a specified serum 25OHD level associated with the health outcome. In fact, however, the examination of data related to dietary recommendations about vitamin D is complicated by the confounding that sun exposure introduces, especially because the factors that affect sun exposure—such as skin pigmentation, genetics, latitude, use of sunscreens, cultural differences in dress, etc.—are not clearly measured and controlled for in research studies and in some cases not fully understood. Further, and just as critically, vitamin D requirements cannot be based on an accepted or "recommended" level of sun exposure as a means to meet vitamin D requirements, because existing public health concerns about sun exposure and skin cancer preclude this possibility. The absence of studies to explore whether a minimal-risk ultraviolet B (UVB) exposure relative to skin cancer exists to enable vitamin D production has been noted (Brannon et al., 2008).

Instead, the best remaining approach is to describe the relationship between total intake and serum 25OHD levels under conditions of minimal sun exposure. In doing so, the committee made the assumption that the outcomes, therefore, would reflect only a very small component attributable to sun exposure as would occur naturally in free-living individuals in winter in the northern hemisphere. This approach to DRI development requires that persons who use the DRI values for health policy or public health applications adjust their considerations relative to adequacy of the diet based on whether the population of interest is minimally, moderately, or highly exposed to sunlight. As mentioned previously, the potential contribution from body stores remains unknown and thus introduces uncertainty. Further, the application of the DRIs relative to assessing the

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adequacy of vitamin D intake/exposure for the population (foods, supplements, and sun exposure) would benefit from consideration of the serum 25OHD concentrations in the population of interest.

The committee examined information from controlled trials in younger and older adults and in children that could be used in the simulation to describe the relationship between vitamin D intake and changes in serum 25OHD concentrations. Of interest was the condition of minimal sun exposure, which occurs in northern latitudes and in Antarctica during their respective winters. The focus was clinical trials in Europeans or North Americans in which baseline total intake was measured or could be reliably estimated using peer-reviewed published data on baseline intakes of the population studied. In this way, the total intake of vitamin D (baseline plus supplement) was known or could be reliably estimated at latitudes greater than 50°N during late fall (October) through early spring (April) or in Antarctica during its fall (March) through its winter (October). These studies are summarized in Table 5-4. Studies needed to report measured serum 25OHD levels as means or medians with estimates of variance (standard deviation [SD], CI, or inter-quartile ranges) are included. Some studies in the United State at 40°N to 46°N were identified that met all inclusion criteria except that of latitude. These are also included in Table 5-4.

In reviewing these studies, most of which were published in the past 2 years, the committee noted the variability in the declines in serum 25OHD levels during the winter seasons in the respective hemispheres and the existence of a non-linear response to doses of vitamin D. These are discussed below prior to the description of the simulated dose–response analysis.

Winter season change in serum 25OHD levels across age groups As shown in Figure 5-2, the serum 25OHD levels of the placebo groups in the studies conducted with children (Viljakainen et al., 2006) and with younger, middle-aged, and older adults (Cashman et al., 2008, 2009; Smith et al., 2009) decreased over a wide range during the winter season at each latitude. In one study where participants started the season with lower baseline serum 25OHD levels (i.e., 36 nmol/L), the concentrations decreased only slightly (i.e., to 34 nmol/L) (Smith et al., 2009). However, in other studies where participants began the season with higher baseline serum 25OHD levels (i.e., 57 to 66 nmol/L, respectively) the serum 25OHD levels decreased more (i.e., to 34 and 43 nmol/L, respectively) (Viljakainen et al., 2006; Cashman et al., 2008, 2009), compared with those participants with lower baseline levels. In short, the decline in serum 25OHD levels in the placebo arm of these studies appears to be greatest when initial serum 25OHD levels are higher. Slightly higher intake of vitamin D (of approximately 10 to 150 IU/day, compared with other studies) in the study with

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TABLE 5-4 Key Studies on the Response of Serum 25OHD Levels to Total Dietary Vitamin D Intake in Children and Adolescents, Young and Middle-Aged Adults, and Older Adults During the Winter at High Northern Latitudes and in Antarctica When Sun Exposure Is Minimal and at Lower Northern Latitudes When Sun Exposure Is Reduced

Reference; Type of Study	Location (Latitude)	Season (Duration)	Population Description	Baseline Vitamin D Intake (IU/day)	Baseline 25OHD Level (nmol/L)	Vitamin D Dose (IU/day)	Total Vitamin D Intake (IU/day)	Achieved 25OHD Level (nmol/L)
Children and adolescents								
Latitude ≥ 50°N								
Ala-Houhala et al., 1988 *RCT*	Finland (61°N)	February–March (1 y)	8–10 y boys/girls $n = 60$	200[a]	46.0 ± 15.5 ($n = 27$)	0	200[a]	43.3 ± 19.5
					49.3 ± 19.0 ($n = 24$)	400	600[a]	71.3 ± 23.8
Schou et al., 2003 *Double-blind RCT/crossover*	Denmark (55°N)	November–January (4 wk)	6–14 y boys/girls $n = 20$	96[a]	—	0	96	33.7 ± 3.3 ($n = 10$) 32.3 ± 4.1 ($n = 10$)
						600	696[b]	50.2 ± 4.5 ($n = 10$) 43.4 ± 2.9 ($n = 10$)
Viljakainen et al., 2006 *RCT*	Finland (61°N)	September–March (1 y)	11 y girls $n = 212$	200	47.8 ± 18.2 ($n = 73$)	0	200	42.8
				188	46.3 ± 17.4 ($n = 65$)	200	388	51.7
				196 (FFQ; 10–14% using unspecified supplement)	46.7 ± 15.2 ($n = 64$)	400	596	58.8

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Latitude 40–49° N								
Rajakumar et al., 2008 *Non-RCT*	Pittsburgh, PA (40°N)	December–April (1 mo)	6–10 y boys/girls African American obese and nonobese $n = 41$	218 (obese) 339 (nonobese) (FFQ)	55.5± 24.0 64.8 ± 24.3	400 400	618 738	65.5 ± 20.3 72.8 ± 16.8
Young and middle-aged adults								
Latitudes ≥ 50°N and Antarctica								
Cashman et al., 2009 *Double-blind RCT*	Cork, Ireland (51°N) Cochrane, Northern Ireland (55°N)	October/November–February/April (22 wk)	mean 29.9 ± 6.2 y range 20–40 y men/women	135 (80–200)	65.7 (58.4–94.1) ($n = 57$)	0	135	37.4 (31.4–47.9)
				172 (88–228)	60.0 (50.0–89.7) ($n = 48$)	200	372	49.7 (44.6–60.9)
				140 (92–188)	72.2 (55.7–81.9) ($n = 57$)	400	540	60.0 (51.0–69.1)
				144 (72–232) (FFQ)	76.9 (55.9–89.3) ($n = 53$)	600	744	69.0 (59.1–84.2)
Smith et al., 2009 *Double-blind RCT*	Antarctica (78°S)	June/July–August (5 mo with 0, 18 wk, and 25 wk samples)	39–44 y men/women	302 (235–302)	44 ± 18 ($n = 18$)	400	659	55 ± 19 (18 wk) 57 ± 15 (25 wk)
				329 (328–352)	44 ± 19 ($n = 19$)	1,000	1,342	63 ± 20 (18 wk) 63 ± 25 (25 wk)
				356 (276–356) (Diet questionnaire)	45 ± 14 ($n = 18$)	2,000	2,305	71 ± 20 (18 wk) 71 ± 23 (25 wk)

continued

TABLE 5-4 Continued

Reference; Type of Study	Location (Latitude)	Season (Duration)	Population Description	Baseline Vitamin D Intake (IU/day)	Baseline 25OHD Level (nmol/L)	Vitamin D Dose (IU/day)	Total Vitamin D Intake (IU/day)	Achieved 25OHD Level (nmol/L)
Viljakainen et al., 2009 *Double-blind RCT*	Helsinki, Finland (61°N)	November–April (25 wk)	21–49 y men	264 ± 112	64.7 ± 18.5 (n = 16)	0	264	52.2 (Δ −12.5 ± 9.1)
				304 ± 2,202	60.3 ± 11.6 (n = 16)	412	716	75.4 (Δ +15.3 ± 2.3)
				344 ± 252 (FFQ)	62.3 ± 13.6 (n = 16)	760	1,104	90.1 (Δ +27.8 ± 17.5)
Latitudes 40–49°N								
Biancuzzo et al., 2010 *Double-blind RCT*	Boston, MA (42°N)	February–May (11 wk)	mean 29–41 y range 18–79 y men and women including European Americans, Asian Americans, African Americans, North Americans, Hispanic Americans	200[c]	49.5 ± 24 (n = 15)	0	200	45.3 ± 16.0
					49.0 ± 27.8 (n = 20)	1,000 D_3	1,200	70.0 ± 27.5
					41.5 ± 24.8 (n = 16)	1,000 D_2	1,200	68.5 ± 26.3
Harris and Dawson-Hughes, 2002 *RCT*	Boston, MA (42°N)	December–April (8 wk)	18–35 y	132	48.9 ± 17.2	0	132	53.5 (Δ −4.6 ± 6.5) (n = 13)
				71 (FFQ)	59.9 ± 16.4	800	871	82.4 (Δ 22.5 ± 14.7) (n = 14)
Heaney et al., 2003[d] *RCT*	Omaha, NE (41.2°N)	October–March (120–140 d)	38.7 ± 11.2 y	< 200 (estimated from milk consumed)	70.1 ± 5.8	0	< 200	52
					72.0 ± 4.0	1,000	< 1,200	77.1
					69.3 ± 4.2	5,000	< 5,200	150
					65.6 ± 6.3	10,000	< 10,200	212

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Holick et al., 2008 *Double-blind RCT*	Boston, MA (42°N)	February–May (11 wk)	mean 35.5–40.5 y men and women	316[c] (Diet questionnaire used, but baseline intakes not reported)	46.5 ± 22.2 (n = 55) 49.0 ± 27.8 (n = 20) 42.3 ± 26.3 (n = 16) 50.5 ± 26.0 (n = 18)	0 1,000 (D_3) 1,000 (D_2) 500 D_2 + 500 D_2	316 1,316 1,316 1,316	47.0 ± 19.8 72.3 ± 27.5 67.0 ± 24.0 71.0 ± 19.3
Li-Ng et al., 2009 *Double-blind RCT*	Long Island, NY (40.7°N)	December–March (3 mo)	mean 58.1–59.3 y range 18–80 y including European Americans, African Americans, Asian Americans	168.0 ± 146.5 147.3 ± 182.3 (FFQ)	63.0 ± 25.8 (n = 70) 64.3 ± 25.4 (n = 78)	0 2,000	168 2,147	61.9 (Δ –2.1) 88.5 ± 23.2
Nelson et al., 2009 *Double-blind RCT*	Bangor, ME (44.5°N)	September–February	19–35 y women	140 ± 104 140 ± 124 (4 d food records)	61.9 ± 22.5 62.1 ± 24.0 (n = 31)	0 800	140 940	72.7 ± 27.8 97.4 ± 31.3
Older adults								
Latitude ≥ 50°N								
Cashman et al., 2009 *Double-blind RCT*	Cork, Ireland (51°N) Cochrane, Northern Ireland (55°N)	September/November–February/April (22 wk)	mean 70.7 ± 5.4 y > 64 y men/women	188 164 168 192 (7 d diet record)	58.8 (43.6, 78.5) (n = 55) 51.8 (41.3, 68.7) (n = 48) 54.3 (42.6, 72.0) (n = 53) 55.1 (39.6, 70.4) (n = 48)	0 200 400 600	188 364 568 792	41.6 (28.0–55.4) 53.2 (45.6–68.7) 69.5 (58.0–81.4) 73.8 (62.0–89.2)

continued

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TABLE 5-4 Continued

Reference; Type of Study	Location (Latitude)	Season (Duration)	Population Description	Baseline Vitamin D Intake (IU/day)	Baseline 25OHD Level (nmol/L)	Vitamin D Dose (IU/day)	Total Vitamin D Intake (IU/day)	Achieved 25OHD Level (nmol/L)
Honkanen et al., 1990 *RCT*	Kuopio, Finland (62.9°N)	November/December–February/March (11 wk)	67–72 y women	380[a]	36.2 ± 2.7 (n = 26)	0	380	23.3 (18–28, CI)
					42.8 ± 3.5 (n = 25)	1,800	2,180	80.7 (75–86, CI)
Larsen et al., 2004 *RCT*	Randers, Denmark (56°N)	January–June (1 mo)	mean 74–74.9 y range 65–103 y men/women	136[a] for women	33 ± 19 (n = 37)	0	136	34 ± 19
					37 ± 19 (n = 67)	400	536	46 ± 17
					49.0 ± 14.2 (n = 22)	350	414	59 ± 20 (combined 350–400 IU)
					50.0 ± 15.9 (n = 23)	400	464	
Van Der Klis et al., 1996 *RCT*	Groningen, Netherlands (53.2°N)	April–May (5 wk)	61 y Dutch women	64[e] (n = 20)	61.2 ± 2.4	0	64	NS from baseline
						400	464	87.9 ± 26.9 (n = 19)
						800	864	87.9 ± 26.9 (n = 19)
Viljakainen et al., 2006 *RCT*	Helsinki, Finland (61°N)	January–April (12 wk)	65–85 y women	436	52.2 ± 19.9 (n = 12)	0	436	43.9 (Δ −8.3 ± 13.2)
				388	46.0 ± 14.3 (n = 13)	200	588	56.9 (Δ +10.9 ± 8.9)
				424	46.5 ± 10.2 (n = 11)	400	824	60.9 (Δ +14.4 ± 4)
				388 (FFQ)	44.1 ± 13.5 (n = 13)	800	1,188	67.8 (Δ 23.7 ± 11.9)

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Latitudes 40–49°N								
Dawson-Hughes et al., 1991 *Double-blind RCT*	Boston, MA (42°N)	February–May (winter period of 12-mo study)	mean 61–62 y women	90 110 (FFQ)		0 400	90 510	60.6 (55.6–65.6, CI) (n = 125) 92.1 (87.9–96.3, CI) (n = 121)
Harris and Dawson-Hughes, 2002 *RCT*	Boston, MA (42°N)	December–April (8 wk)	62–79 y	115 142 (FFQ)	53.8 ± 18.2 61.5 ± 15.7	0 800	115 942	49.3 (Δ −4.5 ± 6.5) (n = 11) 83.6 (Δ −22.1 ± 13.4) (Δ + 22.1 ± 13.4) (n = 14)

NOTE: BMD = bone mineral density; FFQ = food frequency questionnaire; IU = International Units; mo = month(s); NS = not significant; RCT = randomized controlled trial; wk = week(s); y = year(s).

[a]Baseline intakes from Andersen et al. (2005).

[b]Baseline intake from Ambroszkiewicz et al. (2007).

[c]NHANES intake data for 2005–2006.

[d]Achieved serum 25OHD levels for Heaney et al. (2003) were extracted from their graphic presentation in the article, and no variance could be extracted.

[e]Bergink et al. (2009).

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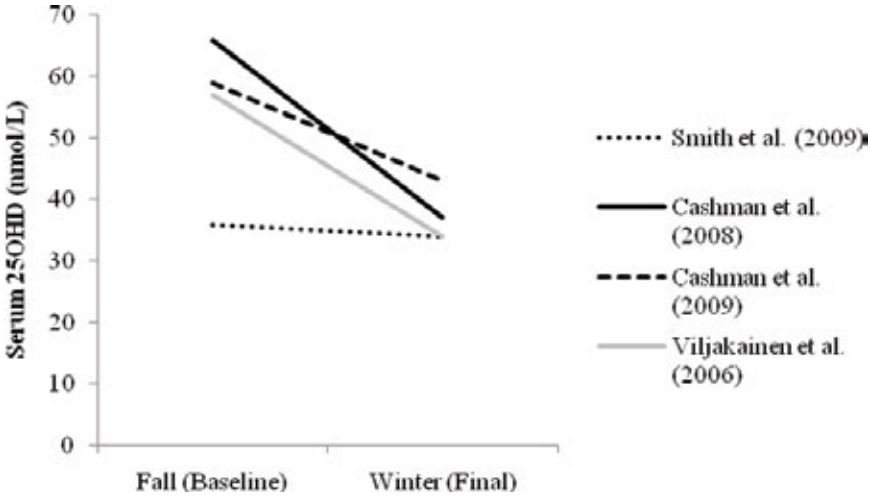


FIGURE 5-2 Fall (baseline) and winter (final) values of serum 25OHD concentrations in non-supplemented placebo (or no pills) groups measured during minimal sun and UVB exposure (Cashman et al., 2008, 2009; Smith et al., 2009) or at the same season for the year-long trials in children (Viljakainen et al., 2006) at latitudes above 50°N or in Antarctica.
NOTE: Values in the graph from Viljakainen et al. (2006) differ from values listed in Table 5-4 due to the use of a subgroup (6-month values for girls recruited in the fall) for graphing purposes.

the lowest baseline serum 25OHD levels (Smith et al., 2009) may have accounted for the attenuated reduction in serum 25OHD level.

A similar trend exists across many of the studies with a placebo group, as summarized in Table 5-4. Declines of 3 to 13 nmol/L in serum 25OHD level are reported for those with baseline levels from 36 to 47 nmol/L. Larger declines in serum 25OHD levels of 8 to 62 nmol/L are reported for those with baseline levels of 64 to 96 nmol/L. However, considerable variability exists in the seasonal decline in serum 25OHD level in winter months, as demonstrated by the increases of 1 nmol/L in some participants with baseline serum 25OHD levels of 33 nmol/L at latitudes above 50°N (Larsen et al., 2004), and increases of 4.6 to 10.8 nmol/L from a baseline of 48.9 to 61.9 nmol/L in some participants at latitudes above 42°N (Harris et al., 2002; Nelson et al., 2009).

These observations suggest that the assumption of minimal sun exposure was met. Further, they suggest that during the winter season small intakes of vitamin D may play a role in attenuating the winter decline in serum 25OHD levels in those with lower baseline serum 25OHD levels. They

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also suggest that the kinetics of vitamin D turnover or mobilization from stores may differ in those who have lower baseline serum 25OHD levels. Further, it is possible that the greater decline of serum 25OHD levels in those with higher baseline levels could, perhaps, also represent regression to the mean, at least in part. At this time, it is not possible to clarify which of these possibilities occur.

Non-linear response to vitamin D dosing The available data suggest a non-linear response of serum 25OHD above baseline levels to doses of vitamin D for all age groups. Non-linear response to doses of vitamin D (total or IU/kg) is also reported in mice (Fleet et al., 2008) and rats (Anderson et al., 2008; Fleet et al., 2008), demonstrating the biological plausibility of a non-linear response of serum 25OHD concentrations to vitamin D intake. It is noted that AHRQ-Ottawa and Heaney et al. (2003) reported a linear relationship between serum 25OHD levels and vitamin D dosing that ranges from 0.7 nmol/L per 40 IU (Heaney et al., 2003) to 1 to 2 nmol/L per 100 IU (AHRQ-Ottawa). Notably, AHRQ-Ottawa found heterogeneity that remained after adjusting for dose. However, in the studies considered by the committee, there is a steeper rise in serum 25OHD levels when vitamin D dosing is less than 1,000 IU/day of vitamin D. A slower, more flattened response is seen when doses of 1,000 IU/day or higher are administered. In short, regardless of baseline intakes or serum 25OHD levels, under conditions of dosing the increment in serum 25OHD above baseline differs depending upon whether the dose was above or below 1,000 IU/day. This is evidenced by examining several studies in young, middle-aged and older adults.

Smith et al. (2009) in Antarctica found a low serum 25OHD level of 37 nmol/L in men and women during the winter season (June to September). The rise in serum 25OHD levels with doses of 400, 1,000, and 2,000 IU/day after 13 and 20 weeks was 2.1, 0.8 and 0.54 nmol/L per 40 IU/day, respectively. In two other studies at latitudes of 52°N to 55°N during winter, the rise in serum 25OHD levels in response to 200, 400, or 600 IU of vitamin D per day with serum 25OHD baseline levels of 37 to 42 nmol/L was examined in young and older individuals. The average rise in serum 25OHD levels was equivalent to approximately 2.3 nmol/L for an intake of 40 IU vitamin D_3 per day without a difference due to age (Cashman et al., 2008, 2009). Others also found that age does not influence the change in serum 25OHD level in response to vitamin D intake (Harris and Dawson-Hughes, 2002). When the dose is 1,000 IU/day or higher, the rise in serum 25OHD level in individuals of all ages is approximately 1 nmol/L for a 40 IU/day intake, which is similar to the response to vitamin D intake found in the AHRQ-Ottawa analysis.

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Analysis and Outcomes

A regression analysis of the relationship between serum 25OHD level and total intake of vitamin D during the winter season at latitudes above 49.5°N or in Antarctica, a period of low sun and UVB exposure, was carried out for each of three age groups—children and adolescents, young and middle-aged adults, and older adults. This approach differs from the others such as the study reported by Heaney et al. (2003) in that total vitamin D intake and not just a supplemental dose of vitamin D was considered, and because we show a non-linear response to total intake rather than the linear response published previously. The interest for this report was an approach that would be relevant to determining the intake needed to achieve the serum 25OHD levels consistent with an EAR- and RDA-type value. The regression analysis using a mixed effect model was preceded by a log transformation of the total vitamin D intake data because the log transformation was the best curvilinear fit. The model controlled for the effect of study clustering by including study as a random effect. Controlling for study effect using a random effect was needed because the interclass correlation of the variance due to study effect compared with the total variance was very high, approximately 95 percent overall, with about 88 percent for children and adolescents, 95 percent for young and middle-aged adults, and 96 percent for older adults. The regression was set for a y_0 intercept of 0 nmol of 25OHD per liter of serum, consistent with the biological reality preventing a negative value for achieved serum 25OHD levels. Baseline serum 25OHD levels did not have significant effect, and was, therefore, not included in the analysis.

The outcome is presented in Figure 5-3. Importantly, age did not significantly affect the response of serum 25OHD level to log vitamin D intake. Neither the main effect of age ($p = 0.162$) nor the interaction term between age and the log of total vitamin D intake ($p = 0.142$) was significant. Thus, there was no effect of age in the response of serum 25OHD level to total intake among the three age groups—children and adolescents, young and middle-aged adults, or older adults. This finding suggests that across ages under conditions of minimal sun exposure, similar intakes of vitamin D result in similar serum 25OHD concentrations, as shown in Figure 5-4.

Because there was no age effect in the response of serum 25OHD level to total intake of vitamin D, a single, combined regression analysis with study as a random effect was carried out. This resulted in the predictive equation of achieved 25OHD in nmol/L = 9.9 ln (total vitamin D intake) with predicted CIs of $y = 8.7$ ln total vitamin D intake) and upper interval of $y = 11.2$ ln (total vitamin intake), as specified in Figure 5-4.

The committee also analyzed the achieved 25OHD with total vitamin D intake at latitudes between 40°N to 49°N during the winter (data shown

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in Table 5-4 above) for which assumption of minimal sun exposure may not be as fully met as at latitudes above 49.5°N or in Antarctica during the winter. The approach was the same as described above for the simulated dose–response in which achieved serum 25OHD level was analyzed at latitudes above 49.5°S. The interclass correlation was large, approximately 80 percent, and study effect was again included as a random effect in the mixed effects model. Age did not affect achieved serum 25OHD level relative to log total vitamin D intake ($p = 0.09$ for main effect and $p = 0.6$ for the interaction of age and log total vitamin D intake), although the data available for children was limited to one study. Therefore, a combined analysis of all age groups at the lower latitudes was conducted. The predicted achieved serum 25OHD level was $y = 12.3$ ln (total vitamin D intake), which explained 45 percent of the within-study variability and 96.6 percent of the between-study variability. The predicted upper and lower CIs for achieved serum 25OHD levels were $y = 10.1$ ln (total vitamin D intake) and $y = 14.5$ ln (total vitamin D intake). There was a significant difference between lower and higher latitudes ($p = 0.000$ for the main effect and $p = 0.021$) for the interaction of latitude and ln (total vitamin D intake). Compared to the simulated dose–response at higher latitudes, the achieved serum 25OHD level at lower latitudes was 24 percent greater for the same total intake as that achieved at higher latitudes. Of note, less of the within-study variance at lower latitudes was explained by the total vitamin D intake (45 percent) compared to that explained (72 percent) for the higher latitudes. Taken together, these results suggest that sun exposure may be more than minimal at lower latitudes, as anticipated. Thus, the committee used the simulated dose–response at the higher latitudes to ensure minimal sun exposure to ensure as little contribution from endogenous production as the evidence allows.

Given the lack of an age effect in the response of the achieved serum 25OHD levels to any total intake of vitamin D, the intake to achieve the EAR-type value of 40 nmol/L was the same across all groups. An intake of 400 IU is associated with a predicted mean circulating 25OHD level of 59 nmol/L in children and adolescents, young and middle-aged adults, and older adults with a lower predicted CI of approximately 52 nmol/L. An intake of 600 IU/day predicts a mean serum 25OHD level of 63 nmol/L in children, adults, and older adults with a lower predicted CI of 56 nmol/L. Although this suggests that intakes of 400 and 600 IU would over-shoot the targeted serum 25OHD concentrations, there is considerable uncertainty in this simulated dose–response relationship that needs to be taken into account. This includes: (1) the large inter-study variance, which is most pronounced in older persons; (2) predicted lower CIs for each age group resulting in an achieved serum 25OHD level of 36 to 46 nmol/L for a 400 IU/day intake and a 38 to 49 nmol/L for a 600 IU/day intake (as

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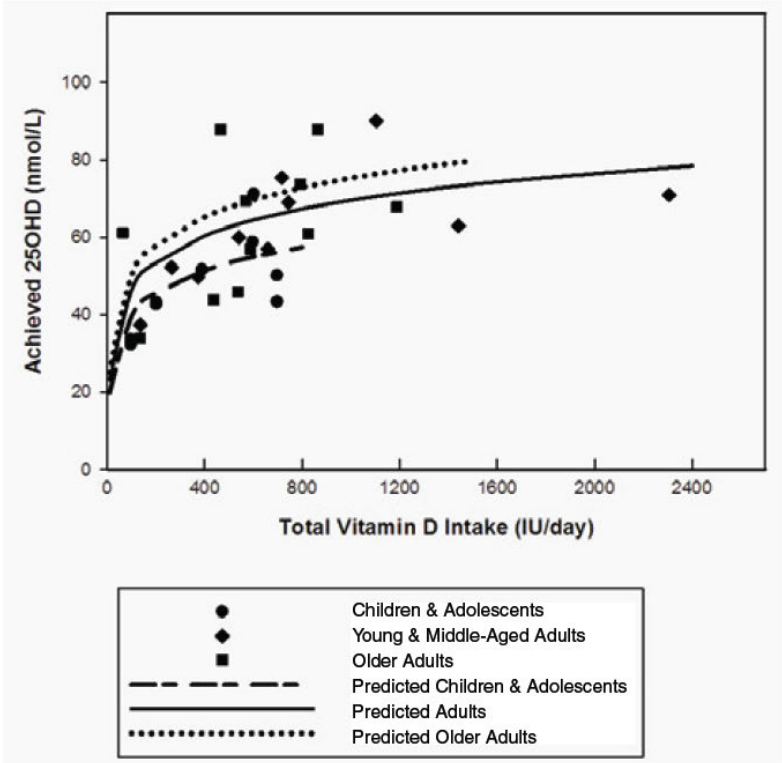


FIGURE 5-3 Response of serum 25OHD level to total intake of vitamin D in northern latitudes in Europe and Antarctica during their respective winter seasons

shown in Figure 5-4), even though there is no significant age effect; (3) the uncertainties in the comparability of the serum 25OHD levels measured with different assays across these studies; and (4) the uncertainty surrounding the predicted CIs of this relationship. Given these limitations and the uncertainties, the committee selected the estimated intakes needed in a fashion that would err on the side of the specified intake "overshooting" the targeted serum value to ensure that the specified levels of intake achieved the desired serum 25OHD levels of 40 and 50 nmol/L. This approach is used despite possible contributions to serum 25OHD from sun exposure that could not be taken into account.

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when effective sun exposure for endogenous vitamin D synthesis is minimal. Mean or median responses of serum 25OHD level to total intake in the winter seasons at northern latitudes (> 49.5°N) and in Antarctica (78°S) (summarized in Table 5-4) were analyzed using a mixed effect model by regression following log transformation with study in a random effects model to control for the large study residual variability for: (1) children and adolescents (boys and girls) ages 6 to 14 years in Finland (Ala-Houhala et al., 1988); (2) young and middle-aged adults ages 19 to 59 years from men in Antarctica (Smith et al., 2009), Ireland (Cashman et al., 2008, 2009), and Finland (Viljakainen et al., 2006, 2009), and Denmark (Schou et al., 2003); and (3) older adult women and men > 60 years of age in Ireland (Cashman et al., 2009), the Netherlands (Van Der Klis et al., 1996), Finland (Viljakainen et al., 2006), and Denmark (Larsen et al., 2004). The relationship of serum 25OHD level to total intake of vitamin D is:

- For children and adolescents: achieved serum 25OHD = 8.6 ln(total vitamin D intake), which explains 68.8 percent of the within-subject variability and 98.3 percent of the between-study variability. Predicted CIs were y = 6.0 ln (total vitamin D intake) for lower limit, and y = 11.3 ln (total vitamin D intake) for upper limit.
- For young and middle-aged adults: achieved serum 25OHD = 10.1 ln (total vitamin D intake), which explained 70.3 percent of the within-study variability and 98.4 percent of the between-study variability. Predicted CIs were y = 6.3 ln (total vitamin D intake) for lower limit, and y = 13.8 ln (total vitamin D intake) for upper limit.
- For older adults > 71 years: achieved 25OHD = 10.9 ln (total vitamin D intake), which explains 77.5 percent of the within-study variability and 92.2 percent of the between-study variability. Predicted CIs were y = 7.7 ln (total vitamin D intake) for lower limit and y = 14.2 ln (total vitamin D intake) for upper limit.
- The interaction term between age and the log of total vitamin D intake (p = 0.142), as well as the main effect of age (p = 0.162) were not significant.

NOTE: log(total vitamin D) has been back-transformed to total vitamin D for presentation in this figure.

Specification of Vitamin D Dietary Reference Intakes for Adequacy

The DRIs for adequacy for vitamin D have been introduced previously in Table 5-3. The rationale for each is presented in the discussions below.

Infants 0 to 12 Months of Age

Infants 0 to 6 Months of Age	
Infants 6 to 12 Months of Age	
	AI 400 IU (10 µg)/day Vitamin D

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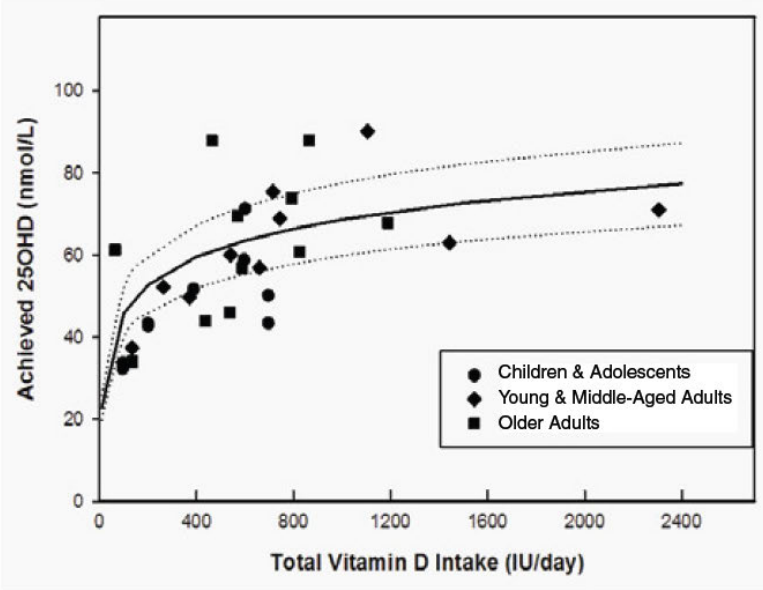


FIGURE 5-4 Response of serum 25OHD level to total intake of vitamin D in all age groups in northern latitudes in Europe and Antarctica during their respective winter seasons when effective sun exposure for endogenous vitamin D synthesis is minimal. Mean responses of serum 25OHD level to total vitamin D intake in the winter seasons at latitudes 49.5°N (Europe) and 78°S (Antarctica) for ages 6 to > 60 years (Ala-Houhala et al., 1988; Van Der Klis et al., 1996; Schou et al., 2003; Larsen et al., 2004; Viljakainen et al., 2006, 2009; Cashman et al., 2008, 2009; Smith et al., 2009; see Table 5-4 for summary of studies) were analyzed by regression using mixed effect model following log transformation controlling for study effect by a random effects model because there was no effect of age on the response of serum 25OHD level to total intake of vitamin D. The relationship for achieved vitamin D is y achieved 25OHD in nmol/L = 9.9 ln (total vitamin D intake) (shown as solid line) with predicted CIs (shown as two dashed lines) for lower interval of $y = 8.7$ ln (total vitamin D intake) and upper interval of $y = 11.2$ ln (total vitamin D intake). This regression explains 72 percent of the within-study variability and 96.4 percent of the between-subject variability.

NOTE: Log (total vitamin D intake) was back-transformed to total vitamin D intake for presentation in this figure.

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Data are not sufficient to establish an EAR for infants less than 1 year of age, and therefore an AI has been developed. Unlike the case for calcium, the content of human milk does not shed light on the vitamin D requirements of infants, as breast milk is not a meaningful source of vitamin D.

The AI for the 0 to 6 months and 6 to 12 months life stage groups is set at 400 IU of vitamin D per day. There are very limited data beyond the conclusion that maintaining serum 25OHD concentrations in this life stage group above 30 nmol/L, and more likely closer to 50 nmol/L, appears to cover adequately the needs of the majority of the infants and support normal bone accretion. There are no data to suggest that older infants would benefit from higher intakes.

Intakes in the range of 400 IU/day appear consistent with maintenance of the desirable serum 25OHD concentrations. There are no reports of a clinical deficiency in infants receiving 400 IU of vitamin D per day, and an intake of 400 IU/day appears to maintain a serum 25OHD level generally above 50 nmol/L in infants (Greer et al., 1982; Rothberg et al., 1982; Ala-Houhala, 1985; Ala-Houhala et al., 1988; Greer and Marshall, 1989; Hollis and Wagner, 2004). There are differences in the volume of milk or formula intake during this 12-month period, with newborns taking in less than older infants. The AI of 400 IU/day, therefore, represents an overall intake for the first year of life, and may vary across the life stages; it also assumes early introduction of a supplement for breast-fed babies. In the case of exclusive formula feeding, there is an assumption of a gradual increase in intake to 800 to 1,000 mL/day during infancy, which for most standard formulas provides about 400 IU/day. Note is made of the case reports concerning the development of rickets among dark-skinned infants who are exclusively breast-fed and not provided a vitamin D supplement (see Chapter 8).

Children and Adolescents 1 Through 18 Years of Age

Children 1 Through 3 Years of Age	
Children 4 Through 8 Years of Age	
Children 9 Through 13 Years of Age	
Adolescents 14 Through 18 Years of Age	
	EAR 400 IU (10 µg)/day Vitamin D
	RDA 600 IU (15 µg)/day Vitamin D

For these life stage groups, ensuring normal, healthy bone accretion is central to the DRI values. The requirement distribution developed using serum 25OHD concentrations and the intakes estimated to achieve such concentrations are the basis for the reference values.

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For very young children in this life stage group, virtually no data are available to link vitamin D nutriture directly to measures related to bone health outcomes. AHRQ-Ottawa examined the relationship between vitamin D and rickets in children 0 to 5 years of age but found no studies that evaluated BMC, BMD, or fractures in comparison with measures of vitamin D intake. Likewise, AHRQ-Tufts found no studies that update AHRQ-Ottawa.

AHRQ-Ottawa did consider serum 25OHD concentrations in the context of the onset of rickets in newborns through children 5 years of age and identified serum concentrations below 27.5 nmol/L as being consistently associated with rickets. However, many of the relevant studies were from developing countries where calcium intake is low; therefore, for these studies, the onset of rickets was associated with higher levels of 25OHD in serum, likely due to low calcium intakes. Specker et al. (1992) has concluded that serum concentrations of approximately 27 to 30 nmol/L places the infant at an increased risk for developing rickets, although the measure is not diagnostic of the disease.

Although the prevention of rickets can be a factor in establishing reference values, it is important to seek measures that are consistent with favorable bone health outcomes. Maximizing calcium absorption, especially for this life stage group, is therefore a reasonable parameter to take into account. Here, as with rickets, serum 25OHD measures are the only data available and there are no direct measures of vitamin D intake. Abrams et al. (2009) conducted calcium absorption studies in 251 children ranging in age from 4.9 to 16.7 years and found that children with serum 25OHD levels of 28 to 50 nmol/L had higher fractional calcium absorption than children with serum 25OHD levels at or greater than 50 nmol/L, suggesting again at the least that maximal calcium absorption is reached at 50 nmol/L. Fractional calcium absorption did not increase with serum 25OHD concentration levels above 50 nmol/L. The findings are consistent with the conclusions reached previously concerning serum 25OHD levels associated with maximum population coverage. Further, as rickets in populations that are not calcium deficient occurs at serum 25OHD levels below 30 nmol/L, it is reasonable to assume that 40 nmol/L is associated with an average requirement.

Serum 25OHD concentrations of 40 to 50 nmol/L would ideally coincide with bone health benefits such as positive effects on BMC and BMD. AHRQ-Ottawa found that there was *fair evidence* that circulating 25OHD levels are associated with a positive change in BMD and BMC in studies in older children and adolescents. The serum 25OHD concentrations varied from 30 to 83 nmol/L. A study conducted by Viljakainen et al. (2006) reported that vitamin D intakes of 200 and 400 IU/day in adolescent girls were associated with positive BMC measures at serum 25OHD levels

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of 50 nmol/L and above. This is consistent with conclusions inferred from calcium absorption studies and, in turn, with the ability to cover the requirements for nearly all in the population. A relatively wide range of total vitamin D intakes reportedly achieved serum 25OHD concentrations between approximately 40 and 60 nmol/L, but most intakes were between about 350 and 600 IU/day. The variability in the data cannot be readily attributed to differences in sun exposure because the studies were all conducted in northern locations during primarily winter months.

Taken as a body of evidence and in the absence of measures that directly relate total intake to health outcomes, the information concerning serum 25OHD concentrations associated with rickets prevention, calcium absorption, and positive effects on BMC measures are consistent with discussions above concerning a requirement distribution based on serum 25OHD concentrations. They support the conclusion that an average requirement for vitamin D for these life stage groups is associated with the achievement of concentrations of 25OHD in serum of 40 nmol/L. Further, they support the conclusion that the requirements for nearly all children and adolescents are covered when serum 25OHD concentrations reach 50 nmol/L. These findings are universally applicable across all children and adolescents from 1 to 18 years of age.

The analysis conducted, described above, indicates that an intake of vitamin D of 400 IU/day achieves serum concentrations of 40 nmol/L, and this intake is therefore set as the EAR for persons 1 to 3 years, 4 to 8 years, 9 to 13 years, and 14 to 18 years of age. As this requirement distribution appears to be normally distributed, the assumption of another 30 percent to cover nearly all the population (i.e., 97.5 percent) is appropriate and consistent with a serum 25OHD level of approximately 50 nmol/L as the target for an RDA value. Based on the same analysis relating serum 25OHD levels to intake, an intake of 600 IU/day is set as the RDA. These reference values assume minimal sun exposure.

Adults 19 Through 50 Years of Age

Adults 19 Through 30 Years of Age Adults 31 Through 50 Years of Age	
	EAR 400 IU (10 μg)/day Vitamin D
	RDA 600 IU (15 μg)/day Vitamin D

For these life stage groups, bone maintenance is the focus. The requirement distribution based on serum 25OHD concentrations and the intakes estimated to achieve such concentrations are the basis for the reference values. As described below, the available data have provided more

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information about intakes and serum 25OHD levels consistent with an RDA value than they have for an EAR value.

Data relating bone health outcomes to vitamin D intake are generally limited for adults 19 to 50 years of ages. Although bone mass measures are, of course, studied in this population, consideration of the dose–response relationship between vitamin D and bone health are not usually included in such studies. In fact, there are no randomized trials in this age group, and whatever data are available come from association studies. The results are inconsistent, in part because the confounding inherent in observational studies.

Serum 25OHD concentrations relative to calcium absorption, therefore, provide an important basis for DRI development for vitamin D for these life stage groups. The conclusions described above indicating that calcium absorption is maximal at serum 25OHD concentrations between 30 and 50 nmol/L with no consistent increase in calcium absorption above approximately 50 nmol/L are informative in estimating the relevant EAR and RDA values for vitamin D for these life stage groups.

In contrast, although data from a very recent study (Priemel et al., 2010) based on post-mortem analysis of the relationship between serum 25OHD levels and osteomalacia and re-examined by the committee (as described above) suggest a serum 25OHD level that would cover the needs of approximately 97.5 percent of the population, they also reveal that a level of serum 25OHD consistent with an average requirement is somewhat elusive. That is, serum 25OHD levels of approximately 40 nmol/L to even 30 nmol/L might be expected to be consistent with coverage for no more than half of the population (i.e., a mean/median value). But, in the Priemel et al. (2010) report, even at serum 25OHD levels well below 30 nmol/L more than half of the population studied failed to demonstrate osteomalacia as defined histologically in the study. In essence, these data, which admittedly have limitations, suggest that for some adults the need for vitamin D is extremely low. This is likely due to the very strong interrelationship between calcium and vitamin D; it may even suggest that calcium is the "driver" nutrient relative to bone health, and that calcium is able to more readily overcome lower levels of vitamin D for the purposes of bone health, while vitamin D is likely unable to compensate for a lack of calcium. This finding underscores the uncertainties that are introduced by the calcium-vitamin D interrelationship.

For the purposes of ensuring public health in the face of uncertainty and providing a reference value for stakeholders, a prudent approach is to begin the consideration of the DRIs for these age groups with the level of 25OHD in serum that is consistent with coverage of the requirement of nearly all adults in this age range, that is, 50 nmol/L. Taken together with calcium absorption and BMD, and assuming a normal distribution

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of requirements, given no evidence that the distribution is not normal, a serum level of 40 nmol/L can be set as consistent with a median requirement. This modified approach is bolstered by—and consistent with—the relationship between serum 25OHD levels and calcium absorption, in which serum 25OHD levels of between 30 and 50 nmol/L were consistent with maximal calcium absorption. Based on these considerations as well as the intake versus serum response analysis described above, an EAR of 400 IU/day and an RDA of 600 IU/day are established for adults 19 to 50 years of age. These DRI values assume minimal sun exposure.

Adults 51 Years of Age and Older

Adults 51 Through 70 Years of Age	
	EAR 400 IU (10 μg)/day Vitamin D
	RDA 600 IU (15 μg)/day Vitamin D
Adults >70 Years of Age	
	EAR 400 IU (10 μg)/day Vitamin D
	RDA 800 IU (20 μg)/day Vitamin D

For persons in these life stage groups of 51 through 70 years and >70 years, the ability to maintain bone mass and reduce the level of bone loss is the primary focus for DRI development. Evidence related to fracture risk becomes central. For this reason, DRIs for adults >70 years of age are discussed first, followed by DRIs for adults 51 through 70 years of age.

Adults 70 Years of Age and Older

The discussions above concerning serum 25OHD levels in relation to bone health indicate that several newer studies have helped to elucidate a relationship between serum 25OHD concentrations and bone health benefits based on measures of calcium absorption and osteomalacia for a wide age range of adults. These data when used for the purposes of DRI development—coupled with the approximation of intake associated with serum 25OHD concentrations derived from the simulation analysis carried out by the committee—provide a basis for an EAR for young and middle-aged adults of 400 IU/day vitamin D consistent with a serum 25OHD concentration of 40 nmol/L, and for an RDA of 600 IU/day consistent with a serum 25OHD concentration of 50 nmol/L. However, for adults more than 70 years of age, the number of unknowns associated with the physiology of normal aging, coupled with the level of variability around the average requirement for this group that such factors may introduce, all of which may affect the estimation of the RDA (the level of intake needed to cover

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97.5 percent of the population) causes a closer examination of the level of intake appropriate for an RDA value.

For this life stage group (> 70 years), the reduction in fracture risk is the most important indicator of interest, not only because of the actual event, but also because of the high mortality and morbidity associated with fractures. The factors that may have an impact on fracture risk range from functional status to neurological, metabolic, and physical determinants. Such factors enhance uncertainties about vitamin D nutriture. Changes such as impaired renal function, less efficient synthesis of vitamin D in skin, lower endogenous production of active vitamin D, increased PTH as well as age-related changes in body composition affect the daily requirement of vitamin D. Moreover, a sizeable proportion of this population can be categorized as frail compared with other age groups, and the concerns for bone health are increased. Factors of increased institutionalization also come into play. Although there is insufficient evidence to point to any one of these factors as a contributor to increasing the variability at which 97.5 percent coverage of the population occurs, when taken as a group of unknowns, it would be inappropriate to ignore the concern when considering the level of vitamin D commensurate with an RDA for this group.

For this reason, the level of uncertainty should be taken into account during the specification of the RDA for vitamin D for persons more than 70 years of age. There are very few data that are relevant to adjusting for such uncertainty. There are no dose–response data that would allow comparisons for adults more than 70 years of age regarding the effects of intakes of 600 IU of vitamin D per day with that of a higher level of intake such as 800 or 1,000 IU/day. Moreover, the evidence for fracture risk in relation to vitamin D intake for this older life stage is confounded by study protocols that do not allow separation of the effect of calcium from vitamin D; as discussed previously there is reasonably compelling evidence that calcium alone in this age group can modestly reduce the risk of fracture. Therefore, it is not surprising that the inclusion of calcium with vitamin D treatment generally, albeit not consistently, reduces the risk of fractures among the oldest adults, especially when vitamin D nutriture is considered in the context of serum 25OHD concentrations (Tang et al., 2007; Avenell et al., 2009; AHRQ-Tufts, Tang et al., 2007). Even the 10 trials that examined vitamin D alone (Lips et al., 1996; Peacock et al., 2000; Meyer et al., 2002; Trivedi et al., 2003; Avenell et al., 2004; Harwood et al., 2004; Grant et al., 2005; Law et al., 2006; Lyons et al., 2007; Smith et al., 2007), when pooled by Avenell et al. (2009), showed no statistically significant effect on fracture risk. As shown in Table 5-5, which is focused on studies with subjects more than 70 years of age and vitamin D intakes as opposed to serum 25OHD concentrations, such studies are generally non-significant for fracture risk on the basis of both vitamin D alone and vitamin D with

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TABLE 5-5 Randomized Trials on Fracture Risk Associated with Vitamin D and Calcium or Vitamin D Alone in Older Men and Women

Author, Date	Gender/ Mean Age	Vitamin D Dose (IU/day)	Calcium Dose (mg/day)	Relative Risk of Fracture
Vitamin D plus Calcium				
Chapuy et al., 2002	F, 85 y	800	1,200	0.85 NS
Harwood et al., 2004	F, 81 y	800	1,000	0.49 NS
Grant et al., 2005	M/F, 77 y	800	1,000	0.94 NS
Porthouse et al., 2005	F, 77 y	800	1,000	0.96 NS
Vitamin D Alone				
Lips et al., 1996	M/F, 80 y	400	—	1.1 NS
Meyer et al., 2002	M/F, 85 y	400	—	0.92 NS
Trivedi et al., 2003*	M/F, 75 y	800	—	0.67 Significant
Lyons et al., 200)	M/F, 84 y	800	—	0.96 NS

*100,000 IU every four months.
NOTE: NS = Non-significant

calcium. The exception is Trivedi et al. (2003), which examined vitamin D supplementation and fracture risk in a population of men and women of average age 75 years. In any case, interpretation of these data is complicated by the unknowns surrounding the background intake of vitamin D over and above the supplemented dose.

The large study (n = 2,686) carried out by Trivedi et al. (2003) included more men than women (suggesting that the included population was actually at lower risk for fracture than would have been the case if the study had focused predominantly on women) and was longitudinal (5 years), including repeat measures on the same individual. The amount of vitamin D used for treatment was the equivalent of 800 IU/day, although it was administered as a 100,000 IU dose every 4 months for the duration of the study. Although this may limit somewhat the applicability of the study for DRI purposes, it is not as large as the 500,000 IU dose once yearly used by others (e.g., Sanders et al., 2010). Under these circumstances, the work of Trivedi et al. (2003) is helpful in taking uncertainty into account.

The reason not to dismiss the effect of 800 IU of vitamin D per day as an aberration because of a lack of dose–response data, even in the face of data generally not supportive of an effect of vitamin D alone regarding reduced fracture risk for the oldest adults, is that persons more than 70 years are a very diverse group. This group is undergoing a number of physiological changes with aging that could have an impact on and increase the variability around an average requirement, particularly in light of the known and high variability of these physiological changes among aging individuals. If this is assumed to be the case, then it is likely that the

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RDA for persons more than 70 years of age would be higher due to this variability. In addition, there is insufficient evidence to provide assurances that 600 IU/day vitamin D is as effective as 800 IU/day. By comparing the projected RDA based on the simulation analysis (600 IU/day) with the available evidence indicating benefit at 800 IU of vitamin D per day, taking into account the uncertainties would result in an estimation of an RDA of approximately one-third higher than the simulation analysis suggests. Overall, this is a small increase that is not known to increase the possibility of adverse events while providing a certain level of caution for this particularly vulnerable and potentially frail segment of the population. This approach is predicated on caution in the face of uncertainties, and it is anticipated that newer data in the future will help to clarify the uncertainties surrounding the level of intake of vitamin D that could be expected to cover 97.5 percent of persons over the age of 70 years.

The EAR of 400 IU/day and RDA of 800 IU/day for this life stage group, consistent with the DRIs for other life stage groups, assume minimal sun exposure.

Adults 51 Through 70 Years of Age

A question in establishing an EAR and RDA for this life stage group is the relevance of vitamin D in affecting bone loss due to the onset of menopause. Men in this life stage group have not yet reached the levels of bone loss and fracture rates associated with aging as manifested in persons more than 70 years of age and, unlike their female counterparts, they are not experiencing significant bone loss due to menopause. However, a portion—in fact perhaps the majority—of women in this life stage group are likely to be experiencing some degree of bone loss due to menopause.

As discussed above for adults more than 70 years of age, the available data do not suggest that median requirements increase with aging, resulting in support for an EAR of 400 IU/day, the same as for younger adults. Likewise, the EAR for both women and men in the 51 through 70 year life stage group is set at 400 IU of vitamin D per day.

With respect to women 51 through 70 years of age, fracture risk is lower than it is later in life; and as such, it is not entirely congruent with the situation for adults more than 70 years of age. Further, findings for this age group are at best mixed, but are generally not supportive of an effect of vitamin D alone on bone health. Although the AHRQ analyses of studies using vitamin D alone found the results to be inconsistent for a relationship with reduction in fracture risk, more recent studies have trended toward no significant effects (Bunout et al., 2006; Burleigh et al., 2007; Lyons et al., 2007; Avenell et al., 2009b). For those studies showing benefit for BMD with a vitamin D and calcium combination, interpretation

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is confounded by the effects of calcium especially since calcium alone appears to have at least a modest effect on BMD. The report from the WHI (Jackson et al., 2006), a very large cohort study, has limited applicability to the question of the effect of vitamin D on bone health among women because of relatively high levels of calcium intake (baseline mean calcium intake of approximately 1,150 mg/day at randomization plus 1,000 mg/day supplement) and the confounding due to hormone replacement therapy. Given these data plus the inability to extrapolate the variability seen in the requirements surrounding persons 70 or more years of age to this life stage group, the RDA for women 51 through 70 years of age is set at 600 IU of vitamin D per day, the same level as that for younger adults. With respect to men 51 through 70 years of age, there is also no basis to deviate from the RDA set for younger adults. The available evidence for men is extremely limited, and there are not data to suggest that bone health is enhanced by vitamin D intake among men in this life stage group. An RDA of 600 IU/day is established for these men.

The DRIs for these two life stage groups assume minimal sun exposure.

Pregnancy and Lactation

Pregnant 14 Through 18 Years of Age	
Pregnant 19 Through 30 Years of Age	
Pregnant 31 Through 50 Years of Age	
	EAR 400 IU (10 µg)/day Vitamin D
	RDA 600 IU (15 µg)/day Vitamin D
Lactating 14 Through 18 Years of Age	
Lactating 19 Through 30 Years of Age	
Lactating 31 Through 50 Years of Age	
	EAR 400 IU (10 µg)/day Vitamin D
	RDA 600 IU (15 µg)/day Vitamin D

Pregnancy The EAR for non-pregnant women and adolescents is appropriate for pregnant women and adolescents based on: (1) AHRQ-Ottawa's finding of insufficient evidence on the association of serum 25OHD level with maternal BMD during pregnancy and (2) the 1 available RCT (Delvin et al., 1986) and 14 observational studies reviewed in Chapter 4 regarding vitamin D deficiency and genetic absence of the vitamin D receptor (VDR) or 1α-hydroxyalase, which all demonstrate no effect of maternal 25OHD level on fetal calcium homeostasis or skeletal outcomes. Of the limited number (i.e., four) of observational studies that suggest an influence of maternal serum 25OHD levels on the offspring's skeletal outcomes later in life (so-called developmental programming), one study reports associa-

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tions consistent with an EAR-type value of approximately 40 nmol/L below which negative fetal skeletal outcomes were reported (Viljakainen et al., 2010), and another reports an RDA-type value of 50 nmol/L late in gestation above which reduced skeletal BMC was not seen in offspring at 9 years of age (Javaid et al., 2006). In addition, development of the fetal skeleton without dependence on maternal vitamin D is also biologically plausible as indicated by the studies in animal models in rats, mice, pigs, and sheep (see review in Chapter 3). Finally, there is no evidence that the vitamin D requirements of pregnant adolescents differ from those of non-pregnant adolescents.

The EAR is thus 400 IU of vitamin D per day for pregnant women and adolescents. Likewise, the RDA values for non-pregnant women and adolescents are applicable, providing an RDA of 600 IU/day for each group.

Lactation The EAR for non-lactating women and adolescents is appropriate for lactating women and adolescents based on evidence from RCTs (Rothberg et al., 1982; Ala-Houhala, 1985; Ala-Houhala et al., 1988; Kalkwarf et al., 1996; Hollis and Wagner, 2004; Basile et al., 2006; Wagner et al., 2006; Saadi et al., 2007), which are consistent with observational data (Cancela et al., 1986; Okonofua et al., 1987; Takeuchi et al., 1989; Kent et al., 1990; Alfaham et al., 1995; Sowers et al., 1998) that increased maternal vitamin D intakes increase maternal serum 25OHD levels, with no effect on the neonatal serum 25OHD levels of breast-fed infants unless the maternal intake of vitamin D is extremely high (i.e., 4,000 to 6,400 IU/day) (Wagner et al., 2006). Observational studies report no relationship between maternal serum 25OHD levels and BMD (Ghannam et al., 1999) or breast milk calcium content (Prentice et al., 1997). Also, there is no evidence that lactating adolescents require any more vitamin D or higher serum 25OHD levels than non-lactating adolescents. The EAR is thus 400 IU of vitamin D per day for lactating women and adolescents. Likewise, the RDA values for non-lactating women and adolescents are applicable, providing an RDA of 600 IU/day for each group.

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6

Tolerable Upper Intake Levels: Calcium and Vitamin D

The Tolerable Upper Intake Level (UL) is not a recommended intake. Rather, it is intended to specify the level above which the risk for harm begins to increase, and is defined as the highest average daily intake of a nutrient that is likely to pose no risk of adverse health effects for nearly all persons in the general population. As intake increases above the UL, the potential risk for adverse effects increases. In short, the UL is a reference value intended to guide policy-makers and scientists charged with ensuring a safe food supply and protecting the health of the U.S. and Canadian populations. It applies to intakes on a chronic basis among free-living persons. Those responsible for determining the appropriate dosages of nutrients to be studied in carefully controlled experimental trials conducted in clinical or community settings have the opportunity to bring other considerations into play when deciding on the acceptable levels of nutrients that are appropriate for subjects taking part in such studies. ULs are not designed to address experimental protocols in which safety monitoring occurs.

This chapter is organized to include hazard identification (indicator review and selection) and hazard characterization (intake–response assessment and reference value specification), the first two steps of the general risk assessment approach for Dietary Reference Intake (DRI) development. Therefore, compared with the discussions presented in the two chapters on reference values for adequacy (Chapters 4 and 5), the discussions for ULs are contained in a single chapter. This chapter addresses adverse effects of excess intakes of calcium and vitamin D. Although adverse effects are also associated with deficiencies of calcium and vitamin D, those concerns are

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incorporated into the previous discussions focused on establishing reference values for adequacy.

There are often ethical issues associated with conducting clinical trials designed to study the adverse effects of substances that can limit the types of data available for DRI development. For this reason, the derivation of ULs for DRI purposes necessarily relies more heavily on observational data and information derived from animal models than does the approach for the determination of levels of intake for nutritional adequacy. Thus, the emphasis on causality and strength of evidence needed for establishing reference values for adequacy is difficult to apply to the derivation of ULs.

At the outset, it is important to distinguish between the relatively "acute" toxic effects of excess intake and the "chronic" adverse effects of high levels of intake that may manifest in other ways including disease risk. When the ULs for calcium and vitamin D were originally established in 1997, it was noted that the available data were limited relative to adverse outcomes and dose–response relationships (IOM, 1997). In that report, adverse effects from excess intakes of calcium and vitamin D were considered primarily in terms of acute toxicity, which was defined as the condition of hypercalcemia or, in some cases, hypercalciuria with or without hypercalcemia.

The conditions associated with the intoxication syndrome for calcium and vitamin D are informative, but avoiding acute toxicity is not the ideal basis for a UL, a reference value with the larger purpose of public health protection over a life time of chronic intake. Although information concerning chronic excess intakes remains limited, data have emerged recently that may warrant caution about the levels of vitamin D that are consumed and raise questions about the long-term effects of high intakes that are less than those associated with toxicity and that may result in an increase in serum 25-hydroxyvitamin D (25OHD) levels into upper ranges previously considered physiological. Caution may also be warranted in comparing the effects at these high physiological levels of 25OHD achieved through supplementation versus sun exposure, and further research is needed to clarify the relative adverse effects of different sources of vitamin D.

The model developed for UL derivation was summarized in 1998 (IOM, 1998), and it acknowledged that the lack of data would affect the ability to derive precise estimates. Specifically: "Several judgments must be made regarding the uncertainties and thus the uncertainty factor (UF) associated with extrapolating from the observed data to the general population." Although a number of reports describe the underlying basis for uncertainty factors (Zielhuis and van der Kreek, 1979; Dourson and Stara, 1983), the strength of the evidence supporting the use of a specific UL undoubtedly varies. The summary of the 2007 workshop focused on enhancing DRI development (IOM, 2008) and pointed out the need for uncertainty fac-

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BOX 6-1
Potential Indicators of Adverse Outcomes for Excess Intake of Calcium and Vitamin D

Calcium
- Hypercalcemia
- Hypercalciuria
- Vascular and soft tissue calcification
- Nephrolithiasis (kidney stones)
- Prostate cancer
- Interactions with iron and zinc
- Constipation

Vitamin D
- Intoxication and related hypercalcemia and hypercalciuria
- Serum calcium
- Measures in infants: retarded growth, hypercalcemia
- Emerging evidence for all-cause mortality, cancer, cardiovascular risk, falls and fractures

tors, but also indicated that the scientific judgment involved should be described. In developing ULs for calcium and vitamin D, the limited nature of the data resulted in the committee using UFs to adjust for uncertainties in the data. These were necessarily qualitative adjustments rather than quantitative adjustments. As suggested repeatedly during the 2007 workshop on DRIs (IOM, 2008), an educated guess for a reference value is more useful to stakeholders than the failure to set a reference value in the face of uncertainty.

Discussions related to calcium ULs are provided first, and then vitamin D ULs are considered. At the start, the committee identified potential indicators to assess adverse effects for excess intakes of calcium and vitamin D based on the available literature, as described below. The potential indicators considered are presented in Box 6-1.

CALCIUM UPPER LEVELS: REVIEW OF POTENTIAL INDICATORS AND SELECTION OF INDICATORS

Excess calcium intake from foods alone is difficult if not impossible to achieve. Rather, excess intakes are more likely to be associated with the use of calcium supplements. However, the potential indicators for the adverse outcomes of excessive calcium intake are not characterized by a robust

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data set that clearly provides a basis for a dose–response relationship. The measures available are confounded by a range of variables including other dietary factors and pre-existing disease conditions.

The "classic" toxicity state of hypercalcemia is seen with either calcium or vitamin D excess, although it appears that the symptoms of hypercalcemia are manifested at relatively lower intake of calcium compared with vitamin D, for which high intakes are required to reach a toxic state. In the discussions below, hypercalcemia, as well as, hypercalciuria is described first as general conditions associated with the toxicity of either nutrient, followed by a discussion of adverse outcomes associated with excess calcium intake.

The Toxic Condition of Hypercalcemia and Hypercalciuria

Hypercalcemia occurs when serum calcium levels are 10.5 mg/dL (also expressed as 2.63 mmol/L) or greater depending on normative laboratory values. It can be induced by excess intake of calcium or vitamin D, but it is more commonly caused by conditions such as malignancy and primary hyperparathyroidism (Moe, 2008). Clinical signs and symptoms of hypercalcemia may vary depending on the magnitude of the hypercalcemia and the rapidity of its elevation; they often include anorexia, weight loss, polyuria, heart arrhythmias, fatigue, and soft tissue calcifications (Jones, 2008). When serum calcium levels rise above 12 mg/dL, the kidney's ability to reabsorb calcium is often limited; in turn, hypercalciuria can occur, particularly with increased calcium or vitamin D intake. Hypercalciuria is present when urinary excretion of calcium exceeds 250 mg/day in women or 275-300 mg/day in men. Often, urinary calcium excretion is expressed as the ratio of calcium to creatinine excreted in 24 hours (milligrams of calcium per milligram of creatinine). Values above 0.3 mg/mg creatinine are considered to be within the hypercalcuric range.

Hypercalcemia, in addition to leading to hypercalciuria, can cause renal insufficiency, vascular and soft tissue calcification including calcinosis leading to nephrocalcinosis, and nephrolithiasis. Nephrolithiasis, often referred to as kidney stones, can also be caused by hypercalciuria. Hypercalciuria may occur in the absence of hypercalcemia and is related to either hyperabsorption of calcium in the gut or a renal leak whereby calcium excretion is enhanced. Both etiologies can lead to nephrocalcinosis.

In the North American population, as many as 30 percent of persons ages 60 years or older have some degree of renal insufficiency (Coresch et al., 2005; Szczech et al., 2009). Decreased renal functioning may make persons more sensitive or susceptible to the effects of excess calcium or vitamin D intake. Urinary calcium excretion decreases in older adults, although it is not clear the extent to which this may be due to decreased

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calcium intake as compared to decreased renal function. However, if due to decreased renal function, older persons may be at higher risk for adverse effects derived from excess intakes. Moreover, decreased renal function simultaneously increases cardiovascular disease (CVD) risk and impairs calciuric responses and calcium and phosphate homeostasis. Likewise, those using thiazide-based diuretics—a sizeable proportion of older adults—are more readily challenged by excess calcium and vitamin D due to reduction in calcium excretion from the kidney (Medarov, 2009).

Excess Calcium and Hypercalcemia Leading to Renal Insufficiency

Prior to the introduction of histamine-2 blockers and proton pump inhibitors, liquid formulations that contained high calcium levels and absorbable alkali were used to treat gastric and duodenal ulcers. High intake of these formulations, however, caused a variety of adverse effects including hypercalcemia and renal failure. The syndrome became known as "milk-alkali syndrome" or MAS (Hardt and Rivers, 1923; Burnett et al., 1949) and was originally associated with men with peptic ulcer disease. In this context, hypercalcemia causes emesis and natriuresis, which result in significant drops in extracellular blood volume. This contraction worsens the hypercalcemia. Decreases in blood volume also induce an alkalotic state that causes an increase in proximal tubular bicarbonate resorption. Also, high serum calcium levels worsen the alkalosis through suppression of parathyroid hormone (PTH), which physiologically enhances bicarbonate excretion. Although the availability of absorbable alkali in the diet may enhance the alkalotic state, it is not the major pathogenic factor in MAS.

Recently, Patel and Goldfarb (2010) suggested renaming MAS as "calcium-alkali syndrome" to better reflect the current understanding of the disorder, which now has shifted to be more prevalent in other groups including postmenopausal women. The earlier MAS presented with hyperphosphatemia after prolonged ingestion of phosphorus-containing milk with cream (Patel and Goldfarb, 2010). In contrast, the modern version of the syndrome is associated with hypophosphatemia or low-normal serum phosphorus levels as a result of the phosphorus-binding properties of calcium carbonate. The hypophosphatemia is more pronounced in elderly patients or those with eating disorders, who tend to have relatively low consumption of protein and therefore phosphorus (Picolos et al., 2005; Felsenfeld and Levine, 2006; Medarov, 2009). Confounding this, however, is the chronic renal insufficiency that often accompanies MAS; in that case, serum phosphorus levels may be normal or high. Available case reports tend to provide only serum calcium levels and do not specify calcium intakes per se, or serum phosphate, associated with the condition.

As shown in Table 6-1, a number of recent case reports have been iden-

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TABLE 6-1 Case Reports of Calcium-Alkali Syndrome

Reference	Patient Gender/Age (years)	Calcium Carbonate Intake (mg/day)	Duration	Serum Calcium Level (mmol/L) mg/dL	Creatinine Level (μmol/L) mg/dL
Javed et al., 2007	Male/70	> 1,000	1 year	(3.43) 13.7	(344.8) 3.9
Nabhan et al., 2004	Female/61	1,500 + 600 IU vitamin D	Several years	(6.43) 25.7	(106.1) 1.2
Caruso et al., 2007	Male/60	> 2,000 + 800 IU vitamin D	NR	(3.08) 12.3	(530.4) 6.0
Gordon et al., 2005	Female/35	3,000	1 month	(2.64) 10.6	(190.0) 2.1
Shah et al., 2007	Female/47	3,000 + 200 IU vitamin D	NR	(4.13) 16.5	(362.4) 4.1
Kaklamanos and Perros, 2007	Female/76	5,500	2 years	(3.45) 13.8	(124.0) 1.4
Grubb et al., 2009	Female/51	7,200	NR	(5.70) 22.8	(185.6) 2.1
Ulett et al., 2010	Male/46	> 7,500	NR	(3.98) 15.9	(406.6) 4.6
Irtiza-Ali et al., 2008	Case 1: Female/48 Case 2: Male/74 Case 3: Male/51	Case 1: ~ 8,000 Case 2: ~ 18,000 Case 3: ~ 44,000	Case 1: 19 years Case 2: several weeks Case 3: NR	Case 1: (3.25) 13.0 Case 2: (3.31) 13.2 Case 3: (2.97) 11.9	Case 1: (737) 8.3 Case 2: (245) 2.8 Case 3: (1,013) 11.5
Jousten and Guffens, 2008	Male/66	~ 10,000	Several months	(4.15) 16.6	(459.7) 5.2
Bailey et al., 2008	Female/40	~ 11,000	NR	(4.71) 18.8	(164.0) 1.9
Waked et al., 2009	Male/81	~ 12,500	NR	(3.65) 13.8	(733.7) 8.3

NOTE: To convert mmol/L to mg/dL, multiply by 0.25; IU = International Units; NR = not reported.

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tified for calcium-alkali syndrome. For these individuals, a calcium intake of 3,000 mg/day was associated with the onset of hypercalcemia. However, in every case except one, all outcomes were found in individuals with impaired renal function and high serum creatinine levels. The one exception (Nabhan et al., 2004) was a patient who was using hydrochlorothiazide as a diuretic and was hypoparathyroid. Although these data cannot be applied directly to the normal, free-living population, they are informative and indicate that calcium levels of 3,000 mg/day are problematic for these compromised persons.

Patel and Goldfarb (2010) suggested that the incidence of calcium-alkali syndrome is growing as a result of the widespread use of over-the-counter calcium and vitamin D supplements, particularly among older persons. The basis for the suggestion is that while healthy younger adults rely on the bone reservoir to buffer excess calcium, the net flux of calcium is out of the bone for older persons, thereby making the bone less functional as a reservoir. These older persons are more susceptible to the syndrome when they begin taking supplemental calcium. Patel and Goldfarb (2010) also noted that the excess ingestion of calcium with or without vitamin D is an integral feature of this syndrome, making it potentially relevant to the consideration of upper levels of calcium intake.

Excess Calcium and Soft Tissue Calcification

Associated with Hypercalcemia

Calcification of soft tissues—calcinosis—occurs as a result of long-standing hypercalcemia, increased serum phosphate levels, or local abnormality in the affected tissues. Clinically, the condition is linked to metabolic disorders such as hyperparathyroidism, sarcoidosis, or connective tissue disease such as scleroderma.[1]

Calcification of kidney tissues, or nephrocalcinosis, results in symptoms similar to those of renal dysfunction, ranging from painful and frequent urination to nausea, vomiting, and swelling. Although nephrocalcinosis has been reported to be induced by calcium intake in rats (Peterson et al., 1996), no data link calcium intake or the use of calcium supplements in humans to the onset of nephrocalcinosis. Nephrocalcinosis may be associated with calcium nephrolithiasis under particular conditions (Vervaet et al., 2009).

Relative to hypercalcemia and calcification of vascular tissue, there is

[1]Soft tissue calcifications are more severe in hypocalcemic disorders such as hypoparathyroidism and renal failure, but in such cases it is the associated hyperphosphatemia that is causing the calcifications.

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experimental evidence in humans and laboratory animals indicating that hypercalcemia can lead to vascular calcification in the setting of renal insufficiency as a result of elevated calcium and phosphate concentrations (Reynolds et al., 2004; Yang et al., 2004; Cozzolino et al., 2005). However, this has not been demonstrated clinically.

Associated with Calcium Supplements

Calcification of vascular tissues has been reported with high calcium intake (Goodman et al., 2000; Asmus et al., 2005; Block et al., 2005; Raggi et al., 2005); however, the reports are based on individuals with compromised kidney function. No link has been clearly established for a general population.

Bolland et al. (2008), in a recent randomized, placebo-controlled trial, found that cardiovascular events may be slightly more prevalent in older women on calcium supplementation. Reid and Bolland (2008), in a subsequent companion publication, suggested among other possibilities that vascular calcification may be relevant to their finding of an upward trend in cardiovascular event rates in healthy postmenopausal women supplemented with calcium. These findings were contrary to the purported benefits of calcium supplementation and CVD.

A more recent meta-analysis conducted by Bolland et al. (2010) examined 11 randomized controlled trials of calcium supplements in 12,000 older patients and found that there was a 30 percent increased risk of heart attack independent of age, gender, and type of supplement. Although this report is of concern, there are several relevant limitations. The studies included are small, the event frequency is low, and most outcomes have confidence intervals (CIs) that overlap. Moreover, cardiovascular events were not a primary outcome, the events may not have been well adjudicated, and renal function was not considered as a covariate. Many of the studies supplemented with 1,000 to 1,200 mg of calcium per day and did not report the total calcium intake (supplement plus diet). The events may therefore be associated with intakes higher than the supplemented dose, perhaps 2,000 mg of calcium per day or more, as reported, for example, by Jackson et al. (2006). Under these circumstances, it is difficult to conclude that calcium intakes per se in the range of 1,000 to 1,200 mg/day can be associated with cardiovascular events. In addition, some questions remain as to whether the addition of this amount of calcium to a baseline diet as a calcium supplement may have adverse consequences.

Excess Calcium and Nephrolithiasis (Kidney Stones)

More than 12 percent of men and 6 percent of women in the general population will develop kidney stones (Stamatelou et al., 2003). The mor-

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bidity of kidney stones is not limited to the pain of stone passage; stones increase the risk of renal and urinary tract infections as well as renal insufficiency. A contributing factor in stone formation is hypercalciuria from any cause; another is hyperabsorption of calcium from the gut. Hypercalciuria increases the risk for nephrolithiasis (Pak and Holt, 1976). Hypercalciuria can be present in the absence of hypercalcemia and may reflect routine excretion of excess calcium intake.

Incidence rates for kidney stones vary by age and gender. The rates are highest in men, rising after age 20, peaking between 40 and 60 years, and then beginning to decline (Johnson et al., 1979; Hiatt et al., 1982; Curhan et al., 1993). For women, incidence rates seem to be higher in the late 20s, decreasing by age 50, and then remaining relatively constant (Johnson et al., 1979; Hiatt et al., 1982; Curhan et al., 1997, 2004).

Although calcium is present in approximately 80 percent of kidney stones (Coe et al., 1992), the role of calcium and other nutrients, acting alone or in concert as risk factors, is not completely understood and may be a function of physiological context. Various dietary and non-dietary factors are associated with stone formation, making data difficult to interpret. Rodent models that have been used to explore the effect of dietary factors on the propensity to form calcium oxalate and calcium phosphate stones suggest that the role of supplemental calcium in determining risk for nephrolithiasis varies by interaction with a given dietary component. One study in rats compared renal oxalate crystallization relative to the consumption of calcium-supplemented or oxalate-rich diets as well as control diets. The study found that rats fed the calcium-supplemented diet had enhanced calcium and oxalate accumulation as well as crystallization in renal tissues, even though urinary oxalate and citrate excretion was not significantly different in rats fed the control diet (Mourad et al., 2006). In this study, measures of renal function, including glomerular filtration rate, fractional excretion of urea, and fractional reabsorption of water and magnesium were not affected by the calcium-supplemented diet, and calciuria was only slightly increased.

Nephrolithiasis in Adults

Recently, a study using data from the Women's Health Initiative (WHI) trial, which recruited more than 36,000 post-menopausal women ages 50 to 79 years (mean age 62 years), reported findings on the incidence of kidney stones (Jackson et al., 2006). Participants were randomly assigned to receive a placebo or 1,000 mg of elemental calcium (calcium carbonate) per day with 400 International Units (IU) of vitamin D_3. The primary outcome focus was fractures and measures of bone density. Mean baseline intake of calcium was approximately 1,100 mg/day and the supplement added another 1,000 mg/day, for a total average calcium intake of about

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2,100 mg/day for the experimental group. The mean baseline intake for vitamin D was about 365 IU/day, which, when combined with the vitamin D supplement, resulted in an approximate vitamin D intake of 765 IU/day for the experimental group. The rate of adherence (defined as use of 80 percent or more of the assigned study supplements) ranged from 60 to 63 percent during the first 3 years of follow-up, with an additional 13 to 21 percent of the participants taking at least half of their study pills. At the end of the trial, 76 percent were still taking the study supplements, and 59 percent were taking 80 percent or more of the supplements.

Among the healthy postmenopausal women in the WHI study, the doses of calcium and vitamin D resulted in an increased risk (17 percent) of kidney stones. Kidney stones were reported by 449 women in the supplemented group, compared with 381 women in the placebo group. With respect to the intention to treat, the reported hazard ratio (HR) was 1.17 (95% CI: 1.02–1.34). Although this study did not focus on calcium intake alone, the total vitamin D intakes were around 800 IU/day, a level that is not associated with either hypercalcemia or hypercalciuria. Therefore, it is reasonable to consider the possibility that total calcium intake of 2,100 mg per day were associated with increased kidney stones in this population. Although the kidney stone events were not adjudicated specifically, adjudication problems should be randomly distributed and thus not a contributing factor to the outcome.

The WHI reflects a large, well-designed cohort study. There is also a report from a small, short trial (covering 4 years) of 236 elderly women with a baseline calcium intake of 800 mg/day and with calcium supplementation of 1,600 mg/day for 1 year (total calcium intake of approximately 2,400 mg/day) (Riggs et al., 1998). In this study, 50 percent of subjects receiving supplemental calcium and 8 percent of placebo controls had urinary calcium levels exceeding 350 mg/day, but no subjects in the calcium group experienced nephrolithiasis, nephrocalcinosis, or a decrease in glomerular filtration rate. Other smaller trials among older subjects have shed little light on the issue of nephrolithiasis and calcium intake, either because the doses were relatively low or because subjects were recruited on the basis of having had previous incidence of kidney stones (Levine et al., 1994; Williams et al., 2001; Borghi et al., 2002).

Curhan et al. (1997) examined the risk for kidney stones in women 34 to 59 years of age, using data from the Nurses' Health Study (NHS), a notably younger group of subjects than those included in the WHI study. They reported an inverse association between calcium intake from foods, but a positive relationship between risk and intake of calcium from supplements (Curhan et al., 1997). In a 2004 study, Curhan and colleagues (Curhan et al., 2004) prospectively examined data again from the NHS for an 8-year period relative to dietary factors and the risk for kidney stones in women 27 to 44 years of age. In this analysis, the inverse relationship between calcium

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intake from foods and the risk of kidney stone formation remained, but there was no apparent relationship between supplement use and risk. In a study of 50,000 men 40 to 75 years of age (Curhan et al., 1993), the same relationship was evident: reduced risk with increased intake of calcium from food sources, but no association with use of calcium supplements.

The suggested discrepancy between the risks from food sources of calcium and from calcium supplements may in part be due to the timing of the supplement intake (Curhan et al., 2007). Calcium present in the food will bind oxalate, a known contributor to kidney stone formation, and prevent its absorption. If taken between meals, the calcium would have less opportunity to bind oxalate, and so oxalate absorption would be increased. These observations suggest that taking calcium supplements with meals should reduce the formation of kidney stones, but this has not been tested.

Overall, the data indicate that the calcium content of foods does not cause stone formation, but may be protective against it. On the other hand, calcium supplements are emerging as a concern based on observational data, at least for some groups under certain circumstances. Further, individuals with a history of kidney stones are at increased risk if they obtain their calcium from supplements rather than food sources. There is, however, limited evidence from small, short-term trials suggesting that supplemental calcium in moderate doses may not increase risk for stone recurrence. The most important evidence to date is from the WHI trial (Jackson et al., 2006), which indicated that a mean calcium intake from foods and supplements that totaled about 2,150 mg/day—plus a vitamin D supplement of 400 IU/day, a level low enough to avoid potential confounding effects for adverse events given the mean total vitamin D intake of approximately 750 IU/day—resulted in a 17 percent increased incidence of kidney stones among postmenopausal women, regardless of whether the subjects had experienced previous clinical events related to urinary calculi formation.

Nephrolithiasis in Children

Hypercalciuria, as a secondary outcome to high calcium intake, can occur in children as well as in adults. However, the incidence of kidney stones in children is rare. There is limited evidence concerning high calcium intakes in young children relative to calcium excretion. In a study of children ages 1 to 6 years and designed to test the effects of 1,800 mg/day total calcium (supplementation adjusted on the basis of dietary calcium questionnaire), the calcium intake of 1,800 mg/day calcium did not cause urinary calcium/creatinine ratios to differ significantly from those of placebo controls (Markowitz et al., 2004).

A study by Sargent et al. (1999) provides information relevant to infants and calcium excretion. This study supplemented the formula of full-term

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infants with calcium glycerophosphate, providing 1,800 mg of calcium (and 1,390 mg of phosphate) per liter of formula. The mean calcium intake for infants receiving the supplemented formula was more than 4 times that of children in control groups at months 4 and 9, with a mean calcium intake of 1,563 ± 703 mg/day at 9 months. Although the focus of the study was lead absorption, the data demonstrated that total calcium intakes of about 1,550 to 1,750 mg/day did not affect urinary calcium excretion. The data are somewhat limited in that younger infants were not studied; further, the contribution from solid foods in older infants was not clearly tracked. With these limitations, the authors' conclusion that this level of intake probably would not increase the likelihood of nephrolithiasis is reasonable.

Excess Calcium and Prostate Cancer

The vast majority of the data relating to prostate cancer and calcium intake are derived from observational studies, and the ability to sort the effect of dairy products from that of calcium is challenging. Some observational data suggest a role for dairy products as a risk factor for prostate cancer (Tominaga and Kuroishi, 1997; Grant, 1999). A recent case–control study examined associations between dairy products and dietary calcium and prostate cancer risk among men ages 35 to 84 years with a histological diagnosis of prostate cancer (Raimondi et al., 2010). Intake of dairy products, in particular milk consumption, was associated with a two-fold increased risk for prostate cancer, whereas consumption of other dairy products (cheese, yogurt, and cream) suggested no association for increased risk. Total calcium intake was not significantly associated with risk for prostate cancer ($p = 0.09$).

Other observational studies evaluating associations between milk or dairy product intake and overall risk for prostate cancer have suggested that supplemental calcium intake may be a stronger risk factor for prostate cancer than calcium from foods, particularly for aggressive prostate cancer with high mortality. Studies of associations between calcium supplement use and risk for incident prostate cancer provide mixed results. A small case–control study assessed men ages 40 to 64 years with newly diagnosed prostate cancer for multivitamin and supplement use by questionnaire (Kristal et al., 1999). Although about a third of both cases and controls reported using multivitamins, only 5 percent reported taking calcium supplements. No association was found between calcium supplement use and risk for incident prostate cancer in this relatively young and low-risk population. A prospective study of male participants ages 50-74 years from the Cancer Prevention Study II examined associations between calcium and dairy product intake and risk for incident prostate cancer (Rodriguez et al., 2003). This analysis of 65,321 men found a small increase in overall pros-

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tate cancer risk for calcium intakes of 2,000 mg/day and higher compared with intakes less than 700 mg/day. High calcium intake (≥ 2,000 mg/day), however, was significantly associated with risk for advanced prostate cancer. When calcium supplements (≥ 500 mg/day) were analyzed, controlled for total calcium intake, a weak association was found for prostate cancer risk. Dairy product intake was not associated with risk for prostate cancer. The report from the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR, 2007) concluded that the relationship between prostate cancer and milk and dairy product intake is inconsistent from both cohort and case–control studies, and there is limited evidence suggesting that milk and dairy products are a cause of prostate cancer. A food-use questionnaire administered at baseline to participants in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study explored associations between intake of certain foods and nutrients and risk for incident prostate cancer in a large cohort of male smokers ages 50 to 69 years. This study analyzed intake of calcium and dairy foods and found no associations with development of prostate cancer (Chan et al., 2000). In a longitudinal follow-up of this cohort, Mitrou et al. (2007) found a graded positive association between increasing total calcium intake and total prostate cancer risk. A prospective study of male participants ages 40 to 75 years from the Health Professionals Follow-Up Study (HPFS) examined whether calcium and fructose intake were risk factors for prostate cancer (Giovannucci et al., 1998). Calcium intake exceeding 2,000 mg/day was found to be associated with higher risk for total, advanced, and metastatic prostate cancer. Further, supplemental calcium intake above 900 mg/day was associated with metastatic prostate cancer risk at all levels of total calcium intake. In a follow-up analysis of this cohort, Giovannucci et al. (2006a) found a significantly increased risk for advanced prostate cancer associated with increasing total calcium intake and for fatal prostate cancer associated with supplemental calcium intakes of 401 mg/day and above. The WCRF/AICR (2007) concluded that there is a probable association between diets high in calcium and prostate cancer.

In the case of intervention studies, one randomized controlled multicenter clinical trial based on 672 men (mean age 61.8 years) living in the United States examined risk for prostate cancer from supplemental calcium intake. Participants received either 3 g of calcium carbonate or placebo daily for 4 years and were followed for up to 12 years for prostate cancer diagnosis (Baron et al., 2005). Over the entire study period, risk for prostate cancer was lower in the calcium-supplemented group than in controls (relative risk [RR] = 0.83; 95% CI: 0.52–1.32) but was not statistically significant. For specific years in the study, increase in prostate cancer risk was statistically significant between baseline and year 6 (RR = 0.52; 95% CI: 0.28–0.98) and between years 2 and 6 (RR = 0.44; 95% CI: 0.21–0.94).

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No significant differences were found for total calcium intake and prostate cancer risk.

The 2009 analysis from the Agency for Healthcare Research and Quality (Chung et al., 2009; hereafter referred to as AHRQ-Tufts) examined 12 cohort studies that reported on the association between calcium intake and the risk of prostate cancer (Schuurman et al., 1999; Chan et al., 2001; Rodriguez et al., 2003; Baron et al., 2005; Tseng et al., 2005; Giovannucci et al., 2006a; Koh et al., 2006; Mitrou et al., 2007; Park et al., 2007a,b; Rohrmann et al., 2007; Kurahashi et al., 2008). One of the studies also provided a post hoc analysis of a randomized controlled trial on calcium supplementation. The incidence of prostate cancer in these studies ranged from 0.008 to 0.10. Most of the studies were conducted in Europe or North America, and one study was conducted in Japan. The mean age of the subjects ranged from 53 to 67 years, but did include men 51 to 70 years of age. No study specifically targeted men older than 70 years of age. Total calcium intake ranged from less than 500 mg/day to at least 2,000 mg/day. The time between dietary assessment and the diagnosis of prostate cancer varied from 1 to 17 years. AHRQ-Tufts rated the studies for methodological quality as follows: four studies were rated A, seven studies were rated B, and one study was rated C. The studies included participants in the age range of 51 to 70 years. Seven studies did not find an association between calcium intake and the risk of prostate cancer (Baron et al., 2005; Koh et al., 2006; Mitrou et al., 2007; Park et al., 2007a,b; Kurahashi et al., 2008). The remaining five studies found that the risk was higher in the groups that took more calcium compared with those that took a lower amount; the higher amount ranged from 921 to at least 2,000 mg of calcium per day.

Overall, data in this area are at best emerging. Although observational studies suggest that total calcium intake of 2,000 mg/day or higher may be associated with increased risk for prostate cancer and particularly with advanced and metastatic cancer, these data are not sufficiently robust to serve as an indicator for a UL. The one available trial was negative. The observations, however, are notable for levels of intake that are less than those that produce hypercalcemia and hypercalciuria.

Excess Calcium and Nutrient Interactions: Iron and Zinc

Despite the absence of clinically or functionally significant depletion of relevant mineral nutrients, calcium interaction with other minerals in the diet has been considered a potential risk related to high calcium intakes. The 1997 DRI report (IOM, 1997) specifically called for increased study in this area. However, data remain limited.

With respect to iron, Ilich-Ernst et al. (1998) carried out a placebo-

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controlled randomized trial assessing the effects of calcium supplementation on bone mass in adolescent girls ages 8 to 13 years (n = 354). A secondary analysis at year 4 of this 7-year trial found that girls in the supplemented group achieved a total calcium (food plus supplements) intake of 1,500 mg/day. When assessed for interactions between calcium and iron, measures of iron status—hemoglobin, hematocrit, and corpuscular indexes—were not significantly different from those of girls in the placebo group who reached a calcium intake of 800 mg/day. Ames et al. (1999) found no effect of a calcium intake of approximately 1,200 mg/day compared with 500 mg/day for 5 weeks on iron absorption in children 3 to 5 years of age.

With respect to zinc, McKenna et al. (1997) conducted a calcium and zinc balance study on a subset (n = 26) of participants in a longitudinal clinical trial of the effects of calcium supplementation on bone mass in girls with a mean age of 11 years. Trial participants received either 1,000 mg/day of supplemental calcium or a placebo. Mean calcium intake reached 847 ± 287 and 821 ± 224 mg/day from diet for placebo and intervention groups, respectively, at 6 months. With the additional supplement, the mean calcium intake in the intervention group exceeded 1,700 mg/day. The results of the balance study found no effect in the intervention group from intake of approximately 1,700 mg of calcium per day on net zinc absorption, zinc excretion, or zinc balance compared with intakes of approximately 800 mg/day in the placebo group.

Taken together, the studies suggest that calcium intakes of 1,500 to 1,700 mg/day do not interfere with iron or zinc absorption in adolescent girls. However, as calcium intakes among this age group could be higher than those studied, there is little evidence to shed light on the larger issue.

Excessive Calcium and Constipation

Calcium supplement intake has long been associated with constipation. In fact approximately 1 of every 10 participants in the WHI calcium–vitamin D supplementation trial reported moderate to severe constipation (Jackson et al., 2006). If a food source of calcium is the problem, the constipation is likely due to the components of dairy products (Anthoni et al., 2009) rather than to the calcium in food. Calcium supplements, which are regarded as "binding," can cause side effects for some people, such as constipation and gas (Jackson et al., 2006; Prince et al., 2006), which varies greatly from person to person. Usually the constipation is alleviated by increasing intakes of water or fiber-rich foods, or by trying another form of supplement (calcium citrate may be less constipating than calcium carbonate, for example). Although such conditions warrant attention, the utility of constipation as an indicator for DRI development is doubtful.

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Selection of Indicator for Calcium UL

The risk assessment framework, as described in Chapter 1, specifies that, in the case of ULs, the available data pertaining to adverse effects be first examined for evidence of a benchmark intake (BI). Alternatively, either a no observed adverse effect level (NOAEL) or a lowest observed adverse effect level (LOAEL) is considered. In the case of calcium, limited new information has become available since 1997. The indicators selected are calcium excretion for younger age groups, and kidney stone formation for older age groups. The calcium excretion data provide information for a group for which no data were available in 1997. The newer data on kidney stone formation form a basis for a UL that is more akin to conditions experienced by the normal, healthy population than is calcium-alkali syndrome, although the cautions expressed by Patel and Goldfarb (2010) concerning the vulnerability of older persons to calcium-alkali syndrome with the use of calcium supplements are worthy of note.

The available data could not offer a BI or be used to estimate a dose–response relationship. The basis for the ULs is a NOAEL for infants and a LOAEL for adults, as described further below for specific life stage groups.

CALCIUM UPPER LEVELS: INTAKE-RESPONSE ASSESSMENT AND SPECIFICATION OF UPPER LEVELS

The ULs for calcium established for the DRI life stage groups are shown in Table 6-2. These values suggest that the levels of intake regarded as consistent with a UL are relatively close to the levels of intake considered to be appropriate for nutritional adequacy.

ULs for Infants 0 to 12 Months of Age

Infants 0 to 6 Months of Age	UL 1,000 mg/day Calcium
Infants 6 to 12 Months of Age	UL 1,500 mg/day Calcium

In the previous 1997 DRI report (IOM, 1997), a UL for calcium for infants was not specified owing to lack of data. The 1997 report noted a small, randomized trial of 81 infants (103 at baseline, ages 2.5 to 5.0 months at entry) designed to examine the tolerance of calcium-supplemented infant formula through 9 months of age (Dalton et al., 1997). The data, as analyzed in 1997 (IOM, 1997), indicated only that infants fed calcium at up to approximately 1,750 mg/day experienced no adverse effect on iron status.

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TABLE 6-2 Calcium Tolerable Upper Intake Levels (UL) by Life Stage

Life Stage Group	UL
Infants	
0 to 6 mo	1,000 mg
6 to 12 mo	1,500 mg
Children	
1–3 y	2,500 mg
4–8 y	2,500 mg
Males	
9–13 y	3,000 mg
14–18 y	3,000 mg
19–30 y	2,500 mg
31–50 y	2,500 mg
51–70 y	2,000 mg
> 70 y	2,000 mg
Females	
9–13 y	3,000 mg
14–18 y	3,000 mg
19–30 y	2,500 mg
31–50 y	2,500 mg
51–70 y	2,000 mg
> 70 y	2,000 mg
Pregnancy	
14–18 y	3,000 mg
19–30 y	2,500 mg
31–50 y	2,500 mg
Lactation	
14–18 y	3,000 mg
19–30 y	2,500 mg
31–50 y	2,500 mg

Using these same data, Sargent et al. (1999) later reported on calcium excretion measures, and this measure serves as the UL indicator for infants. This 1999 report has provided the ability to estimate a NOAEL for calcium intake for infants based on calcium excretion. Within the confines of the limitations of the data, they suggest that infants can tolerate approximately 1,750 mg of calcium per day with no noted adverse effects. A NOAEL of 1,750 mg/day is therefore established for infants on this basis.

Infants 0 to 6 Months of Age

The presumed sensitivity of the young infant to excess intakes of any substance, as well as the lack of direct evidence to clarify the nature of adverse effects for this group, warrants a cautious approach. Quantitative factors relative to metabolic differences between younger infants and older

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infants in terms of handling excess calcium cannot be derived based on the literature, and little is available to inform the scientific judgment for public health protection except body weight. According to the Centers for Disease Control and Prevention (CDC) growth charts,[2] infants should increase their weight between birth and 3 months of age from about 7.0 pounds (3.5 kg) to 13.0 pounds (6 kg), and then to about 17.5 pounds (8 kg) by 6 months of life. The NOAEL of 1,750 mg/day—which is derived from one study within the age range of 3 to 9 months (Sargent et al., 1999)—is reduced by an uncertainty factor of 2 to adjust for this weight difference and rounded to 1,000 mg of calcium per day to serve as the UL for this life stage group. This is admittedly a cautious approach but, by establishing a UL for infants, their safety is more readily ensured than would be the case in the absence of a UL, and the value is reasonable in view of the available data and current biological understandings. The 1997 IOM report on calcium DRIs did not establish a UL for infants (IOM, 1997).

Infants 6 to 12 Months of Age

The NOAEL of 1,750 mg/day is a reasonable starting point for the UL for older infants. Consistent with general principles of human physiology and toxicology, the committee considered that an infant's capacity to handle excess nutritional substances is increased with increased body size. Presumably in the case of calcium, which is a critical requirement during these periods of bone development, the infant's ability to tolerate higher levels of intake is greater as the infant grows and develops skeletal structure. Therefore, the NOAEL of 1,750 mg/day is not unreasonable as the basis for a UL. However, given the paucity of data, a slight uncertainty correction is warranted, and the UL is set at 1,500 mg/day for infants 7 to 12 months of age. No UL for this age group was established in 1997 (IOM, 1997).

ULs for Children and Adolescents 1 Through 18 Years

Children 1 Through 3 Years of Age Children 4 Through 8 Years of Age	UL 2,500 mg/day Calcium
Children 9 Through 13 Years of Age Adolescents 14 Through 18 Years of Age	UL 3,000 mg/day Calcium

[2]Available online at http://www.cdc.gov/growthcharts/ (accessed July 19, 2010).

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New data on adverse outcomes due to excess calcium intake among children and adolescents—specifically data that would identify a NOAEL or LOAEL—have not emerged since the last DRI report on calcium in 1997 (IOM, 1997). At that time, it was noted that the safety of excess calcium intake in children and adolescents had not been studied. A UL of 2,500 mg of calcium per day was established in 1997 for all children and adolescents in these life stage groups, largely on the basis of the UL established for adults (i.e., 2,500 mg/day) (IOM, 1997).

There is currently no evidence that the 1997 level is too low to provide public health protection for this group; further, when compared with the new UL set for infants, the level of 2,500 mg of calcium per day is a reasonable increase given the expected increases in body weight and metabolic capacities, especially for younger children between the ages of 1 and 8 years.

However, for older children it is also appropriate to take into account the likely increases in tolerated intakes as metabolic demands increase and the pubertal growth spurt associated with bone accretions sets in, primarily between 9 and 18 years of age. Again, there are no data to allow quantitative uncertainty factors to be developed to mathematically correct for the likelihood of increased capacities during the bone growth spurt, but to do so in some fashion is consistent with a general toxicological approach. An added level of 500 mg/day is reasonable, resulting in a UL of 3,000 mg of calcium per day for children 9 to 13 years of age and adolescents 14 to 18 years of age.

The UL for children 1 through 8 years of age is the same as that established for these life stage groups in 1997 (IOM, 1997). However, the UL has been increased by 500 mg/day for older children and adolescents compared with 1997 (IOM, 1997). This is based on a biologically reasonable adjustment intended to take into account increased need and therefore increased capacity to tolerate a slight increase in a UL value.

ULs for Adults 19 or More Years of Age

Adults 19 Through 30 Years of Age Adults 31 Through 50 Years of Age	UL 2,500 mg/day Calcium
Adults 51 Through 70 Years of Age Adults >70 Years of Age	UL 2,000 mg/day Calcium

The onset of hypercalcemia is clearly an adverse outcome. However, it was not selected as an indicator for ULs for adults because it reflects an

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extreme pathological condition, and the ability to consider other adverse events associated with sustained, high levels of intake has emerged. Specifically, kidney stone formation is an adverse outcome, notably among post-menopausal women. Although there is also evidence related to calcium-alkali syndrome among adults, most of the data relate to those with compromised kidney function. Vascular calcification in postmenopausal women has emerged as an interesting hypothesis, but available data are conflicting, and threshold levels for intake are unknown. Evidence related to prostate cancer, although concerning, was too confounded to allow this disease risk to serve as an indicator for establishing ULs for calcium intake. Further, neither constipation nor nutrient interactions were associated with data to suggest that these outcomes would serve as indicators for UL development.

Given the size and quality of the WHI trial, its outcome relative to the incidence of kidney stones (Jackson et al., 2006) results in the selection of kidney stones as the indicator for adults for DRI purposes. The levels of calcium intake that may cause kidney stones within a normal population cannot be specified with certainty and are known to be variable depending upon a number of factors, including baseline renal function, pre-existing disease conditions, and interactions with drugs. Based on the findings of Jackson et al. (2006), and with the understanding that the data are derived from women between the ages of 50 and 79 years, there is a concern for kidney stone risk at total calcium intakes of approximately 2,000 mg/day. Underpinning the concern is the recognition that intakes of calcium from food do not readily result in excess intakes and are not associated with adverse effects; rather, the adverse effects appear to be a function of calcium supplementation added to baseline intake. The level of 2,000 mg of calcium per day is established as the LOAEL for adults, including men, more than 50 years of age. The very limited data available for adults 19 to 50 years of age do not allow the specification of a LOAEL or NOAEL for this younger group and the UL for this group is derived from considerations used for the UL for persons above 50 years of age. For this reason the ULs for older adults are discussed first below, followed by adults 19 to 50 years of age.

Adults 51 Years of Age and Older

The committee considered the option of applying an uncertainty factor to lower the LOAEL, given the limited data. However, the unknowns surrounding the precision of the LOAEL coupled with the observation that the LOAEL is very close to intakes that are considered adequate and recommended, caused the committee to conclude that, until there are

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better data related to calcium intakes from supplements and the incidence of kidney stones or other relevant health outcomes, establishing a UL of 2,000 mg of calcium per day is justified and provides a reasonable degree of public health protection without overly restricting the intake of calcium (notably from calcium supplements) for both men and women. There is no apparent reason to conclude that men in this age group are more sensitive than women. Although one 1993 observational study does not support the potential for increased kidney stone formation with supplement use among men, public health protection warrants caution for this older group. Moreover, the value of 2,000 mg of calcium per day is also somewhat below the 3,000 mg/day associated with calcium-alkali syndrome among persons with waning kidney function, the only other potential indicator with an estimate of threshold levels for effect.

The new UL of 2,000 mg of calcium per day for persons 51 to 70 years of age and for persons more than 70 years of age is lower than the 1997 UL of 2,500 mg/day for these groups (IOM, 1997). The newer data related to kidney stone formation are the primary basis for the new UL. It is extremely difficult to reach the UL on the basis of food sources of calcium. Rather, the excess intake comes about from the use of calcium supplements. Special considerations about the use of high level calcium supplements and the timing of supplement intake are discussed in Chapter 8.

Adults 19 Through 50 Years of Age

Although the LOAEL (which is also the UL, as described above) for older adults more than 50 years of age is established at 2,000 mg/day, it can only serve as a starting point for UL consideration for adults 19 to 50 years of age given the observations that kidney stone formation in younger adults does not appear to be driven by calcium supplement use, and, as a rule, calcium supplement use is not as prevalent among younger adults. However, kidney stone formation is notable among younger persons; as discussed previously, the incident rate is actually higher among younger adults than among older adults. Given the UL of 3,000 mg/day for calcium set for adolescents up to the age of 18 years (based on high rate of bone accretion) as well as the likelihood that younger adults are able to tolerate higher maximal levels of calcium than other adults for whom kidney function may be slowly decreasing, an interpolation approach is used to establish a UL of 2,500 mg/day for adults 19 to 30 and 31 to 50 years of age, based on the mid-point between the UL of 2,000 mg of calcium per day set for persons more than 50 years of age and the UL of 3,000 mg/day set for adolescents 14 to 18 years of age. Further, concerns about the

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timing of calcium supplement intake would still be relevant to this group and are discussed in Chapter 8.

ULs for Pregnancy and Lactation

Pregnant or Lactating 14 Through 18 Years of Age	UL 3,000 mg/day Calcium
Pregnant or Lactating 19 Through 30 Years of Age	
Pregnant or Lactating 31 Through 50 Years of Age	UL 2,500 mg/day Calcium

Hypercalciuria is often present during normal pregnancy as a consequence of the doubling of intestinal calcium absorption that occurs, and pregnancy itself increases the risk of kidney stones. Consequently, excess intakes of calcium during pregnancy will aggravate hypercalciuria and possibly increase the risk of kidney stones. During lactation, the serum calcium (both ionized and albumin-corrected total calcium) level rises and usually remains within the normal range (although hypercalcemia can occur during normal lactation), and urinary excretion of calcium is reduced to the low-normal range or below. Consequently, higher intakes of calcium during lactation could potentially increase the risk of hypercalcemia. However, there is no evidence to suggest that the risk manifests itself at intakes lower than the UL for non-pregnant or non-lactating women, although it is acknowledged that relevant studies have not been rigorously carried out for pregnancy and lactation. Given that available evidence suggests that requirements for calcium among pregnant and lactating females are similar to those of non-pregnant and non-lactating females, and lacking data to suggest a basis for a different UL, the ULs for calcium for pregnancy and lactation have been kept the same as those for their non-pregnant and non-lactating counterparts.

VITAMIN D UPPER LEVELS: REVIEW OF POTENTIAL INDICATORS AND SELECTION OF INDICATORS

Few studies have been designed to specifically evaluate the safety of vitamin D intake, and there is not general agreement about the intake levels at which vitamin D may cause harm. A recent National Institutes of Health conference highlighted the lack of knowledge about mechanisms of action and toxic forms of the vitamin as well as the many limitations in the available evidence. Conference participants noted that available randomized controlled trials designed to illuminate health benefits likely

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underestimate the true potential for risk because: (1) for ethical reasons, adverse outcomes are secondary outcomes, (2) studies are of relatively short duration, (3) adverse outcomes are not always adequately monitored or completely reported, and (4) adverse outcomes generally lack adequate statistical power for detection (Brannon et al., 2008). Further, inclusion and exclusion criteria prevent persons at greatest risk from being study participants (Yetley et al., 2009).

Over the years, excess intake of vitamin D has been considered in the context of "intoxication" or "hypervitaminosis D"; as such, the condition is perhaps best regarded as a relatively acute response. Symptoms can appear in less than 4 weeks of continual excess ingestion. The hallmark of vitamin D intoxication is hypercalcemia, which is associated with a rise in serum 25OHD levels. The conditions of hypercalcemia and hypercalciuria were described previously in the section on calcium. Vitamin D intoxication generally presents with non-specific symptoms that may vary and often include anorexia, weight loss, polyuria, and heart arrhythmias (Jones, 2008). The condition eventually leads to vascular and tissue calcification with subsequent renal and cardiovascular damage.

Although data about vitamin D intoxication are informative, avoiding this relatively acute toxicity is not the intended purpose of a UL. Rather, the UL reflects a long-term level of intake that will not cause harm to the normal, free-living population. The 2007 AHRQ analysis (Cranney et al., 2007; hereafter referred to as AHRQ-Ottawa) concluded that few adverse outcomes could be identified for intakes "above current recommended levels," but it raised concerns about potential previously unrecognized adverse effects, including an increased risk of pancreatic cancer. The later AHRQ-Tufts analysis further identified all-cause mortality as an emerging concern, but the authors also pointed to the dearth of data.

Unfortunately, as pointed out in the earlier IOM report on DRIs for vitamin D, there continues to be a large uncertainty about the progressive health effects for regular ingestion of even moderately high amounts of vitamin D over several decades (IOM, 1997). Most available evidence is based on short-term exposures (less than 6 months). Generalization to long-term exposures—as would occur during a lifetime—is challenging. Also, most evidence is derived from adult populations with few data specific to children or vulnerable groups. For the purposes of an overview of the literature concerning adverse effects of excess vitamin D, the effects related to vitamin D intoxication (hypervitaminosis D) are discussed first and are based on a paper prepared for the committee by Hector DeLuca (DeLuca, 2009). They can provide a starting point for UL considerations. The emerging concerns about the adverse effects at higher intakes that are less than those associated with the toxicity are discussed next and are

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examined in the context of the appropriateness of introducing caution into the specification of ULs for vitamin D.

Vitamin D Intoxication and Related Hypercalcemia

Etiology and Effects of Vitamin D Intoxication

Increased serum 25OHD levels and resulting hypercalcemia are the hallmarks of vitamin D toxicity (Jones, 2008). Although intakes of either vitamin D_2 or vitamin D_3 can cause toxicity, there is evidence that higher levels of vitamin D_2 can be tolerated (Hunt et al., 1972; Stephenson and Peiris, 2009). Similarly, in laboratory animal experiments, vitamin D_3 has been reported to be more toxic (Roborgh and de Man, 1960).

The hypercalcemia that occurs from a rise in serum 25OHD level is due to increased bone resorption (Jones, 2008). In the early stages of intoxication, hypercalcemia may be modest and the renal glomerular filtration rate (GFR) remains stable. As bone resorption continues, however, the increasing blood levels of calcium lead to suppression of PTH production. The function and activity of the parathyroid–kidney–bone axis have thus emerged as contributors to the "set point" for toxicity of excess vitamin D and calcium. Decreased renal function simultaneously increases CVD risk and impairs calciuric responses and calcium phosphate homeostasis. Thus, elderly people represent a high-risk group for both extant CVD and impaired parathyroid–kidney–bone interactions that preserve normal calcium–phosphate homeostasis. Eventually, there is a loss of urinary concentrating mechanisms of the kidney tubule as well as a decrease in GFR (Towler, 2009). Hypercalciuria results from the hypercalcemia and the disruption of normal reabsorption processes of the renal tubules (IOM, 1997). As renal function declines (as occurs in disease and, to a lesser extent, with aging), there is additional loss of homeostatic control of serum calcium and phosphorus levels. Failure of the kidney and cardiovascular system is likely the ultimate cause of death in vitamin D intoxication. The prolonged ingestion of excess amounts of vitamin D and the accompanying hypercalcemia can cause metastatic calcification of soft tissues (IOM, 1997). Calcification of vascular tissue has long been known to be associated with vitamin D toxicity (Taussig, 1966; Bajwa et al., 1971; Kamio et al., 1979). Major perturbations in calcium–phosphate homeostasis may increase the risk of CVD, related in part to arterial calcium deposition (Hruska et al., 2009). The hypothesis that excess vitamin D intake may be associated with kidney stone formation is not supported by the available data.

Animal models of vitamin D toxicity reveal symptoms that are almost identical to those described for humans and have provided useful information (Shephard and DeLuca, 1980; Littledike and Horst, 1982a,b;

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Tryfonidou et al., 2003; Harmeyer and Schlumbohm, 2004). Rats, in particular, have been used to study vitamin D toxicity. The form of vitamin D that rises exponentially in plasma following overdose is 25OHD, not calcitriol (Vieth, 1990; Jones, 2008; Stephenson and Peiris, 2009). Shephard and DeLuca (1980) administered graded doses of either vitamin D_3 or calcitriol to rats for a 2-week period. The results indicated that frank toxicity was achieved at 650 nmol of vitamin D_3 per day or 50,000 IU/kg body weight, producing a blood 25OHD level of 1,607 nmol/L, while calcitriol levels were markedly reduced. These results support 25OHD and not calcitriol as the likely toxicant. In fact, in most species, vitamin D intoxication is accompanied by a *decrease* in plasma calcitriol level (Hughes et al., 1977; Shephard and DeLuca, 1980; Harrington and Page, 1983). Nonetheless, a case has been made that the "free" calcitriol level in the plasma—that is, the metabolite displaced from the plasma transport protein, vitamin D binding protein, by other accumulating metabolites—increases in vitamin D intoxication (Vieth, 2007; Jones, 2008). Overall, however, the accumulating 25OHD appears to be the critical factor in triggering the intoxication.

Serum 25OHD Concentrations as Indicative of Toxicity

In the absence of well-controlled studies, the serum 25OHD level representing the vitamin D toxicity threshold in humans is not readily defined. Similarly, the vitamin D intakes required to trigger toxicity symptoms are not precisely known. Moreover, even though the physiological changes that occur with vitamin D toxicity are correlated to serum 25OHD levels, they may not be precisely aligned (Towler, 2009) and may vary from subject to subject and among sub-populations. Appendix G summarizes a number of human vitamin D toxicity case studies gathered from the scientific literature from early in the 20th century to the present. Some of these reports originated from a time before vitamin D metabolism was discovered and hence lack confirmation that the causative agent was vitamin D overdose. Table 6-3 contains case reports from the past 35 years in which the data are supported by vitamin D dose administered, serum calcium levels, and serum 25OHD levels. Also provided are data from several month-long studies with a range of vitamin D supplements in which fully documented vitamin D intoxication was not identified. It is concluded that occasional reports of hypercalcemia in these studies are not related to vitamin D.

As shown in Table 6-3, the literature contains evidence that a range of vitamin D supplements from 800 to 300,000 IU/day have been used for periods ranging from months to years. Doses below 10,000 IU/day are not usually associated with toxicity, whereas doses equal to or above 50,000 IU/day for several weeks or months are frequently associated with toxic side effects including documented hypercalcemia.

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TABLE 6-3 Case Reports of Vitamin D Intoxication: Intake and Plasma Measures

Vitamin D Intake (IU/day)	Duration	Serum Calcium (mg/dL)	Serum 25OHD (nmol/L)	Serum Creatinine (μmol/L)	Urinary Calcium (mmol/L GFR)	Reference
Vitamin D supplementation studies without documented hypercalcemia						
800	4–6 mo	NCa[a]	60–105[b]	—	—	Byrne et al., 1995[e]
1,800	3 mo	NCa	65, 80[c]	—	—	Byrne et al., 1995[e]
1,800	3 mo	NCa	57–86	82.4–3.8	—	Honkanen et al., 1990[f]
2,000	6 mo	NCa	—	—	—	Johnson et al., 1980[g]
10,000	4 wk	—	105[d]	—	—	Stamp et al., 1977
10,000	10 wk	—	110[d]	—	—	Davie et al., 1982
20,000	4 wk	—	150[d]	—	—	Stamp et al., 1977
Vitamin D supplementation studies reporting hypercalcemia						
50,000	6 wk	15.0	320	388	—	Schwartzman and Franck, 1987
50,000	15 y	12.5	560	—	—	Davies and Adams, 1978
100,000	10 y	12.8	865	215	0.508	Selby et al., 1995
200,000	2 y	15.1	1,202	207	—	Selby et al., 1995
300,000	6 y	13.2	1,692	184	0.432	Rizzoli et al., 1994
300,000	3 wk	11.3	800	339	0.065	Rizzoli et al., 1994
Accidental vitamin D intoxication						
~ 1,131,840; vitamin D overdose	—	15.0	1,171	265	—	Klontz and Acheson, 2007

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TABLE 6-3 Continued

~ 1,700,000; vitamin D poisoning	—	15.3	1,555	442	—	Vieth et al., 2002
~ 9,000,000; vitamin D overdose	—	11.3	> 375	159	—	Chiricone et al., 2003
~ 18,000,000; vitamin D overdose	—	15.3	> 375	329	—	Chiricone et al., 2003
Vitamin D poisoning	—	13.8–18.4 (n = 11)	847–1,652	—	—	Pettifor et al., 1995
Overfortification of milk	—	13.1 (n = 35)	560	—	—	Blank et al., 1995
Reference levels	—	8.6–10.6	20–100 (10)	18–150	< 0.045	Blank et al., 1995
			25–200 (9)			Haddad, 1980

NOTE: IU = International Units; GFR = glomerular filtration rate; mo = month(s); wk = week(s); y = year(s).

[a]NCa = normocalcemic.

[b]Five studies; n = 188.

[c]Two studies; n = 55.

[d]Indicates extrapolation from graphic data.

[e]Byrne et al. (1995) reported that 3 of 449 subjects had hypercalcemia, but 2 were deemed to be non–vitamin D related.

[f]Honkanen et al. (1990) measured serum 25OHD levels but observed no side effects of the vitamin D or calcium supplements.

[g]Johnson et al. (1980) reported that 2 of 63 subjects developed hypercalcemia but provided no details of the 2 subjects and did not measure serum 25OHD levels in their study protocol.

DeLuca (2009) concluded that, overall, the toxicity of hypercalcemia becomes evident at vitamin D intakes above 25,000 IU/day, corresponding to a serum 25OHD level of about 500 nmol/L. Hathcock et al. (2007), following an analysis of more than 20 publications, concluded that there was no association between harm and intakes of 10,000 IU/day. Although toxic effects associated with 400 IU/day seem implausible,[3] the diverse range of intakes and serum 25OHD levels is notable. Most reports suggest that the toxicity threshold is between 10,000 and 40,000 IU of vitamin D per day. Also, most do not identify toxicity until serum 25OHD levels of 500 to 600

[3]It is noted that Adams and Lee (1997) reported toxicity at a serum 25OHD level of 160 nmol/L, but this is based on a single patient with an elevated urinary calcium level that was corrected by withdrawing a vitamin D supplement of 1,200 IU/day.

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nmol/L or higher are reached; frank toxicity has been associated with a serum 25OHD level of 750 nmol/L (Jones, 2008; Deluca, 2009).

There have been no reports of vitamin D intoxication by ultraviolet B light alone (Webb et al., 1989). Davie et al. (1982), using high-performance liquid chromatographic analysis, found that skin irradiation reached a plateau after 5 to 6 weeks of exposure and achieved a plasma $25OHD_3$ level of no more than 45 nmol/L. In a study of healthy men ($n = 26$) who had just completed a summer of extended outdoor activity, Barger-Lux and Heaney (2002) found that the median serum 25OHD level was 122 nmol/L in late summer and decreased to 74 nmol/L by late winter. Binkley et al. (2007) found that among subjects ($n = 93$) with habitually high sun exposure (~ 29 hours/week), the mean serum 25OHD level was 79 nmol/L, with the highest reported level of 155 nmol/L. In a study of subjects ($n = 50$) who used a tanning bed on a regular basis (at least once a week), serum 25OHD levels were 90 percent higher in tanners than in controls ($n = 106$) (115.5 ± 8.0 nmol/L vs. 60.3 ± 3.0 nmol/L) (Tangpricha et al., 2004).

Conclusion

Clearly, there are a number of variables that may affect the onset of toxic symptoms in the face of excess vitamin D intake. There has been a paucity of longer-term studies that have investigated the effects of doses over 10,000 IU or the maintenance of serum 25OHD levels above 250 nmol/L. What the data do suggest is that it would be unlikely to observe symptoms of toxicity at daily intakes below 10,000 IU, while it is possible that daily intakes above 10,000 IU could be associated with toxicity. In any case, such short-term findings related to the extreme conditions of toxicity are not the ideal basis for setting ULs for the general population, which apply to long-term (essentially lifetime) exposures. Thus, additional considerations were evaluated, as discussed next.

Excess Vitamin D and Serum Calcium

The 1997 IOM report on DRIs for vitamin D used the effect of vitamin D intake on serum calcium level in humans as the basis for developing ULs for vitamin D (IOM, 1997). The work of Johnson et al. (1980) and most notably that of Narang et al. (1984) were taken into account. In the Narang et al. (1984) study, serum calcium levels in humans (with and without tuberculosis) were measured as a function of daily vitamin D doses of 400, 800, 1,200, 2,400, and 3,800 IU for 3 months. Thirty healthy men and women ranging in age from 21 to 60 years and without tuberculosis were included in the study. Hypercalcemia with vitamin D supplementation was reported in 63 percent of the patients with active tuberculosis, consis-

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tent with the known effect of granulomatous diseases on enhancement of 1α-hydroxylase activity. In the 30 subjects reported to be normal, statistically significant increases in serum calcium level were observed with vitamin D doses of 2,400 and 3,800 IU/day; however, only at the dose of 3,800 IU/day did the serum calcium level exceed the upper limits of normal (i.e., 10.5 mg/dL). Moreover, there were only five subjects in the highest dose group, the duration of the effect was not reported, and the heterogeneity within that subgroup was reflected by the large standard error.

Although increased serum calcium levels are of concern, the Narang et al. (1984) study is likely too small to allow any conclusions to be drawn beyond the potential risk of hypercalcemia during vitamin D supplementation in patients with tuberculosis. More recently, Aloia et al. (2008) conducted a 6-month dose–response study using 138 white and African American adults to determine the intake of vitamin D_3 needed to achieve a targeted plasma 25OHD level. Doses of vitamin D varied but reached means of 3,915 ± 840 IU/day for blacks and 3,040 ± 1,136 IU/day for whites. No patient presented with a serum calcium level above 265 mmol/L (or 10.6 mg/dL).

Excess Vitamin D and Measures in Infants

Jeans and Stearns (1938) found a retarded linear growth rate in 35 infants up to 45 weeks of age who received daily doses of 1,800 to 4,500 IU of vitamin D as supplements (without regard to sun exposure), for a minimum of 6 months, compared with infants receiving supplemental doses of 340 IU/day or less. Fomon et al. (1966), in a similar study, explored the effects of vitamin D on linear growth in infants ($n = 13$) ingesting 1,380 to 2,170 IU/day (mean = 1,775 IU/day) of vitamin D from fortified evaporated milk formulas as the only source of vitamin D, compared with infants receiving 350 to 550 IU/day ($n = 11$) from another batch of formula. No effect was found in infants who were enrolled in the study from the first 9 days after birth up to 6 months of age. Newer data to better elucidate the relationship between vitamin D and retarded linear growth in infants have not emerged in recent years.

Reports in Britain in the 1950s, when foods were being liberally fortified with vitamin D, indicated an unusually large number of cases of "idiopathic hypercalcemia" (British Paediatric Association, 1956). Given the number of foods fortified at the time, the British Paediatric Association (1956) estimated an intake of about 4,000 IU of vitamin D per day for an infant who consumed a typical diet of milk (1.5 pints), cereal (1 ounce), and cod liver oil (1 teaspoon). The outbreak of idiopathic hypercalcemia that took place was attributed to vitamin D supplementation, but the cause cannot be determined with certainty. Survey data apparently reported a

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marked decline in hypercalcemia in infants, from 7.2 cases per month in a 1953 to 1955 survey, to 3.0 cases per month in a 1960 to 1961 survey (British Paediatric Association, 1956, 1964). This change occurred at the time new guidelines were introduced for fortification of food products with vitamin D. Data from the British Paediatric Association (1956) and Bransby et al. (1964) also suggested that the estimated total vitamin D intake in infants at the 75th percentile declined from 4,000 IU/day to a range of 724 to 1,343 IU/day between the two surveys.

Other Adverse Effects of Excess Vitamin D: Mortality, Chronic Disease, Falls and Fractures

The committee reviewed the evidence emerging from observational/association studies and a limited number of clinical trials related to vitamin D intake and a diverse set of health outcomes, ranging from breast cancer to falls and fractures. The purpose was not to determine that certain levels of intake definitively cause harm, but rather to decide whether the emerging data were sufficiently compelling to warrant caution relative to vitamin D intakes and associated serum 25OHD concentrations that may be less than those associated with the more widely known acute toxicity but still associated with adverse effects that may occur as a result of chronic intake. The potential adverse effects are considered in alphabetical order.

All-Cause Mortality

All-cause mortality data emerging from the examination of national survey data as well as observational studies suggest adverse effects at serum 25OHD levels much lower than those associated with the toxicity demonstrated by hypervitaminosis D. The AHRQ-Tufts analysis identified four cohort studies (Sambrook et al., 2004, 2006; Visser et al., 2006; Jia et al., 2007; Melamed et al., 2008) that focused on the relationship between serum 25OHD level and all-cause mortality. In general, these studies, as expected, indicated that low serum 25OHD levels akin to deficiency states (< 30 nmol/L) are associated with an increased risk of mortality. Further, as serum 25OHD levels increase—up to a point—mortality is lowered.[4] However, some, but not all, of the studies have observed a troubling U-shaped (or perhaps more appropriately a reverse-J-shaped) relationship. For example, Jia et al. (2007) found a statistically significant trend between increasing serum 25OHD levels and lower odds ratios for all-cause

[4]Note: The studies adjusted for lifestyle factors linked with poor vitamin D nutriture and other factors; not unexpectedly the relationship became weaker, reflecting some confounding factors.

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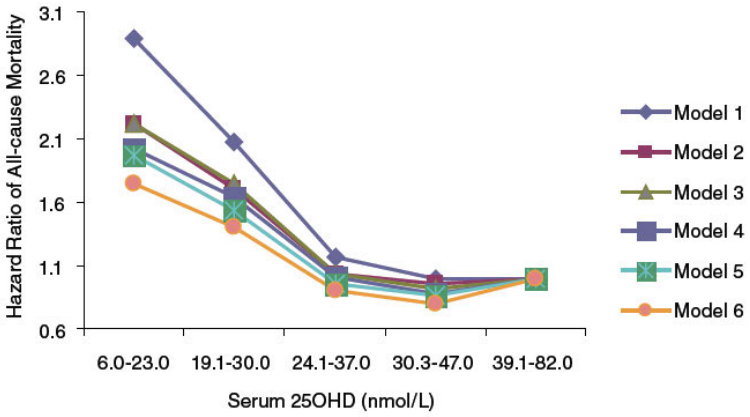


FIGURE 6-1 Hazard ratios of risk of death according to baseline serum 25OHD level (subjects with serum 25OHD levels 39.1–82.0 nmol/L are the referent category).
NOTE: Model 1 is adjusted for age and gender; model 2 is adjusted for model 1 and taking five or more kinds of medicine and self-perceived health status; model 3 is adjusted for model 2 and having heart problem and/or diabetes at baseline; model 4 is adjusted for model 3 and sunlight exposure (i.e., season of blood sampling, sunbathing, and outdoor physical activity); model 5 is adjusted for model 3 and use of a supplement containing vitamin D; model 6 is adjusted for model 3 and variables in models 4 and 5.
SOURCE: Jia et al. (2007).

mortality ($p = 0.03$); however, a U-shaped or reverse-J-shaped relationship between serum 25OHD level and mortality was observed, with the lowest mortality at serum 25OHD levels below 50 nmol/L (see Figure 6-1). Visser et al. (2006) showed a similar pattern, with reduced mortality associated with higher than deficiency levels, but increased mortality at the highest blood 25OHD levels (see Figure 6-2). Melamed et al. (2008), using data from the Third National Health and Nutrition Examination Survey (NHANES III), also suggested a U-shaped or reverse-J-shaped risk curve with increasing risk at about 75 nmol/L (see Figure 6-3). The similar patterns emerging in these studies are of concern and are suggestive of at least a reverse-J-shaped curve, if not precisely a U-shaped curve for risk relative to serum 25OHD levels and all-cause mortality. Of note, Sambrook et al. (2004, 2006) found no relationship between mortality and the log of serum 25OHD levels in a sample ($n = 842$) of frail, institutionalized persons, most

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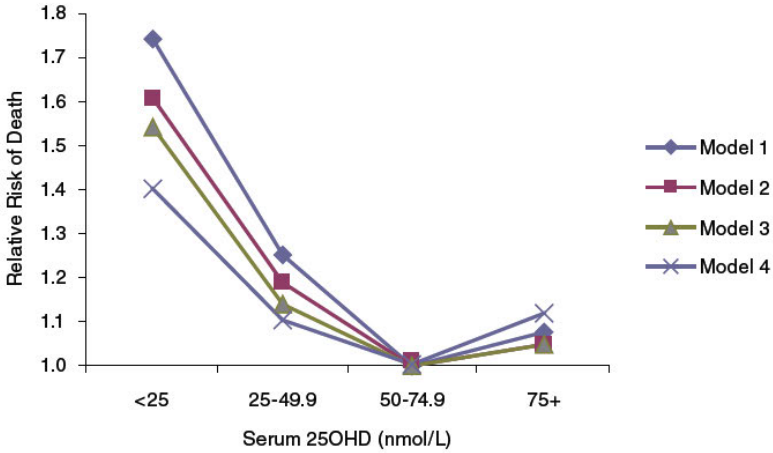


FIGURE 6-2 Risk of death in elderly people according to baseline serum 25OHD level in the Longitudinal Aging Study (subjects with serum 25OHD levels of 50.0–74.9 nmol/L are the referent category).
NOTE: Model 1 is adjusted for gender, age, and education; model 2 is adjusted for model 1 and for chronic disease, serum creatinine concentration, cognitive status, and depressive symptoms; model 3 is adjusted for model 2 and for lifestyle variables including body mass index, smoking status, alcohol consumption, and physical activity; model 4 is adjusted for model 3 and for frailty indicators: mobility performance, low serum albumin concentration, and low serum total cholesterol concentration.
SOURCE: Visser et al. (2006).

over the age of 80 years. Also, the committee identified another cohort study not included in the AHRQ-Tufts report (Semba et al., 2009) that did not observe a U-shaped relationship, but the highest exposure category in this Italian cohort was approximately 64 nmol/L. In addition to these published observational studies, a preliminary analysis of NHANES III data limited to data on non-Hispanic blacks with follow-up as of December 31, 2006, also saw a U-shaped relationship, although the suggested increase in risk was seen at a lower serum 25OHD concentration of approximately 60 nmol/L.[5]

Turning to evidence from vitamin D supplementation trials, AHRQ-Tufts calculated an overall relative risk (RR) for all-cause mortality of 0.97 (95% CI: 0.92–1.02), with no evidence of between-study heterogeneity. The doses studied included 400 and 880 IU of supplemental vitamin D per day,

[5]Personal communication, R. Durazo-Arvizu, Loyola University, Maywood, IL, May 28, 2010.

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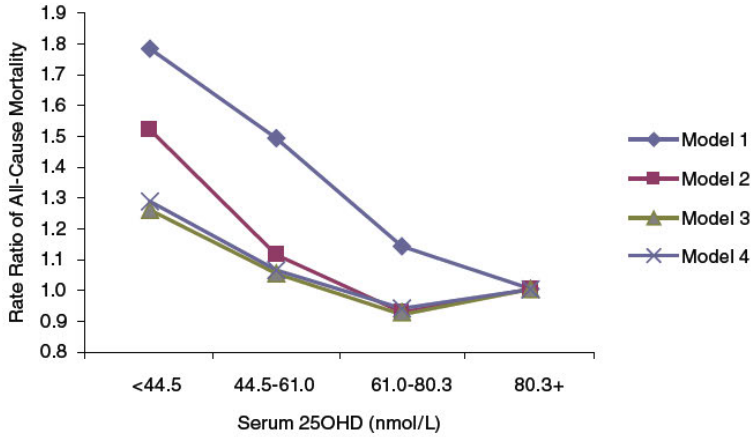


FIGURE 6-3 Rate ratios of all-cause mortality by serum 25OHD level in NHANES III (subjects with serum 25OHD levels above 80.3 nmol/L are the referent category).
NOTE: Model 1 is unadjusted; model 2 is adjusted for age, gender, race, and season; model 3 is adjusted for age, gender, race, season, hypertension, history of prior cardiovascular disease, diabetes, smoking, high-density lipoprotein cholesterol, total cholesterol, use of cholesterol medications, estimated glomerular filtration rate categories, serum albumin, log (albumin-creatinine ratio), log (C-reactive protein), body mass index, physical activity level, vitamin D supplementation, and low socioeconomic status; model 3 is adjusted for age, gender, race, season, cigarette use, body mass index, log (C-reactive protein), serum albumin, physical activity level, vitamin D supplementation, and low socioeconomic status.
SOURCE: Melamed et al. (2008).

and one trial that gave a supplement of 100,000 IU every 3 months, which is roughly equivalent to 1,100/day.

Because the trials did not evaluate particularly high doses and as observational studies are subject to confounding, one cannot interpret conclusively whether or not this U-shaped relationship is real or causal. However, the data are clearly suggestive of a U-shaped or reverse-J-shaped risk curve between serum 25OHD level and all-cause mortality; increases in risk are suggested at thresholds in the range of 75 to 120 nmol/L for the white population, with lower levels for the black population.

Cancer

Breast cancer A study from the randomized, double-blind, placebo-controlled WHI trial (Chlebowski et al., 2008) indicated overall that daily

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supplementation with 1,000 mg of elemental calcium combined with 400 IU of vitamin D_3 had no effect on breast cancer incidence. However, through a stratified analysis, the data demonstrated an increased risk of breast cancer for women who were already consuming 600 IU of vitamin D per day at baseline, to which a supplement of 400 IU/day was added ($P_{\text{interaction}} = 0.003$). Serum 25OHD measures were analyzed by quintile and the highest quintile was 67.6 nmol/L and above.

Pancreatic cancer Some, but not all, observational studies suggest that higher serum 25OHD levels are associated with an increased risk of pancreatic cancer. Beginning with negative studies, Skinner et al. (2006) examined two large cohort populations—the HPFS and the NHS—for associations between pancreatic cancer incidence and vitamin D intake from diet and supplements. Another study of the HPFS cohort examined associations between serum 25OHD level and total cancer mortality or digestive (including pancreatic) cancer incidence (Giovannucci et al., 2006b). Both studies found reduced risk for pancreatic cancer incidence: in one instance associated with higher vitamin D intake (≥ 600 IU/day) (Skinner et al., 2006) and in the other based on a predicted serum 25OHD level (as described by the authors) for which RR was calculated for incremental increases in serum 25OHD level of 25 nmol/L (Giovannucci et al., 2006b).

In contrast, an initial study from Stolzenberg-Solomon et al. (2006), using a nested case–control protocol to evaluate associations between vitamin D nutriture and incidence of pancreatic cancer in subjects from the ATBC Study, found a positive association between higher serum 25OHD levels (highest quintile at 83.2 nmol/L) and risk for pancreatic cancer. In a subsequent nested case–control analysis of a cohort from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, the same investigators found no association between higher serum 25OHD level and increased pancreatic cancer risk as an outcome (Stolzenberg-Solomon, 2009). The difference between the study populations included living at a northern latitude, positive smoking history, and gender (male) in the ATBC Study compared with a mixed gender U.S. population that was controlled for smoking history in the PLCO Cancer Screening Trial.

To address the dissimilarity in results from individual large cohort studies, Stolzenberg-Solomon et al. (2010) conducted a pooled nested case–control analysis of participants from several cohorts (the ATBC Study, CLUE, the Cancer Prevention Study II Nutrition Cohort, the New York University Women's Health Study, the PLCO Cancer Screening Trial, and the Shanghai Women's and Men's Health Studies) to determine associations between serum 25OHD levels pre-diagnosis and risk for incident pancreatic cancer. This large-scale pooled analysis ($n = 2,285$) found a statistically significant two-fold increased risk for pancreatic cancer in participants with

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serum 25OHD levels at or above 100 nmol/L compared with those with levels between 50 to 75 nmol. Further, the association was strongest for whites, participants in northern latitudes (> 35°N), and participants whose blood was collected in summer months. Thus, a pooled analysis of large cohort studies suggests an association for increased risk of pancreatic cancer with serum 25OHD levels greater than 100 nmol/L that is not consistently seen in analyses of individual large cohorts.

Prostate cancer Regarding prostate cancer, Tuohimaa et al. (2004) found a higher risk of prostate cancer for those with serum 25OHD levels above 80 nmol/L. The subjects were 67 men, mostly from Norway. Although another study from Finland (Tuohimaa et al., 2004) also found an association between serum 25OHD levels and prostate cancer at levels above 80 nmol/L, a study conducted by Faupel-Badger et al. (2007) also in Finland did not find a relationship.

Cardiovascular Risk

Although Linden (1974) observed that myocardial infarct patients in Norway were more likely than matched controls to have consumed vitamin D in excess (greater than 1,200 IU/day), two later studies (Schmidt-Gayk et al., 1977; Vik et al., 1979) failed to confirm these results. Melamed et al. (2008) examined data from 3,439 persons in the NHANES 2001 to 2004 surveys to determine the relationship between serum 25OHD level and peripheral arterial disease (defined as an ankle-brachial index < 0.9). The researchers noted that there was a lower risk of CVD mortality in men and women at levels of 75 to 122 nmol/L, but a higher risk of CVD mortality in women at levels above 125 nmol/L. Recently, Ginde et al. (2009), in a prospective cohort analysis of NHANES III data (1988 to 1994) on serum 25OHD levels in adults ages 65 years and older (n = 3,408) over a median 7.3-year follow-up, examined CVD mortality. Analysis of fully adjusted data indicated an inverse relationship between CVD mortality and baseline serum 25OHD level of 50.0 to 74.9 nmol/L. Risk began to increase at approximately 75 nmol/L and then it declined after 100 nmol/L.

Analyses from the Framingham Offspring Study (Wang et al., 2008), which followed 1,739 participants (mean age 59 years) with an average follow-up at 5.4 years, found a significant relationship between low serum 25OHD levels and incident cardiovascular risk. During a mean follow-up of 5.4 years, 120 individuals developed a first cardiovascular event; vitamin D deficiency as defined in the study (serum 25OHD level < 37.5 nmol/L) was associated with increased risk for cardiovascular events. However, a closer look at the individuals with the highest serum 25OHD levels suggests that there was no additional reduction in risk with 25OHD levels above 75 nmol/L and even that the dose–response relationship may be U-shaped or

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reverse-J-shaped, with increased risk not only at low but also at the higher levels of serum 25OHD (i.e., > 75 nmol/L).

Fiscella and Franks (2010) conducted a retrospective cohort analysis also based on NHANES III data. They examined serum 25OHD levels and CVD mortality in participants ages 18 years and older ($n = 15,363$). Analysis of fully adjusted data showed a U-shaped risk profile for CVD mortality, as reported by others. Without consideration of vitamin D status, after adjusting for age, gender, season, and region, non-Hispanic blacks had a 38 percent higher cardiovascular mortality than whites. Adjusting for low serum 25OHD levels reduced the racial difference in risk by about 60 percent (to 23 percent). Including both low serum 25OHD level and poverty level reduced the racial difference in risk to 1.0, suggesting that low serum 25OHD level and poverty capture much of the racial disparity in cardiovascular mortality in blacks compared with whites; however, it must be recognized that the low serum 25OHD level may be a marker for other factors (obesity, inactivity, etc.). Additionally, a cross-sectional study conducted by Freedman et al. (2010) reported a positive association between serum 25OHD level and calcified atherosclerotic plaque in the aorta and carotid arteries of African Americans.

Falls and Fractures

In a recent trial of 2,256 community-dwelling women 70 years of age and older residing in Australia and presenting with high risk of fracture, the women were treated with 500,000 IU of vitamin D annually for 3 to 5 years (Sanders et al., 2010). Sanders et al. (2010) reported that "… participants receiving annual high-dose oral cholecalciferol experienced 15% more falls and 26% more fractures than the placebo group. Women not only experienced excess fractures after more frequent falls but also experienced more fractures that were not associated with a fall. A post hoc analysis found that the increased likelihood of falls in the vitamin D group was exacerbated in the 3-month period immediately following the annual dose and a similar temporal trend was observed for fractures. An increased risk (albeit, not significant because of smaller numbers) of falls and fracture in the vitamin D group was apparent for each year of the intervention. The results were similar after adjustment for baseline calcium intake.…"

The non-physiological nature of a large one-time dose cannot be readily extrapolated to the situation in which smaller daily doses are provided. However, in view of a number of studies in the literature (e.g., Trivedi et al., 2003) in which large bolus doses have been given without apparent adverse effect, the results of the study by Sanders et al. (2010) are unexpected, but not readily dismissed. The study is notable because the adverse effect was demonstrated as a result of the intervention (which was primarily

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for safety) as well as through a measure of interest, serum 25OHD concentration. The median serum 25OHD concentration 1 month after dose for study participants was 120 nmol/L. By 3 months, the median value was approximately 90 nmol/L. One other study (Smith et al., 2007) also reported an increase in fracture associated with vitamin D treatment. Participants were 75 years of age or older (4,354 men and 5,086 women) and received an annual injection of 300,000 IU as ergocalciferol or placebo. In men, treatment had no effect on fractures. However, women treated with vitamin D had increased risk of fractures classified as non-vertebral (HR = 1.21), hip/femur (HR = 1.80), and hip/femur/wrist/forearm (HR = 1.59). No effect on falls was observed; however, falls were a secondary outcome and ascertainment was based on 6-month recall. Baseline serum 25OHD levels and changes in serum 25OHD levels were very similar to the results from Sanders et al. (2010). Another common feature was that calcium supplements were not given.

A recent study reported by Cauley et al. (2009) indicated that in contrast to white and American Indian women, black women and possibly Asian women appeared to be at greater risk of fracture with higher serum 25OHD levels (≥ 75 nmol/L).

Conclusion

Despite the limitations of the evidence, there is a notable congruence across different health indicators—all-cause mortality, some cancers, CVD risk, fractures and falls—for adverse outcomes associated with serum 25OHD levels ranging from about 75 to 120 nmol/L. The U-shaped curve, or possibly a reverse-J-shaped curve, for risk does indeed emerge, with adverse effects reported at either end of the serum 25OHD concentration span. Data for associated intakes of vitamin D are limited.

The committee's approach was to consider whether it was reasonable to use these findings as a basis for adjusting data on the toxicity of vitamin D, discussed above. In doing so, it was aware of recent criticisms related to taking into account these so-called U-shaped serum 25OHD response curves for elucidating levels of vitamin D that may cause adverse effects (e.g., Grant, 2010). However, although these data may be characterized as emerging and in need of further study before firm conclusions can be made, they are not reflective of flawed studies nor are they readily dismissed by other literature. Further, in the absence of data to demonstrate benefit at such serum 25OHD levels, a cautious approach is justified and appropriate given the purpose of the UL. In the committee's view, these emerging relationships do not have to be definitively proven in order to justify a cautious approach that is most likely to ensure safety, and in the absence of data to demonstrate benefit from the higher intake level or higher

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serum 25OHD levels, these signals can be taken into account. Focusing exclusively on vitamin D acute toxicity for the purposes of establishing a UL and ignoring the emerging data related to other adverse events is not in the best interests of public health.

Selection of Indicator for the UL: Vitamin D

The best available data set for establishing a UL for vitamin D is associated with the onset of hypercalcemia and related toxicity. This indicator is selected as the basis for the UL for all age groups except infants, with the caveat that while it serves as a starting point, it is to be subject to adjustment for uncertainty. The adjustment is based on: (1) the recognition of the goal of public health protection, which suggests that avoiding hypervitaminosis D is, of course, desirable, but not necessarily sufficient; and (2) the emerging data concerning other adverse effects at intakes lower than those associated with acute toxicity and at serum 25OHD levels previously considered to be at the high end of physiological values. Taken as a whole, the body of evidence suggests that there is reason to proceed cautiously in assuming that higher levels of vitamin D intake below those expected to cause hypervitaminosis D are harmless, especially in the absence of data to demonstrate benefit at such intake levels. For infants, the long-standing measures related to retarded linear growth serve as the indicator for the UL.

The available data could not offer a BI or be used to estimate a dose–response relationship. The basis for the ULs is a NOAEL as described further below for specific life stage groups.

VITAMIN D UPPER LEVELS: INTAKE-RESPONSE ASSESSMENT AND SPECIFICATION OF UPPER LEVELS

The ULs established for vitamin D are shown in Table 6-4 by life stage group. The ULs for infants are discussed first. This is followed by a discussion of ULs for adults rather than children and adolescents because the UL for adults is used to extrapolate or scale a UL value for children and adolescents. A discussion of ULs during pregnancy and lactation follows.

ULs for Infants 0 to 12 Months of Age

Infants 0 to 6 Months of Age	UL 1,000 IU (25 μg)/day Vitamin D
Infants 6 to 12 Months of Age	UL 1,500 IU (38 μg)/day Vitamin D

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TABLE 6-4 Vitamin D Tolerable Upper Intake Levels (UL) by Life Stage

Life Stage Group	UL
Infants	
0 to 6 mo	1,000 IU (25 µg)
6 to 12 mo	1,500 IU (38 µg)
Children	
1–3 y	2,500 IU (63 µg)
4–8 y	3,000 IU (75 µg)
Males	
9–13 y	4,000 IU (100 µg)
14–18 y	4,000 IU (100 µg)
19–30 y	4,000 IU (100 µg)
31–50 y	4,000 IU (100 µg)
51–70 y	4,000 IU (100 µg)
> 70 y	4,000 IU (100 µg)
Females	
9–13 y	4,000 IU (100 µg)
14–18 y	4,000 IU (100 µg)
19–30 y	4,000 IU (100 µg)
31–50 y	4,000 IU (100 µg)
51–70 y	4,000 IU (100 µg)
> 70 y	4,000 IU (100 µg)
Pregnancy	
14–18 y	4,000 IU (100 µg)
19–30 y	4,000 IU (100 µg)
31–50 y	4,000 IU (100 µg)
Lactation	
14–18 y	4,000 IU (100 µg)
19–30 y	4,000 IU (100 µg)
31–50 y	4,000 IU (100 µg)

NOTE: IU = International Unit.

The work of Fomon et al. (1966) forms the starting point for these life stage group, as it did in the 1997 IOM report (IOM, 1997). Given the small sample size used in the study, the NOAEL for infants is based on the mean intake in this study rather than the high end of the range. The NOAEL was rounded to 1,800 IU/day from 1,775 IU/day. The British Paediatric Association (1956) data and data reported by Bransby et al. (1964) suggested that hypercalcemia could be present at intakes of 4,000 IU/day, but appeared to decline at intakes between 700 and 1,300 IU/day, lending some support to the NOAEL of 1,800 IU/day as reasonable. However, considerable uncertainty surrounds this estimate, and newer data have not

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emerged regarding vitamin D intake and hypercalcemia in infants. Stearns (1968) commented that Fomon et al. (1966) did not study the infants long enough, because the greatest differences in the Jeans and Stearns (1938) study appeared after 6 months. Also, a report from 1959 (Graham, 1959) suggested that a serum calcium level obtained from a study in Glasgow of infants with hypercalcemia ages 3 weeks to 11 months was associated with an estimated vitamin D intake of 1,320 IU/day. Overall, on balance, 1,800 IU/day is reasonable as a NOAEL and offers an appropriate starting point.

Infants 0 to 6 Months of Age

The first order of importance is to protect young infants, and the intake of 1,800 IU/day may not be entirely protective of such young infants. As the UL can reasonably be considered to affect all non-growth retarded infants at greater than 37 weeks gestational age at birth, and given the current practice to begin vitamin D supplementation within days of birth (Wagner and Greer, 2008), it is necessary to ensure an absence of toxicity in infants as small as 2,500 to 3,000 grams who would meet this definition. As such, applying an uncertainty factor of 0.5 and rounding would reasonably give a level of 1,000 IU/day, which is also about 400 IU/kg body weight per day, a dose that can reasonably be considered an upper safety level on a body weight basis. This UL is the same as the UL established in 1997 (IOM, 1997).

Infants 6 to 12 Months of Age

Consistent with general principles of toxicology, the committee considered that an infant's capacity to handle excess substances such as vitamin D is likely increased with increased body size, organ maturation, and growth needs. Therefore, for older infants it is reasonable to consider a higher UL than for younger infants, although available data are inadequate for quantitative risk assessment. Also, the endpoint is acknowledged to be relatively insensitive. The UL for infants 6 to 12 months of age is increased by 500 IU/day from that established for infants 0 to 6 months of age, to a value of 1,500 IU/day. This reflects a more cautious approach than would be taken if the UL were doubled and is consistent with public health protection. This UL is slightly greater than the UL for vitamin D established for this life stage group in 1997, but is consistent with the toxicological principles that older infants are likely to have greater tolerances than younger infants (IOM, 1997).

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ULs for Adults 19 or More Years of Age

Adults 19 Through 30 Years of Age
Adults 31 Through 50 Years of Age
Adults 51 Through 70 Years of Age
Adults >70 Years of Age
UL 4,000 IU (100 µg)/day Vitamin D

The indicator of hypercalcemia for vitamin D toxicity is the starting point for the UL for adults. This condition is at an extreme end of an adverse outcome continuum and it may be appropriate to consider instead as a starting point for other measures, such as hypercalciuria. However, interpretation of measures such as hypercalciuria as a predictor of adverse outcomes is unclear. Therefore, the best available option as an indicator is hypercalcemia. In this case, an intake value of 10,000 IU/day reflects a NOAEL. This NOAEL is initially adjusted for uncertainty to establish a UL of 4,000 IU/day, as described below.

Initially, it should be noted that evidence pertaining to the levels of 25OHD in serum that are associated with adverse effects is less well established than that associated with benefit, and the available literature suggests considerable variability. As shown above in Table 6-3, frank toxicity has been reported to have occurred within a wide range of serum 25OHD levels, from as low as 60 nmol/L (Byrne et al., 1995) to values above 1,500 nmol/L (Rizzoli et al., 1994; Pettifor et al., 1995; Vieth et al., 2002), although the majority of available reports of toxicity involve serum 25OHD values above 350 nmol/L. The variability in the toxicity data may mean that toxicity can be affected by numerous mitigating factors or perhaps may be a function of the diversity in the nature of the available case reports. Reports on maximal sun exposure also described previously (Barger-Lux and Heaney, 2002; Binkley et al., 2007) suggest that serum 25OHD levels under these circumstances generally remain below 125 to 150 nmol/L, although the populations studied are not diverse and generally include younger men. The emerging data related to all-cause mortality, chronic disease risk, and falls would appear to suggest that adverse events may occur with serum 25OHD levels of approximately 75 nmol/L or above (Visser et al., 2006; Ginde et al., 2009), but ranging up to approximately 125 nmol/L (Melamed et al., 2008). The vagaries of serum 25OHD measures in general, the sparse data available, and the uncertainty as to the nature of the adverse effects preclude strong conclusions. On the basis of available reports, the committee considered that serum 25OHD levels above approximately 125 to 150 nmol/L should be avoided. Given the conclusion derived in Chapter 5 that bone health benefit is achieved by 97.5 percent

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of the population at 50 nmol/L, there is a range of serum 25OHD levels between 50 and 150 nmol/L that remains undescribed. The adjustment to the starting point of 10,000 IU/day reflects first data concerning adverse effects related to all-cause mortality, falls and fractures, and CVD risk, which, taken as a total body of evidence, provide reason for caution, as described earlier. More specifically, the evidence from the studies that focused on all-cause mortality, chronic disease, falls and fractures suggested that serum 25OHD levels between 75 nmol/L and approximately 120 nmol/L were associated with the adverse effect. There is considerable uncertainty surrounding such values, and—using information on the serum levels achieved during maximal sun exposure and to avoid being unnecessarily restrictive given the uncertainties—the committee determined that for the purposes of the UL, concern would be for levels above approximately 125 to 150 nmol/L.

Further, there are emerging data concerning the possible differences in adverse event response for African Americans and perhaps other dark-skinned population groups. The cross–sectional study from Freedman et al. (2010) reported a positive association between serum 25OHD levels and calcified atherosclerotic plaque in the aorta and carotid arteries of African Americans, and preliminary reports from NHANES suggest that the risk for all-cause mortality among non-Hispanic blacks compared with whites occurs at a lower serum 25OHD level (60 vs. 75 nmol/L).[6] These data are limited, are not necessarily consistent with other findings, and may eventually be explained by factors other than serum 25OHD levels, but they are concerning. Although data on race and ethnic differences are much too sparse to justify providing different ULs for different racial or ethnic groups, they can be incorporated as a source of uncertainty.

To determine an adjustment from the starting point of 10,000 IU/day as a NOAEL taking into account the uncertainties introduced by reports concerning all-cause mortality and other chronic disease outcomes as well as the possibility that blacks in the North American population may experience adverse effects at lower serum 25OHD concentrations than whites, the committee considered the work of Heaney et al. (2003). As suggested by the study, vitamin D intakes of 5,000 IU/day achieved serum 25OHD levels that range between 100 and 150 nmol/L, but do not surpass 150 nmol/L after 160 days of administration. Almost no other studies have assessed the safety of long-term maintenance of serum 25OHD levels in this range in relation to chronic disease risk and all-cause mortality, so the information about the increases in serum levels is useful for the purposes of establishing a UL. Given the uncertainties surrounding the data and the reliance on a

[6]Personal communication, R. Durazo-Arvizu, Loyola University, Maywood, IL, May 28, 2010.

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single report, the UL is set 20 percent below the level identified by Heaney et al. (i.e., 5,000 IU), specifically at 4,000 IU/day.

This value is greater than that set in 1997 by the previous IOM committee. A UL of 4,000 IU/day is still, however, a reference value that reflects the interest in providing public health protection, especially when existing data do not support benefit above such intakes. Intake values in the range of 4,000 IU/day would not appear to cause serum 25OHD levels to exceed 125 to 150 nmol/L,[7] a concentration which is at the high end of the range of serum levels associated with nadir risk of outcomes such as all-cause mortality.

ULs for Children and Adolescents 1 Through 18 Years of Age

Children 1 Through 3 Years of Age	
	UL 2,500 IU (63 μg)/day Vitamin D
Children 4 Through 8 Years of Age	
	UL 3,000 IU (75 μg)/day Vitamin D
Children 9 Through 13 Years of Age	
Adolescents 14 Through 18 Years of Age	
	UL 4,000 IU (100 μg)/day Vitamin D

No specific data are available for age groups other than adults and infants. In 1997 it was determined that increased rates of bone formation in toddlers, children, and adolescents suggested that the adult UL is appropriate for these age groups (IOM, 1997). The present committee chose to scale down the adult UL for younger children—to 2,500 IU/day for 1- to 3-year-olds and 3,000 IU/day for 4- to 8-year-olds—so as to be more consistent with concepts of graded tolerances with maturity. Although the simulated dose–response relationship between vitamin D intake and serum 25OHD level described in Chapter 5 is not affected by age, the data available did not include any children younger than 6 years old. There is no quantitative basis for such scaling, but it reflects a cautious and prudent approach given current biological understandings. Children and adolescents between 9 and 18 years of age have ULs that are the same as that for adults. All the UL values for children are slightly higher than the values provided in 1997 (IOM, 1997).

[7]Use of the regression model developed to estimate the vitamin D intakes needed to achieve a specific level of 25OHD in serum is not appropriate for this situation, in that the model was derived using data based on minimal sun exposure and did not anticipate estimations of such high levels of intake. However, the use of the related equations suggests that 4,000 IU/day results in a mean serum 25OHD concentration of 91 nmol/L and an upper level of 105 nmol/L.

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ULs for Pregnancy and Lactation

Pregnant or Lactating 14 Through 18 Years of Age
Pregnant or Lactating 19 Through 30 Years of Age
Pregnant or Lactating 31 Through 50 Years of Age
UL 4,000 IU (100 μg)/day Vitamin D

The available data do not indicate a basis for deriving a UL for pregnant and lactating women or adolescents that is different from those for their non-pregnant and non-lactating counterparts.

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7

Dietary Intake Assessment

Conducting an intake assessment—after the available scientific data have allowed the estimation of reference values (see Chapters 5 and 6)—is one of the hallmarks of nutrient risk assessment. Estimates of population intake (i.e., "exposure") are obtained, and these are examined in view of the estimated reference values. When information is available, consideration of biochemical and clinical measures of nutriture is a useful adjunct to the intake assessment and can provide important information about the adequacy of intake as well as excess intake.

In the case of the United States and Canada, data from national government surveys form the basis for the intake assessment. In this chapter, the national surveys are described first. Then information about calcium intake is presented, followed by information about vitamin D intake and serum 25-hydroxyvitamin D (25OHD) concentrations. In this report, the term "dietary intake" includes the intake of foods and supplements and is also referred to as "total intake."

THE NATIONAL SURVEYS AND APPROACH USED

Nutrient intake data for the intake assessment are available through the websites for the national surveys in each country. The U.S. survey data are reported on the basis of Dietary Reference Intake (DRI) life stage groups and are divided by males and females rather than combined. In the case of intake estimates, Canadian data are reported for children ages 1 to 3 and 4 to 8 years without distinction by gender, but they are reported on the basis of males and females for the older groups. Serum 25OHD

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levels for Canadians are collected for persons between the ages of 6 and 79 years. However, in arranging these data from survey age/sex groups into the DRI life stage groups, sample sizes did not allow adequate representation for children less than 9 years of age. Therefore, the data were used to construct values for the DRI life stage groups only between ages 9 to 79 years for Canadians. In addition, neither country reports data for infants 0 to 12 months of age or for pregnant and lactating women; sample sizes for these groups are too low in the surveys to provide nationally representative estimates.

United States: The National Health and Nutrition Examination Survey

Information about the U.S. National Health and Nutrition Examination Survey (NHANES) is available from the survey's main website,[1] and is therefore only summarized here. In the 1960s, the U.S. government initiated the National Health Examination Survey to assess the health status of individuals ages 6 months through 74 years. Nutritional intake was added as a survey component in the 1970s, beginning with the first NHANES, known as NHANES I (1971 to 1974). NHANES II covered the time period 1976 to 1980, and NHANES IIII encompassed 1988 to 1994. NHANES has reflected a continuous and standardized data collection based on a representative sample of the U.S. population and provides critical diet and health measures for federal program planning and policy making. The survey relies on the gold standard for dietary intake measures, two or more 24-hour dietary recalls per person (IOM, 2000). The U.S. Department of Agriculture's (USDA's) food composition database has provided the sources of information that allow the estimates of food intake collected in the NHANES to be translated into quantitative nutrient intake (Bodner-Montville et al., 2006; Briefel, 2006).

In 1999, the survey became a continuous program that has a changing focus on a variety of health and nutrition measurements to meet emerging needs;[2] the survey data are reported on the basis of 2-year periods. The survey now examines a nationally representative sample of about 5,000 persons each year. These persons are located in counties across the country, 15 of which are visited each year.[3] The NHANES and related food intake surveys conducted by the USDA were integrated in 2002; at that time, the

[1]Available online at http://www.cdc.gov/nchs/nhanes.htm (accessed July 23, 2010).

[2]Available online at http://www.cdc.gov/nchs/nhanes/about_nhanes.htm (accessed July 23, 2010).

[3]Available online at http://www.cdc.gov/nchs/nhanes/about_nhanes.htm (accessed July 23, 2010).

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dietary reports from the integrated survey became known as What We Eat in America (WWEIA). For this report data for the 2003 to 2004 and 2005 to 2006 period were used because the data for the 2007 and 2008 period did not become available until after the committee had completed its deliberations. Calcium intake has been estimated since NHANES I. Intakes for vitamin D were first published in 2009 and currently are available for the 2003–2006 survey period. The NHANES is said to "follow the sun" in that the survey is generally conducted in the southern states during the winter months and in the northern states in the summer months.

The NHANES is unique in that it collects and tracks both total intake and health measures in a national sample of Americans, and provides an important aspect of the nation's health monitoring system. As would be expected, total intake estimates are limited by survey respondents' abilities to accurately report foods and amounts consumed and by the accuracy, specificity, and timeliness of the food composition databases linked to foods reported in the survey. Respondents are also prone to under-reporting intake (IOM, 2000). Issues related to estimation of usual intake from WWEIA-NHANES have been reviewed by others (Dwyer et al., 2003), including the challenges of updating food composition tables and addressing the under-reporting of intake amounts by participants.

In the case of nutrient intake data for the United States, calcium and vitamin D intake estimates from the WWEIA report series[4] have formed the basis of an expanded analysis conducted and made available by the National Cancer Institute (NCI) of the National Institutes of Health. The NCI analysis was used in this report, as described below.

Intake Estimates for Calcium and Vitamin D

The USDA has produced the Vitamin D Addendum to the USDA Food and Nutrient Database for Dietary Studies 3.0,[5] and in turn WWEIA reported the vitamin D intake from foods as well as calcium intake from foods.[6] The expanded analysis carried out by NCI has allowed the incorporation of estimates of intake from dietary supplements collected as part of the NHANES but not included in the WWEIA reports, thereby providing an estimate of total calcium and vitamin D intake. Calcium intake data were available for the entire period 2003 to 2006 for the United States. However, although the USDA released vitamin D intake data for the 2003 to 2006

[4]Available online at http://www.ars.usda.gov/Services/docs.htm?docid=13793 (accessed July 23, 2010).

[5]Available online at http://www.ars.usda.gov/Services/docs.htm?docid=18807 (accessed July 23, 2010).

[6]Available online at http://www.ars.usda.gov/Services/docs.htm?docid=18349 (accessed July 23, 2010).

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period in July 2010, vitamin D intake data at the time of the NCI analysis were available only for 2005 to 2006. Therefore, the calcium and vitamin D intake data used in this report reflect overlapping, but not identical, time periods.

Given that there is considerable interest in estimates of total calcium intake and total vitamin D intake from all sources (i.e., foods and supplements), and data on total intake provide the best basis for DRI assessments, the committee relied on the expanded analysis of the NHANES data conducted by NCI and reported by Bailey et al. (2010).[7] Detailed information provided to the committee by NCI staff appears in Appendix H. The related methodologies have been described in detail by Bailey et al. (2010) and provide the opportunity to take into account sources of calcium and vitamin D from supplements.

As described in Bailey et al. (2010), the intake estimates for calcium and vitamin D derived through the NCI method will vary slightly (i.e., by less than 1 percent) from those that appear in the WWEIA. This is because the NCI method uses supplement intake as a covariate in the model for nutrient intake from foods, and because—relative to obtaining usual intake percentiles—a shrinkage estimator approach was incorporated into the analysis rather than a Monte Carlo approach.

Serum 25OHD Concentrations

Measures of serum 25OHD concentrations among survey participants are relevant to the process of a dietary intake assessment in that, whenever possible, the assessment should consider biological parameters thereby basing the assessment on the totality of the evidence and not on intake from foods and supplements alone (IOM, 2000). Also, intake from foods and supplements can often be under-reported by survey participants (IOM, 2000). Analysis of serum 25OHD concentrations has been a component of the NHANES survey since NHANES III. The laboratory methodologies are described on the related website.[8] In 2009, the Centers for Disease Control and Prevention (CDC) posted an Analytical Note[9] regarding the analysis of serum 25OHD levels. Users were cautioned about making direct comparisons between values from NHANES 2000 to 2006 and values obtained in NHANES III. Further, it was noted that serum 25OHD data from the 2000 to 2006 surveys were likely affected by drifts in the assay performance

[7]In addition, the study authors provided tables of intakes arrayed for percentile groupings. These have been made available in the Institute of Medicine public access file available at http://www8.nationalacademies.org/cp/.

[8]Available online at http://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/labcompf.pdf (accessed July 23, 2010).

[9]Available online at http://www.cdc.gov/nchs/nhanes.htm (accessed July 23, 2010).

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(method bias and imprecision) over time. For this reason, the committee used serum 25OHD levels that had been adjusted for this assay drift and posted on the agency's website. This assay drift was discussed in Chapter 3.

Canada: Canadian Health Measures Survey and Canadian Community Health Survey

Data relevant to the Canadian intake of calcium and vitamin D from foods, as well as measures of serum 25OHD concentrations[10] for a representative sample of Canadians, are available from national surveys conducted by the Government of Canada. These are described below.

Intake Estimates for Calcium and Vitamin D

The Canadian Community Health Survey (CCHS) began in 2000, with the goal of providing population-level information on health determinants, health status, and health system utilization.[11] The survey series is a joint effort among Health Canada, Statistics Canada, and the Canadian Institute for Health Information. The CCHS, a nationally representative cross–sectional survey, that operated on a 2-year data collection cycle from 2000 to 2007, and now operates on an ongoing basis, comprises two types of surveys. The first is a general health survey that takes place in the first year of the cycle (i.e., Cycle 1.1, 2.1, etc.). It samples approximately 130,000 Canadians and provides information at the level of regional health units within each province. The second is a focused topic survey that until 2007 took place in the second year of each cycle (i.e., Cycle 1.2, 2.2, etc.), and now takes place every 3 years. It samples approximately 35,000 Canadians, providing information at the national and provincial levels. The focused topic for CCHS 2004 was a food consumption survey and was designed to estimate the distribution of usual total intake in terms of foods, food groups, dietary supplements, nutrients, and eating patterns among a representative sample of Canadians at the national and provincial levels using the same 24-hour recall methodology used in the NHANES. The data from CCHS 2004 were disseminated in three separate releases between 2005 and 2008 (and revised February 2009). The data reflect nutrient intakes from foods only; information on the quantitative contributions from supplement use is not available at this time, but data on the frequency of general supplement use have been collected. Survey methodologies are described

[10] These measures reflect plasma 25OHD concentrations in the case of the Canadian survey data, but for the purposes of this report they are described as serum 25OHD concentrations.

[11] Available online at http://www.hc-sc.gc.ca/fn-an/surveill/index-eng.php (accessed July 23, 2010).

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online.[12] The food composition data used to estimate the nutrient values of the foods consumed are provided by the Canadian Nutrient File (CNF). This database reports the average nutritional values for foods available in Canada. According to the CNF documentation,[13] many of the data in the CNF have been derived from the USDA data base because these foods are available on the Canadian market. Canadian modifications included in the CNF consist of levels of fortification and regulatory standards specific to Canada and certain foods that are unique to the Canadian food supply.

Serum 25OHD Concentrations

Serum 25OHD concentrations have been measured and reported as part of the Canadian Health Measures Survey (CHMS). The CHMS, which was initiated in 2007, collects blood and urine for analysis and also carries out direct physical measurements of blood pressure, height, and weight. Those surveyed are persons 6 through 79 years of age and reflect approximately 97 percent of the population. Participants are those living in privately occupied dwellings in the 10 provinces and the 3 territories; persons living on Indian (First Nation) reserves or Crown land, as well as residents of institutions are excluded. Descriptions of sampling, data sources, error detection, quality evaluation, and laboratory methods can be found online.[14] The currently available data are from the 2007 to 2009 time period and can be accessed online.[15]

Approach Used

An earlier IOM committee addressed applications of the DRIs in dietary assessment and described statistical approaches to estimating the prevalence of inadequate intakes, specifically the probability approach and a shortcut to the probability approach called the Estimated Average Requirement (EAR) cut-point method (IOM, 2000). These approaches are based on a distribution of usual intakes, and by definition the prevalence of inadequate intakes for a group is the *proportion* of the group with intakes below the median requirement (or EAR). The 2000 IOM report also points out that it is inappropriate to compare usual nutrient intakes with the

[12]Available online at http://www.statcan.gc.ca/cgi-bin/imdb/p2SV.pl?Function=getSurvey&SDDS=5049&lang=en&db=imdb&adm=8&dis=2#a2 (accessed July 23, 2010).

[13]Available online at http://www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/user_guide_d_utilisation02-eng.php (accessed July 23, 2010).

[14]Available online at http://www.statcan.gc.ca/cgi-bin/imdb/p2SV.pl?Function=getSurvey&SDDS=5071&lang=en&db=imdb&adm=8&dis=2#b3 (accessed July 23, 2010).

[15]Available online at http://www.statcan.gc.ca/pub/82-623-x/2010002/part-partie1-eng.htm (accessed July 23, 2010).

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Recommended Dietary Allowance (RDA), because this approach will lead to estimates of inadequacy that are too large.

Based on the 2000 IOM report cited above (IOM, 2000), whenever possible, the assessment of apparent dietary adequacy should consider relevant biological parameters. In the case of vitamin D, an important biological parameter reflective of dietary exposure—serum 25OHD concentrations—was available and could be compared to values that the committee estimated to be approximately equivalent to an EAR or an RDA. However, the existing statistical models provided in the 2000 IOM report (IOM, 2000) address only dietary intake data and do not provide a basis for considering a biological parameter such as serum measures in order to specify the prevalence of inadequate intakes in population groups. Further, the apparent discrepancy between the intake data for vitamin D, as described below, and the biological parameter was also concerning and decreased the confidence in the appropriateness of estimating prevalence of inadequacy based on a distribution of vitamin D intakes. Therefore, a descriptive rather than an analytical approach is used for the vitamin D intake assessment.

CALCIUM INTAKE

As presented in Chapters 5 and 6, the Estimated Average Requirements (EARs), Recommended Dietary Allowances (RDAs), Adequate Intakes (AIs), and Tolerable Upper Intake Levels (ULs) for calcium are summarized in Table 7-1. The intake assessment takes into account these reference values.

U.S. Calcium Intake

Estimated calcium intakes from food sources only, by intake percentile groups, are shown as bar graphs in Figure 7-1. The prevalence of dietary inadequacy for a group can be estimated by the proportion of the group with intakes less than the EAR (IOM, 2000). The 5th percentile of intake for children 1 to 3 years of age is approximately equal to their EAR of 500 mg/day, implying a low prevalence of inadequacy (less than 5 percent). However, for all other age and gender groups of children, the prevalence is at least 25 percent, because intake at the 25th percentile is below the EAR. For adults, the prevalence of inadequacy from food sources alone is high.

As shown in Figure 7-2, the addition of information about calcium intake from supplements to the data set, thereby allowing an estimate of total intake, appears to impact primarily women over 50 years of age. All life stage groups show a slight increase when supplements are taken into account, but women 51 to 70 years of age demonstrate an estimated median total calcium intake (i.e., from foods plus supplements) of 1,044 mg/

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TABLE 7-1 Calcium Dietary Reference Intakes by Life Stage (amount/day)

Life Stage Group	AI	EAR	RDA	UL
Infants				
0 to 6 mo	200 mg	—	—	1,000 mg
6 to 12 mo	260 mg	—	—	1,500 mg
Children				
1–3 y	—	500 mg	700 mg	2,500 mg
4–8 y	—	800 mg	1,000 mg	2,500 mg
Males				
9–13 y	—	1,100 mg	1,300 mg	3,000 mg
14–18 y	—	1,100 mg	1,300 mg	3,000 mg
19–30 y	—	800 mg	1,000 mg	2,500 mg
31–50 y	—	800 mg	1,000 mg	2,500 mg
51–70 y	—	800 mg	1,000 mg	2,000 mg
> 70 y	—	1,000 mg	1,200 mg	2,000 mg
Females				
9–13 y	—	1,100 mg	1,300 mg	3,000 mg
14–18 y	—	1,100 mg	1,300 mg	3,000 mg
19–30 y	—	800 mg	1,000 mg	2,500 mg
31–50 y	—	800 mg	1,000 mg	2,500 mg
51–70 y	—	1,000 mg	1,200 mg	2,000 mg
> 70 y	—	1,000 mg	1,200 mg	2,000 mg
Pregnancy				
14–18 y	—	1,100 mg	1,300 mg	3,000 mg
19–30 y	—	800 mg	1,000 mg	2,500 mg
31–50 y	—	800 mg	1,000 mg	2,500 mg
Lactation				
14–18 y	—	1,100 mg	1,300 mg	3,000 mg
19–30 y	—	800 mg	1,000 mg	2,500 mg
31–50 y	—	800 mg	1,000 mg	2,500 mg

NOTE: AI = Adequate Intake; EAR = Estimated Average Requirement; RDA = Recommended Dietary Allowance; UL = Tolerable Upper Intake Level.

day compared with 755 mg/day from foods alone, while median intake for women more than 70 years of age was 983 mg/day compared to 706 mg/day without supplements. Thus, when intake from supplements is considered, the prevalence of dietary inadequacy for older women is approximately 50 percent (i.e., intake at the 50th percentile is approximately equal to the EAR of 1,000 mg/day calcium in Figure 7-2, Panel B).

Total calcium intakes at the 95th percentile are below the UL of 2,000 mg of calcium per day for most of the adult life stage groups, implying that less than 5 percent are at risk of excessive intake. The exception is older women, who have estimated total calcium intakes at the 95th percentile of 2,364 mg/day for those 51 to 70 years of age, and 2, 298 mg/day for those more than 70 years of age, so more than 5 percent are at risk of excessive

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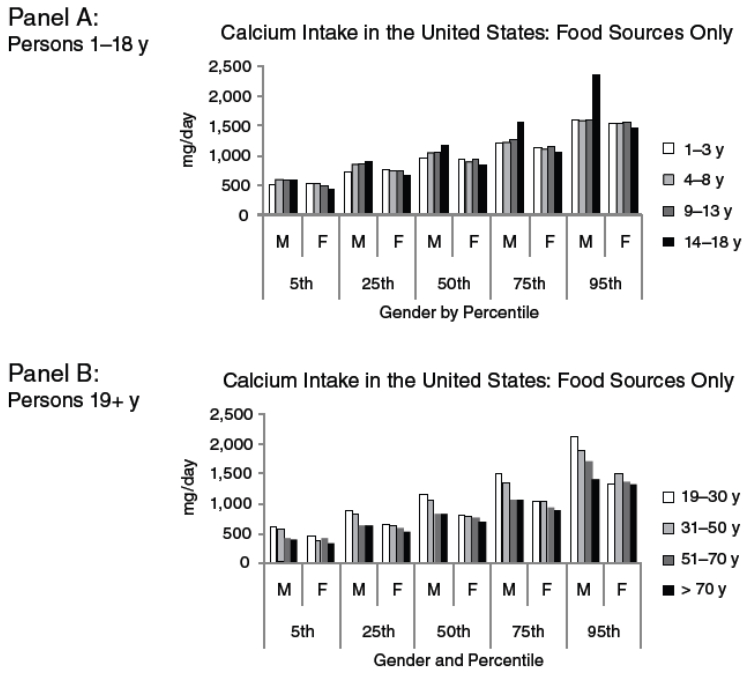


FIGURE 7-1 Estimated calcium intakes in the United States from food sources only, by intake percentile groups, age, and gender.
NOTE: F = female; M = male; y = years.
SOURCE: NHANES 2003–2006 as analyzed by Bailey et al. (2010). Data used to create figure can be found in Appendix H.

intakes. By contrast, the 95th percentile of calcium consumption from food sources alone are 1,353 and 1,337 mg/day for these two life stage groups, respectively.

Canadian Calcium Intake

Estimates of calcium intake from foods for Canadians appear to be similar to those reported for the United States, although the median intake drops at a younger age for men, at the 31- to 50-year life stage as compared to the 51- to 70-year life stage in the United States (Figure 7-3). Overall, estimated intakes of calcium from foods in Canada appear to be slightly lower than those reported for the United States. Although differences in survey

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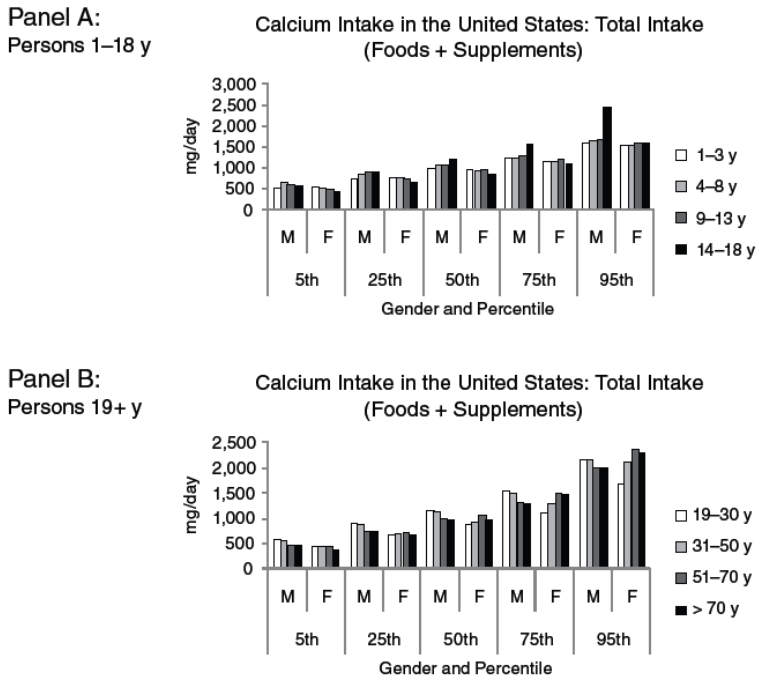


FIGURE 7-2 Estimated total calcium intakes in the United States from food and supplements, by intake percentile groups, age, and gender.
NOTE: F = female; M = male; y = years.
SOURCE: NHANES 2003–2006 as analyzed by Bailey et al. (2010). Data used to create figure can be found in Appendix H.

methodologies could be responsible for some of the difference, the surveys use very similar methodologies and work to ensure uniformity as much as possible. A more likely possibility is that the differences are attributable to food fortification practices. In Canada, calcium may only be added to a limited number of foods. Flour, cornmeal, plant-based beverages, and orange juice may be fortified with calcium, but not breakfast cereals and bread. However, discretionary fortification with calcium is widespread in the United States and can encompass breakfast cereals, breads, and an array of beverages.

At the time of this study, only intake data for foods were available for Canadians; estimates of total calcium intake (i.e., foods plus supplements)

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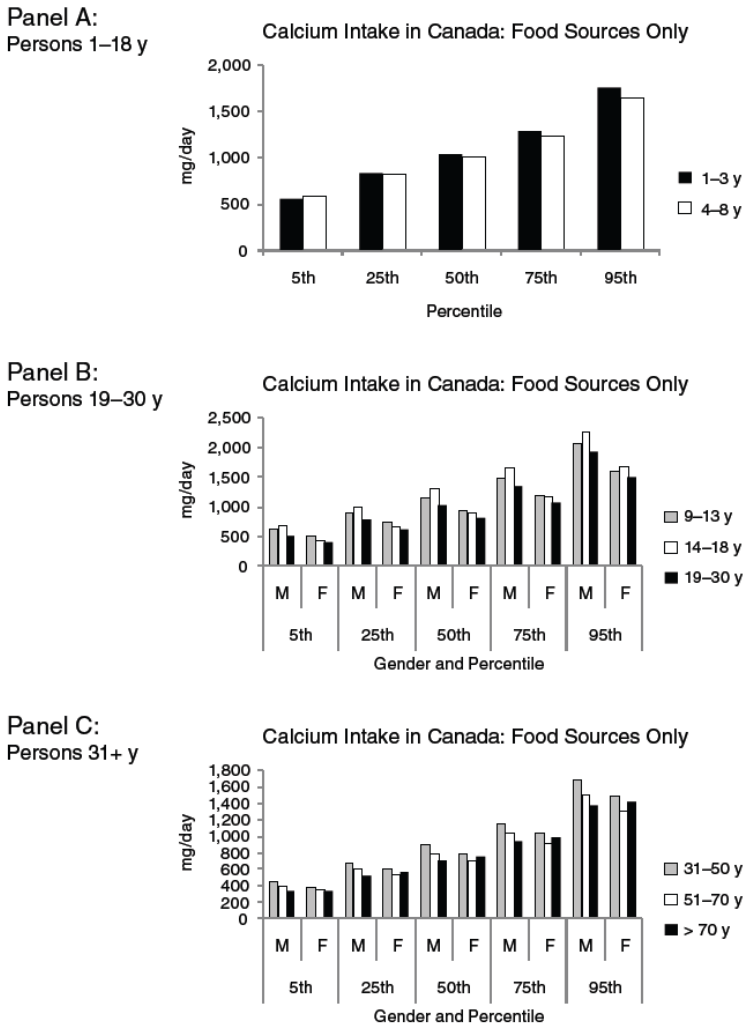


FIGURE 7-3 Estimated calcium intakes in Canada from food sources only, by intake percentile groups, age, and gender.
NOTE: F = female; M = male; y = years.
SOURCE: Statistics Canada, Canadian Community Health Survey (CCHS), Cycle 2.2, Nutrition 2004. Data used to create figure can be found in Appendix H.

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had not yet been compiled. After the completion of the study, information on total intake was published (Garriguet, 2010).

VITAMIN D INTAKE AND SERUM 25OHD CONCENTRATIONS

The EARs, RDAs, AIs, and ULs for vitamin D as presented earlier in Chapters 5 and 6 are relevant to the intake assessment discussions and are shown in Table 7-2.

Considerations about the adequacy of vitamin D intake must be interpreted in view of the fact that these reference values assume that no vitamin D is contributed to the human body by sun exposure. Given the unknowns concerning the contribution from sunlight as well as the inability to recommend an acceptable level of sun exposure, this assumption was necessary. However, it confounds interpretation of the intake assessment. If persons are obtaining some vitamin D from sun exposure, they are less likely to be at risk for inadequacy if their intakes are below the reference value. Although the extent to which this may be the case cannot be determined, a concomitant examination of serum 25OHD levels can assist in better describing the assessment. Moreover, as mentioned earlier, it is an appropriate component of the assessment of dietary adequacy (foods and supplements) because, whenever possible, the assessment should consider biological parameters (IOM, 2000).

U.S. Vitamin D Intakes and Serum 25OHD Concentrations

Figure 7-4 shows U.S. vitamin D intake from foods alone. Median vitamin D intake levels for males ranged from 272 to 396 International Units (IU)/day depending upon life stage group. For females, median vitamin D intakes spanned between 160 and 260 IU/day. When intake from supplements is considered to provide total intakes (Figure 7-5), all life stage groups for both male and female Americans show a slight increase in values. The most marked increase is among older women, as was the case for calcium. For women 51 to 70 years of age, median intake of vitamin D from both food and supplements increases to 308 IU/day, compared with vitamin D intake from foods alone, at 140 IU/day. For women more than 70 years of age, the increase in median intake associated with supplement use is an additional 196 IU/day (356 IU with supplements vs. 160 IUs from foods alone).

As shown in Figure 7-5, the 95th percentiles for total vitamin D (foods plus supplements) for males and females range between 568 and 940 IU/day, with both this high and low value found among the female life stage

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TABLE 7-2 Vitamin D Dietary Reference Intakes by Life Stage (amount/day)

Life Stage Group	AI	EAR	RDA	UL
Infants				
0 to 6 mo	400 IU (10 µg)	—	—	1,000 IU (25 µg)
6 to 12 mo	400 IU (10 µg)	—	—	1,500 IU (38 µg)
Children				
1–3 y	—	400 IU (10 µg)	600 IU (15 µg)	2,500 IU (63 µg)
4–8 y	—	400 IU (10 µg)	600 IU (15 µg)	3,000 IU (75 µg)
Males				
9–13 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
14–18 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
19–30 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
31–50 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
51–70 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
> 70 y	—	400 IU (10 µg)	800 IU (20 µg)	4,000 IU (100 µg)
Females				
9–13 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
14–18 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
19–30 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
31–50 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
51–70 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
> 70 y	—	400 IU (10 µg)	800 IU (20 µg)	4,000 IU (100 µg)
Pregnancy				
14–18 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
19–30 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
31–50 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
Lactation				
14–18 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
19–30 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)
31–50 y	—	400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)

NOTE: AI = Adequate Intake; EAR = Estimated Average Requirement; IU = International Units; RDA = Recommended Dietary Allowance; UL = Tolerable Upper Intake Level.

groups. Persons in the 95th percentile for total intake did not appear to exceed the UL for their group.

The comparison between vitamin D intake estimates and serum 25OHD concentrations is worthy of note, but it is important to recognize that this comparison, although interesting, is somewhat problematic because the only possible comparison is based on group means, rather than on data linked to individuals. Moreover, as pointed out previously (IOM, 2000; Dwyer et al., 2003), estimates of intake tend to reflect an underestimation. With these caveats, the comparison is presented in Table 7-3. Shown are the average intakes for the various life stage groups, along with the average serum 25OHD levels for those life stage groups. For this table, serum 25OHD concentration data from the 2005 to 2006 surveys were used rather

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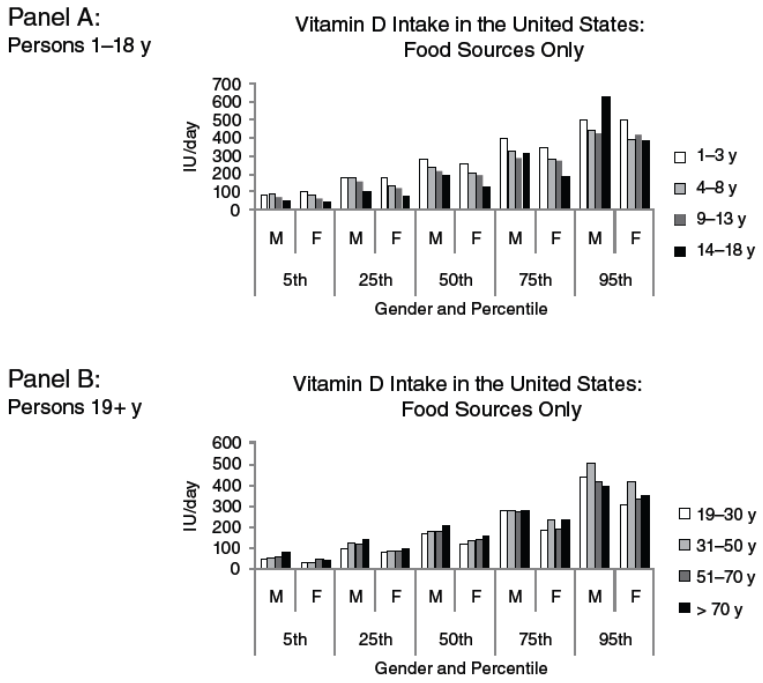


FIGURE 7-4 Estimated vitamin D intakes in the United States from food sources only, by intake percentile groups, age, and gender.
NOTE: F = female; IU = International Units; M = male; y = years.
SOURCE: NHANES 2005–2006 as analyzed by Bailey et al. (2010). Data used to create figure can be found in Appendix H.

than those from the 2003 to 2006 data set because intake estimates for total vitamin D (i.e., the NCI method) are currently available only for the 2005 to 2006 data.

Assuming that a serum 25OHD level of 40 nmol/L is consistent with a desirable median intake,[16] the comparison would suggest that, on average,

[16] As discussed in Chapter 5, measures of 27.5 nmol/L in children, and 30 nmol/L in adults remain a level below which frank deficiency including rickets and osteomalacia may be expected to occur. The vitamin D-related bone health needs of approximately one-half of the population may be expected to be met at serum 25OHD concentrations between 30 and 40 nmol/L; most of the remaining members of the population are likely to have vitamin D needs met when serum concentrations between 40 and 50 nmol/L are achieved. Failure to achieve such serum concentrations place persons at greater risk for less than desirable bone

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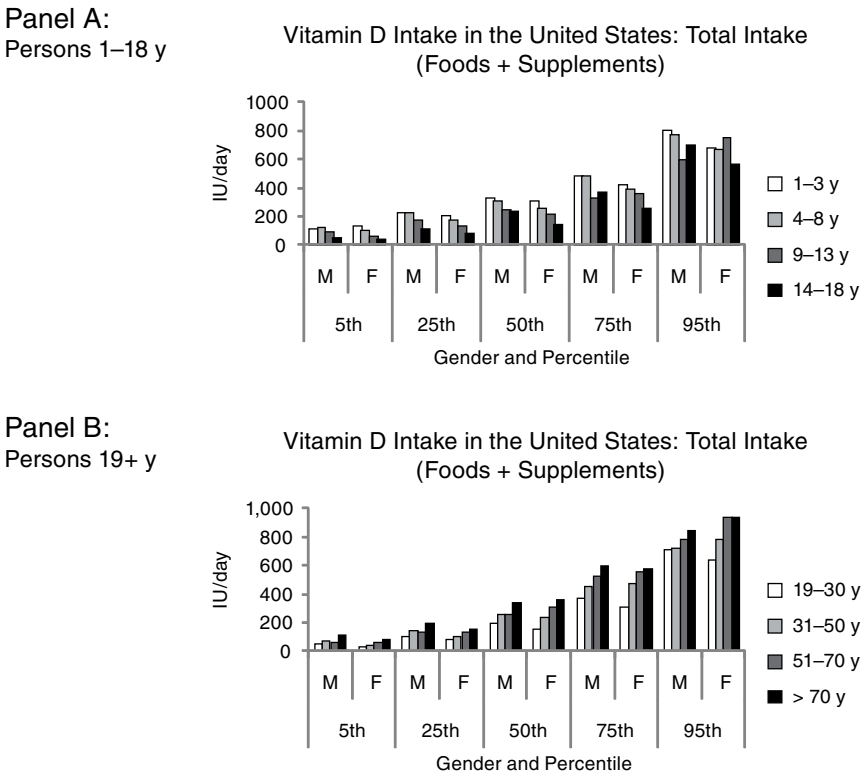


FIGURE 7-5 Estimated total vitamin D intakes in the United States from food and supplements, by intake percentile groups, age, and gender.
NOTE: F = female; IU = International Units; M = male; y = years.
SOURCE: NHANES 2005–2006 as analyzed by Bailey et al. (2010). Data used to create figure can be found in Appendix H.

persons may be experiencing intakes below the reference values, but are exhibiting serum 25OHD levels above 40 nmol/L. In fact, all are above the 50 nmol/L concentration, the level associated with the RDA. There is an additional factor to consider in this comparison, in that the NHANES data are generally collected during the summer months in the northern regions of the United States and in the winter months in the southern regions; this introduces the variable of sun exposure into the comparison in that it decreases the likelihood that individuals surveyed will be experiencing low levels of sun exposure. As an informal conceptual check, it is

health as manifested by, depending upon age, rates of bone accretion, bone mineral density, and fractures.

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TABLE 7-3 Mean Vitamin D Intake and Mean Serum 25OHD Concentrations for the United States, 2005–2006, by Life Stage Groups

Life Stage Group (years)	Vitamin D Intake (IU/day)		Serum 25OHD Levels (nmol/L)	
	Food Alone[a]	Total Intake[b]	Mean[c]	Adjusted for Sun Exposure (Reduced by 1/3)
Males				
1–3	288 ± 8	364 ± 16	71.1 ± 2.0	47
4–8	256 ± 12	372 ± 16	70.5 ± 2.0	47
9–13	228 ± 8	300 ± 28	65.9 ± 2.2	44
14–18	244 ± 16	276 ± 20	60.1 ± 1.9	40
19–30	204 ± 12	264 ± 16	57.9 ± 2.0	38
31–50	216 ± 12	316 ± 12	58.5 ± 1.1	39
51–70	204 ± 12	352 ± 16	57.3 ± 1.8	38
> 70	224 ± 16	428 ± 28	58.9 ± 1.3	39
Females				
1–3	276 ± 16	336 ± 16	71.4 ± 1.9	47
4–8	220 ± 12	316 ± 24	70.5 ± 2.1	47
9–13	212 ± 24	308 ± 40	59.1 ± 1.6	39
14–18	152 ± 8	200 ± 20	57.6 ± 1.9	38
19–30	144 ± 12	232 ± 12	62.7 ± 2.8	41
31–50	176 ± 12	308 ± 20	57.6 ± 1.7	38
51–70	156 ± 16	404 ± 40	57.2 ± 1.5	38
> 70	180 ± 8	400 ± 20	56.5 ± 1.8	37

NOTE: IU = International Units; SE = standard error.

[a]Data are mean ± SE for foods only.

[b]Data are mean ± SE for total intake: foods and dietary supplements.

[c]Data are mean ± SE.

SOURCE: NHANES, 2005–2006; Bailey et al., 2010.

possible to adjust these data so as to roughly simulate a reduction in serum 25OHD levels consistent with the difference between the summer zenith and the winter nadir. Specifically, if the estimate that there is a one-third difference in serum 25OHD levels between the winter nadir and summer zenith as described in Chapter 3 is applied to this comparison, reducing these serum 25OHD levels by one-third results in a range of serum 25OHD levels from a low of 37 nmol/L (women > 70 years) to a high of 47 nmol/L (found in four life stage groups), which are still very close to, and in many cases above, a 40 nmol/L concentration consistent with an estimated average required intake. Given the observation made in Chapter 5 that the seasonal decline during the winter may differ between those with high and low initial baseline values, the correction applied using a 30 percent reduction may overestimate the decline in those at lower baseline 25OHD levels below 50 nmol/L. Moreover, this adjustment is excessive because for those

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persons living in the southern United States, their serum 25OHD measures were taken generally during the winter, not summer, months; the one-third reduction is therefore an over-correction in this case. However, because it is not possible using the data available to the committee to distinguish between values taken in the summer in northern areas and in the winter in southern areas, the adjustment cannot be further refined.

Although serum 25OHD levels from the 2005 to 2006 period in the United States are the data used for Table 7-3 because total intake data are available only for 2005 to 2006, serum 25OHD levels are available for the 2003 to 2006 data set, the two most current surveys, which, when combined, provide a larger data set. For comparison, these are shown in Table 7-4 and appear to reflect values very similar to those reported for 2005 to 2006 alone. No effort has been made to consider vitamin D intake for this period (2003 to 2006) because only data from the WWEIA are available for 2003 to 2006, which would not provide information on total intake (foods plus supplements).

TABLE 7-4 Mean Serum 25OHD Concentrations for the United States, 2003–2006, by Life Stage Group

Life Stage Group (years)	Mean Serum 25OHD Concentration (nmol/L ± SE)
Males	
1–3	71.8 ± 1.4
4–8	70.6 ± 1.2
9–13	64.7 ± 1.4
14–18	60.3 ± 1.4
19–30	57.2 ± 1.3
31–50	59.3 ± 1.1
51–70	59.9 ± 1.2
> 70	59.1 ± 1.0
Females	
1–3	70.4 ± 1.2
4–8	69.3 ± 1.4
9–13	58.9 ± 1.1
14–18	59.9 ± 1.7
19–30	62.2 ± 1.9
31–50	58.1 ± 1.2
51–70	57.6 ± 1.1
> 70	57.4 ± 1.1

NOTE: SE = standard error.
SOURCE: NHANES, 2003–2006.

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Canadian Vitamin D Intakes and Serum 25OHD Concentrations

As previously mentioned, the available Canadian survey data provided information on vitamin D intake from foods alone; quantified information on vitamin D supplement intake among Canadians, and thus on total intake from foods and supplements, was not available at the time of the study. Figure 7-6 outlines the estimated intakes of vitamin D from foods alone, which overall tend to be slightly higher than those reported for the United States. Median vitamin D intakes ranged from a low of 176 IU/day (women 51 to 70 years) to a high of 264 IU/day (boys 9 to 13 years). Similar to the U.S. population, persons in the 95th percentile of intake of vitamin D from foods would be expected to be considerably below the UL for their life stage.

Comparison between mean intakes of vitamin D and mean serum 25OHD concentrations for Canadians is problematic. For Canada, intake estimates are provided for the survey year 2004 based on the CCHS, whereas the serum 25OHD concentrations available reflect data from the 2007 to 2009 CHMS. The mean serum 25OHD levels for Canadians are shown in Table 7-5, and no effort has been made to compare these with intake estimates. As a general matter, average serum 25OHD concentrations of Canadians are above both the 40 and 50 nmol/L concentration levels. Although average intakes of vitamin D among Canadians from foods alone (i.e., not taking into account supplements) are less than the EAR, measures of serum 25OHD levels are well above the 40 nmol/L level consistent with the EAR. Again, as described earlier, the ability to interpret the prevalence of inadequacy based on serum 25OHD concentrations using the methodology as established in the 2000 IOM report (IOM, 2000) is unclear.

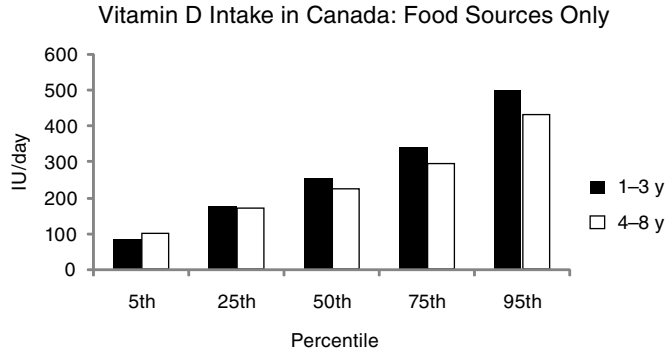
DIFFERENCES BETWEEN THE UNITED STATES AND CANADA: NATIONAL SURVEY DATA FOR CALCIUM AND VITAMIN D

All total intake estimates are subject to uncertainties owing to a variety of factors that affect estimates of food intake, ranging from the depth and nature of the probing carried out to obtain the information on food consumption to the ability of persons to accurately recall and estimate their food intake. Overall, the nature and approach of the national surveys in the United States and Canada are notably similar, which suggest that the small differences seen in intake estimates for calcium and vitamin D may reflect true differences in intake.

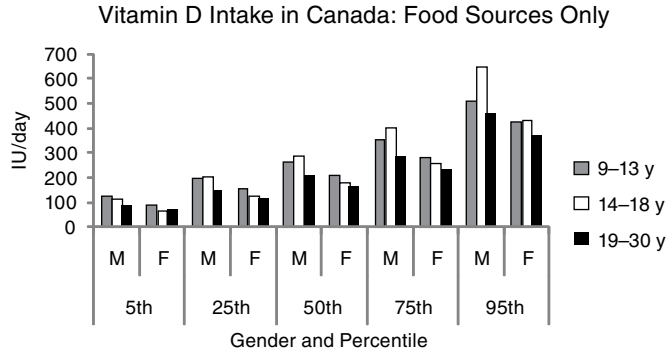
With respect to vitamin D intake from foods alone, to the extent a comparison is appropriate given that they reflect different periods—2004 for Canada and 2005 to 2006 for the United States—Canadian intakes of

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Panel A:
Persons 1–18 y



Panel B:
Persons 19–30 y



Panel C:
Persons 31+ y

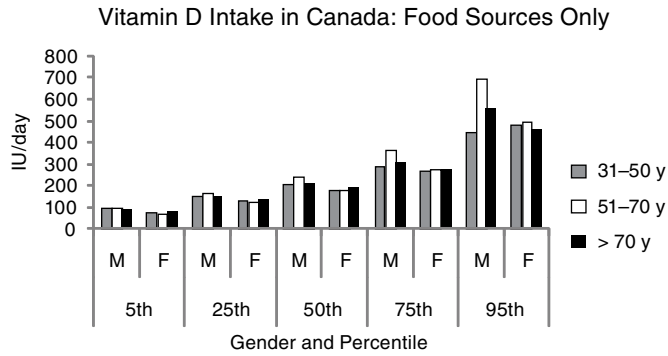


FIGURE 7-6 Estimated vitamin D intakes in Canada from food sources only, by intake percentile groups, age, and gender.
NOTE: F = female; IU = International Units; M = male; y = years.
SOURCE: Statistics Canada, Canadian Community Health Survey (CCHS), Cycle 2.2, Nutrition 2004. Data used to create figure can be found in Appendix H.

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TABLE 7-5 Serum 25OHD Levels for Canadians by Percentile Group, Age, and Gender

Life Stage Group (years)	Mean Serum 25OHD Level (nmol/L) (Confidence Interval)
Males	
9–13	73.4 (69.7–77.2)
14–18	65.2 (57.2–73.1)
19–30	62.5 (53.3–71.7)
31–50	61.6 (57.0–66.2)
51–70	69.2 (65.4–73.1)
71–79	73.7 (67.1–80.3)
Females	
9–13	69.5 (63.6–75.5)
14–18	68.6 (63.0–74.2)
19–30	72.5 (67.2–77.9)
31–50	67.1 (63.7–70.4)
51–70	68.9 (66.3–71.5)
71–79	77.8 (72.6–83.0)

SOURCE: Statistics Canada, Canadian Health Measures Survey (CHMS), Cycle 1, 2007–2009.

vitamin D from food sources are somewhat more than those in the United States. This may be due to the Canadian food supply having mandatory fortification of margarine with vitamin D in addition to fortification of milk.

Further, differences in serum 25OHD concentrations between the United States and Canada are evident. The estimates for Canadians are consistently higher than those for the United States. Although differences in the food supply may account for some of these differences, it is noted that the analyses for the Canadian data are based on the use of the "Liaison" kit,[17] whereas the U.S. data are derived from the "DiaSorin RIA" kit.[18] Direct comparison of the two kits within the CHMS laboratory at Health Canada indicates a 6 to 9 percent difference, with the Liaison measuring values higher than the RIA kit.[19] Other researchers have also performed comparisons with various outcomes. The differences may be laboratory-specific because Wagner et al. (2009) found no difference, although data from Carter et al. (2010) suggest a 5 percent bias, with the RIA kit giving higher values. It is notable that the serum 25OHD levels in Canada are not lower than those in the United States, as would be predicted if higher latitudes were responsible for reduced serum 25OHD levels.

Finally, Appendix I contains information about the proportion of per-

[17]DiaSorin Liaison (Stillwater, MN).
[18]DiaSorin Radio-immunoassay (RIA) (Stillwater, MN).
[19]Personal communication, S. Brooks, Health Canada, August 9, 2010.

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sons in both countries above or below designated levels of serum 25OHD. These data are included as information for the users of this report and have been provided by the U.S. Centers for Disease Control and Prevention and by Statistics Canada. However, these data were not reviewed by the committee given that the analyses did not take place until after the close of the committee deliberations.

SUMMARY

The intake assessment conducted in this report suggests that calcium remains a nutrient of public health concern in some population groups. Girls 9 to 18 years of age, who have a fairly high requirement for calcium, are clearly falling below desirable intake estimates in both countries when only food sources of calcium are considered, as are women over the age of 50 years. On the other hand, available data from the United States on the total intake of calcium when dietary supplements are considered, suggests that older women on average, at least in the United States, have added to their calcium intakes through supplement use. For girls, the increase in intake that might be attributable to supplement use is small. No life stage groups exceeded the UL for calcium when foods alone were considered. However, when supplement use was taken into account (United States only), those women consuming at the 95th percentile of calcium intake appeared to be at risk for exceeding the UL. This suggests that there may be value in underscoring the need for older girls to modestly increase intake of calcium, and in emphasizing that for older women high intakes from supplements may be concerning.

Due to the desirability of considering biological parameters for intake assessments whenever possible (IOM, 2000), the vitamin D assessment presented some challenges. Although median vitamin D intakes from foods in both countries for all life stage groups were below the EAR of 400 IU/day, these data and any future intake analyses conducted using the IOM methodology (IOM, 2000) should be considered in light of the corresponding serum 25OHD concentrations. However, specific prevalence estimates based on serum values are not provided here because the appropriate application of the IOM methodology outlined in 2000 (IOM, 2000), which is focused on use of dietary intake estimates, is currently unclear and may not be appropriate for use with serum values.

Average serum 25OHD concentrations from the NHANES were well above the 40 nmol/L established as consistent with an intake equivalent to the EAR, although a number of North Americans have serum values below 40 nmol/L. All average values were above 50 nmol/L, the level consistent with an intake equivalent to the RDA. When the U.S. data were "adjusted" to simulate conditions more consistent with winter months, at

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least in the more northern parts of the United States, mean serum 25OHD levels hovered around 40 nmol/L, consistent with an EAR intake. Further, this adjustment over-corrects because for persons living in southern parts of the United States—where NHANES generally is conducted during the winter months—their serum 25OHD levels are already reflective of winter and are not appropriately corrected from a summer level to a winter level. In the case of data for Canada from the CHMS, the mean serum 25OHD levels for all life stage groups are at or above 60 nmol/L. The fact that they are higher than those for the U.S. population may be in part a function of differences in the assay methods used, although this is not clearly established. If it is assumed that the Canadian values would be 8 percent lower if analyzed using the same methodology that was used in the U.S. survey, then they would then be quite similar to those for the United States, leaving open the question of whether the latitude difference between the two countries has a meaningful impact on serum 25OHD levels.

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8

Implications and Special Concerns

The last step in the risk assessment process is the step of so-called risk characterization. Its intent is to highlight the nature of the "risks" or public health problems that are relevant to the use of Dietary Reference Intakes (DRIs) and to alert users of the DRI reference values to implications of the assessors' work and to related special issues. This chapter reflects the risk characterization step of the risk assessment approach and is organized to provide: a brief summary of the assessment; discussions about the implications of the committee's work for stakeholders; and discussions to highlight population segments and conditions of interest relative to calcium and vitamin D nutriture.

SUMMARY OF ASSESSMENT

The new DRIs establish, for the first time, an Estimated Average Requirement (EAR) and a Recommended Dietary Allowance (RDA) for calcium and vitamin D. Previously, the DRIs for these nutrients reflected Adequate Intakes (AIs). The ability to set EARs and RDAs rather than AIs enhances the utility of the reference values for national planning and assessment activities. It is important to recognize that these values are intended for the North American population, and also that the requirement for each nutrient is based on the assumption that the requirement for the other nutrient is being met.

Considerable effort was made to ensure that an array of indicators was examined as a possible basis for setting requirements, as well as upper levels of intake. The intent was to fully and objectively examine the scientific

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basis for the suggested benefit before drawing conclusions. Despite the many claims of benefit surrounding vitamin D in particular, the evidence did not support a basis for a causal relationship between vitamin D and many of the numerous health outcomes purported to be affected by vitamin D intake. Although the current interest in vitamin D as a nutrient with broad and expanded benefits is understandable, it is not supported by the available evidence. The established function of vitamin D remains that of ensuring bone health, for which causal evidence across the life stages exists and has grown since the 1997 DRIs were established (IOM, 1997). The conclusion that there is not sufficient evidence to establish a relationship between vitamin D and health outcomes other than bone health does not mean that future research will not reveal a compelling relationship between vitamin D and another health outcome. The question is open as to whether other relationships may be revealed in the future.

Of great concern recently have been the reports of widespread vitamin D deficiency in the North American population. Based on this committee's work and as discussed below, the concern is not well founded. In fact, the cut-point values used to define deficiency, or as some have suggested, "insufficiency," have not been established systematically using data from studies of good quality. Nor have values to be used for such determinations been agreed upon by consensus within the scientific community. When higher cut-point values are used compared with those used in the past, they necessarily result in a larger proportion of the population falling below the cut-point value and thereby defined as deficient. This, in turn, leads to higher estimations of the prevalence of deficiency among the population and possibly to unnecessary intervention incorporating high-dose supplementation in the health care of individuals. National survey data suggest that the serum 25-hydroxyvitamin D (25OHD) levels in the North American population generally exceed the levels identified in this report as sufficient for bone health, underscoring the inability to conclude that there are significant levels of deficiency in the population.

Specifically in terms of the new DRIs and challenges for calcium and vitamin D nutriture, several points can be highlighted, within the context of the limitations of estimates of dietary intake, which tend to be underestimates of actual consumption. First, for calcium, adolescent girls continue to be a group at risk for low intakes from food sources. Older women use calcium supplements in greater proportion, and some may be at risk for excess intake as a result of the use of high-dose supplements. If supplements are needed to ensure adequate calcium intake, it would appear that lower dose supplements should be considered. Many older women have baseline calcium intakes that are close to or just below requirements, and therefore the practice of calcium supplementation at high levels may be unnecessary. This is a special concern for calcium supplement use given

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the possibility that total intakes (diet plus supplements) above 2,000 mg/day may increase the risk for kidney stones, and demonstrate no increase in benefits relative to bone health. There is also some limited evidence that the long-term use of calcium supplements may increase the risk for cardiovascular disease. Although no attempt was made to compare systematically the data used for the North American population that is the subject of this report with data from other countries focused on persons who are genetically and environmentally different from those in the United States and Canada, it should be recognized that calcium requirements may be subject to a variety of factors that have not yet been fully elucidated and so therefore cannot yet be integrated into DRI reviews.

For vitamin D, the challenges introduced by issues of sun exposure cannot be ignored. This nutrient is unique in that it functions as a prohormone, and the body has the capacity to synthesize the nutrient if sun exposure is adequate. However, concerns about skin cancer risk preclude making recommendations about sun exposure; in any case, there are a number of unknowns surrounding the effects of sun exposure on vitamin D synthesis. At this time, the only solution when DRIs are to be set for vitamin D is to proceed on the basis of an assumption of minimal sun exposure and set a reference value assuming that all of the vitamin D must come from the diet. Moreover, the possibility of risk for persons typically of concern because of reduced synthesis of vitamin D, such as persons with dark skin or older persons in institutions, is minimized given the assumption of minimal sun exposure for the DRIs.

One unknown in the process of DRI development for vitamin D is the degree to which waning kidney function with aging may be relevant. It appears that increasing serum 25OHD levels do not typically increase calcitriol levels in aging persons with mild renal insufficiency, and a dietary strategy to address the concern is not evident.

Although ensuring adequacy is important, there is now an emerging issue of excess vitamin D intakes. A congruence of diverse data on health outcomes ranging from all-cause mortality to cardiovascular risk suggests that adverse health outcomes may be associated with vitamin D intakes that are much lower than those classically associated with hypervitaminosis D and that appear to occur at serum 25OHD levels achievable through current levels of supplement use.

IMPLICATIONS

The extensive review of the data required to conduct this study and to determine DRIs for calcium and vitamin D that are consistent with existing scientific understandings has answered many questions. But, the process has also identified or left unanswered other questions due to the limita-

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tions of the available evidence. Because uncertainties exist in the knowledge base related to the role of vitamin D and calcium in health outcomes, it is important to acknowledge that there are uncertainties surrounding these reference values for calcium and vitamin D. The development of any reference value should be viewed as a work in progress, which may be subject to change if there are significant changes in the science base.

Further, an important aspect of DRI development is its grounding in public health applications and the concept of distributions of risk. This approach may appear strange to some and may be disconcerting to those with a clinical orientation who are familiar with the medical model in which the goal is to treat the patient in the most efficacious manner to enhance a positive outcome. The interpretation and use of data in the case of DRI development are within the context of the relevant probability distributions of risk; the DRI task focuses on median requirements and the description of risk, whereas the medical model is based on maximizing effects that ensure beneficial outcomes for all persons. This report, therefore in contrast to a medical model approach, determines dose–response relationships by assessing the level at which 50 percent of the population's needs are met (the EAR) and the level at which approximately 97.5 percent of the population are likely to have their needs met (the RDA). The distribution of dose–response effects is highly relevant to DRI development, compared with information about a maximizing effect for benefit. A difficulty the committee too often faced was studies that included only a placebo or baseline low dose coupled with a relatively large, single supplemental dose, as these are relatively uninformative for DRI development.

Discussions below call attention to the uncertainties surrounding the DRI values for calcium and vitamin D and also highlight important conclusions that stem from the process of developing these DRIs. In addition, given that this report is the first effort to develop DRIs since the 2007 IOM workshop that explored lessons learned and new challenges and outlined the risk assessment approach for DRI development (IOM, 2008; Taylor, 2008), comments are offered about the process. Specific research recommendations for the future development of DRIs related to calcium and vitamin D are presented in Chapter 9.

Assumption of Minimal Sun Exposure

The committee's assumption of minimal sun exposure is a markedly cautious approach given that the vast majority of North Americans appear to obtain at least some vitamin D from inadvertent or deliberate sun exposure. Currently, there is a lack of information about whether certain levels of sun exposure may be experienced without increased risk of cancer and whether such exposure would be consistent with a contribution of vitamin

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D useful to the body. Therefore, at this time, recommendations concerning sun exposure relative to vitamin D requirements cannot and should not be offered; there are no options other than to base dietary recommendations on the assumption of minimal sun exposure. The evidence to indicate that the synthesis of vitamin D from sun exposure is subject to a feedback loop that precludes toxicity from sun exposure is reassuring and, when coupled with the checks and balances introduced into the DRI development process, makes it very unlikely that consumption of the DRI levels of vitamin D, even if combined with high levels of sun exposure, will be problematic to the general population.

However, given that many North Americans appear to obtain at least some vitamin D from inadvertent or deliberate sun exposure, there are implications for the interpretation of intake levels of the vitamin. In short, the intake data for vitamin D cannot stand alone as a basis for public health action on a national population level. Such considerations are consistent with the 2000 IOM report on applications of DRIs in dietary assessment (IOM, 2000), which states: "Whenever possible, the assessment of apparent dietary adequacy should consider biological parameters such as anthropometry, … biochemical indices, … diagnoses, … clinical status, and other factors as well as diet. Dietary adequacy should be assessed and diet plans formulated based on the totality of the evidence, not on dietary intake data alone." In short, for policy making and decisions about the adequacy of the food supply for the general population at the national level, vitamin D must be considered in the context of measures of serum 25OHD, an established biomarker of exposure from endogenous synthesis as well as diet, including supplements. Although the reported estimates of vitamin D intake appear to be less than needed to meet requirements, the serum 25OHD data available—when coupled with the committee's assessment of serum 25OHD levels consistent with EAR and RDA values—suggest that average requirements are being met for the DRI age groups nationally in both countries. That is, although mean total intakes of vitamin D generally are lower than the estimated median requirement (the EAR), the available clinical measures do not suggest widespread deficiency states. This underscores the possibility that sun exposure is contributing generally to the maintenance of adequate serum 25OHD concentrations.

Uncertainties

As discussed in the preceding chapters, there are limited data for many topics of interest in setting DRI values for calcium and vitamin D. Overall, the uncertainties surrounding the DRI values for calcium are less than those for vitamin D, because the evidence base is considerably larger for calcium, and the physiology and metabolism of calcium are better

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understood. The following key issues were identified by the committee as introducing uncertainty into the DRI values for calcium and vitamin D, as based on bone health outcomes:

- The tendency for study protocols to administer a combination of calcium and vitamin D, reducing the opportunity to ascertain the effects of each nutrient independently;
- The lack of data examining the responses and health outcomes due to graded doses of calcium or vitamin D intake so as to elucidate dose–response relationships;
- The interaction between calcium and vitamin D to the extent that it would appear that adequate calcium intake greatly diminishes the need for vitamin D relative to bone health outcomes;
- The unique situation in which a nutrient (vitamin D) physiologically serves as a prohormone introduced a myriad of variables and feedback loops related to its health effects;
- The paucity of data and resulting uncertainty concerning sun exposure that confound interpretation of the dose–response relationship between intakes of vitamin D and various health outcomes. This, coupled with the apparent contribution of sun exposure to overall vitamin D nutriture in North American populations, leads to an inability to characterize and integrate sun exposure with intake recommendations as much as may be appropriate, given the concern for skin cancer risk reduction, which must be paramount. Thus, for individuals who do not follow recommendations to avoid sun exposure, the uncertainty of the DRI values is greater than for those who do;
- The lack of clarity concerning the validity of the serum 25OHD measure as a biomarker of effect;
- The variability surrounding measures of serum 25OHD concentrations as a result of different methodologies used;
- A number of findings suggesting a strong role for metabolic adaptations and controls in the case of vitamin D, which complicates estimations of nutrient requirements. These include: the nonlinear response of serum 25OHD level to vitamin D intake, which, in turn, suggests that it requires proportionately more vitamin D to continue to increase serum 25OHD levels after a certain serum 25OHD level is reached; the observation that seasonal declines in serum 25OHD level are greater if a person begins the winter season with a higher compared with a lower serum 25OHD level; and the lack of effect of age and body size (other than adiposity) on serum 25OHD levels;

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- The limited number of long-term clinical trials related to calcium and vitamin D intakes and health outcomes; and
- The need to set ULs based on limited data in order to ensure public health protection.

An important question that will undoubtedly be asked given this committee's report, is: Why is it that so much information about the positive effects of vitamin D on outcomes such as cancer, diabetes, and immunity is said to exist and is reported almost daily in the press, but this committee found no basis to support these causal relationships? The short answer is that a systematic examination of the evidence, using established guidelines for measuring the strength and quality of studies, revealed that the claimed benefits based on the associations of low or high intakes of vitamin D on non-skeletal health outcomes could not be supported by the studies—the evidence was inconsistent and/or conflicting or did not demonstrate causality. In addition, some effects were not related to setting nutritional requirements for vitamin D. This conclusion, however, does not preclude pursuing investigation of causal relationships.

Moreover, a related question that will be asked is: With the advent of newer studies, why is there still so much uncertainty? At least one reason is that most studies were not designed to seek data maximally useful for DRI development, which is well described by others (Yetley et al., 2009). DRI development fundamentally requires elucidation of dose–response relationships and benefits from data of high quality obtained in randomized controlled trials. In making its conclusions about potential indicators other than bone health, the committee noted the findings previously specified by an IOM committee tasked with examining the evolution of evidence for nutrient and disease relationships (IOM, 2002). That committee concluded that evidence about relationships between specific nutrients and a disease or health outcome typically remains elusive for a number of reasons (IOM, 2002). These include the following:

- Although preliminary evidence, usually from mechanistic studies, experimental animal studies, and observational studies in humans, can generate exciting new hypotheses about nutrient–health relationships, evidence from these studies has limitations. For instance, even in well-designed, large-scale observational studies, it is difficult to isolate the effects of a single nutrient under investigation from the confounding effects of other nutrients and from non-nutrient factors.
- Scientific advances in understanding relationships between specific nutrients and health outcomes do not necessarily emerge

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within a short time, and progress is often erratic. Some gaps are filled, while others are created.

- The etiology of disease–health relationships, especially in the case of chronic disease, is commonly multi-factorial. Even if diet has a prominent role, it is extremely unlikely that a single nutrient is directly responsible for a chronic disease or, conversely, that addition of a single nutrient will eliminate disease risk. It is possible that a focus on specific nutrients as risk factors for diseases in relatively homogeneous or diseased populations can lead to a number of spurious associations.
- Clinical trials, which are generally considered to provide the strongest evidence about the effects of nutrient intake on subsequent disease and health, are complex, expensive, and time-consuming, especially for chronic diseases that develop over decades and are influenced by a host of genetic, physiological, and environmental factors that may also affect risk.

The committee found all of the above findings to be the case for nonskeletal health outcomes for vitamin D, as the discussions of the strength, consistency, and causality of the evidence demonstrate in Chapter 4.

Finally, an important uncertainty focuses on the issue of excess intake. This is particularly true for vitamin D, which has been hypothesized to confer health benefits at relatively high levels of intake. Although the committee's decisions for the ULs made use of emerging data concerning a U-shaped (or perhaps reverse-J-shaped) curve for risk, which suggested adverse effects at levels much lower than those associated with hypervitaminosis D, the lack of data on the safety of higher intakes of vitamin D when used chronically is very concerning. Byers (2010), in a recent editorial commenting on the outcomes of a pooling study focused on vitamin D and six types of cancer in which the only association observed was a doubling of the risk for pancreatic cancer for those in the highest quintile of circulating serum 25OHD levels, offered the following observation: "We have learned some hard lessons…. and we now know that taking vitamins in supernutritional doses can cause serious harm."

Conclusions About Vitamin D Deficiency in the United States and Canada

Serum 25OHD levels have been used as a "measure of adequacy" for vitamin D, as they reflect intake from the diet coupled with the amount contributed by cutaneous synthesis. The cut-point levels of serum 25OHD intended to specify deficiency and sufficiency for the purposes of interpreting laboratory analyses and for use in clinical practice are not specifically

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within the charge to this committee. However, the committee notes with some concern that serum 25OHD cut-points defined as indicative of deficiency (or as reported by some, "insufficient") for vitamin D have been subject to a wide variation in specification without a systematic, evidence-based consensus development process. In order to ensure clarity, the discussion in this section expresses serum 25OHD levels in both nmol/L and ng/mL measures.

From this committee's perspective, a considerable over-estimation of the levels of vitamin D deficiency in the North American population now exists due to the use by some of cut-points for serum 25OHD levels that greatly exceed the levels identified in this report as consistent with the available data. The 1997 IOM report (IOM, 1997) specified a serum 25OHD concentration of 27.5 nmol/L (11 ng/mL) and above as an indicator of vitamin D adequacy from birth through 18 years of age, and a concentration of 30 nmol/L (12 ng/mL) and above as an indicator of vitamin D adequacy for adults. This level (27.5 nmol/L for children, and 30.0 nmol/L for adults) remains a level below which frank deficiency including rickets and osteomalacia may be expected to occur. In recent years, others have suggested different cut-point values as determinants of deficiency (or "insufficiency"). These include values ranging from less than 50 nmol/L (20 ng/mL) to values above 125 nmol/L (50 ng/mL). Based on this committee's deliberations, the vitamin D–related bone health needs of approximately one-half of the population may be expected to be met at serum 25OHD concentrations between 30 and 40 nmol/L (12 and 16 ng/mL); most of the remaining members of the population are likely to have vitamin D needs met when serum concentrations between 40 and 50 nmol/L (16 and 20 ng/mL) are achieved. Failure to achieve such serum concentrations place persons at greater risk for less than desirable bone health as manifested by, depending upon age, increased rates of bone accretion, bone mineral density, and fractures.

Use of higher than appropriate cut-points for serum 25OHD levels would be expected to artificially increase the estimates of the prevalence of vitamin D deficiency. The specification of cut-point values for serum 25OHD levels has serious ramifications not only for the conclusions about vitamin D nutriture and nutrition public policy, but also for clinical practice. At this time, there is no central body that is responsible for establishing such values for clinical use. This committee's review of data suggests that persons are at risk of deficiency at serum 25OHD levels of below 30 nmol/L (12 ng/mL). Some, but not all, persons are potentially at risk for inadequacy at serum 25OHD levels from 30 up to 50 nmol/L (12 to < 20 ng/mL). Practically all persons are sufficient at levels of 50 nmol/L (20 ng/mL) and above. Serum concentrations of 25OHD above 75 nmol/L (30 ng/mL) are not associated with increased benefit. There may be reason

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for concern at serum 25OHD levels above 125 nmol/L (50 ng/mL). Given the concern about high serum 25OHD levels as well as the desirability of avoiding mis-classification of vitamin D deficiency, there is a critical public health and clinical practice need for consensus cut-points for serum 25OHD. The current lack of evidence-based consensus guidelines is problematic and of concern because individuals with levels of 25OHD serum above 50 nmol/L (20 ng/mL) may at times be diagnosed as deficient and treated with high-dose supplements of vitamin D containing many times the levels of intake outlined in this report.

Decisions Regarding Levels of Calcium and Vitamin D to Be Administered in Controlled Clinical Trials

Although this report identifies upper levels of intake below which adverse effects are not expected to arise, ULs are intended to serve as a lifetime public health measure for a free-living, unmonitored population. Those responsible for determining the appropriate dosages of nutrients to be studied in carefully controlled experimental trials conducted with appropriate adverse event and safety monitoring have the opportunity to bring other considerations into play when deciding on the levels of nutrients that are acceptable and appropriate for subjects taking part and being monitored in such studies. Research using intakes higher than those specified in the ULs can be justified under a number of circumstances after careful review of the literature and through the use of appropriate study protocols. Indeed, such studies are likely to be informative to the understanding of dose–response relationships and the health benefits or risks associated with calcium and vitamin D intakes.

The DRI Development Process

As described in Chapter 1, the DRI development process has recently been subjected to a review as well as targeted discussions about the process and ways to enhance it (IOM, 2008). As an overall result of these discussions, DRI development is now placed more clearly in the context of the risk assessment approach—that is, an organizing framework for conducting evaluations with public health implications often made with evidentiary uncertainties. There is also a series of existing "gap issues"—specifically, needed methodologies and guidelines—that have been identified as important to improving and enhancing the process for developing DRIs and would benefit from targeted efforts to resolve the gaps (Taylor, 2008).

The report of this committee is the first DRI report to be completed subsequent to the 2004 to 2008 evaluation of the DRI development process. It has been structured to be consistent with the risk assessment pro-

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cess with the intent of enhancing its transparency, especially in the face of uncertainties. Although this committee was mindful of the identified methodological gaps for enhancing the DRI process, it was not tasked with addressing them; in any case, virtually all of the relevant issues are complex and suggest a need to convene groups of individuals with specific expertise germane to the question at hand. Because this DRI report is an initial effort to set DRI development on the path of a risk assessment approach, its experience points to the importance of addressing several gap issues.

Specifically:

- The identification of dose–response relationships for calcium and vitamin D relative to health outcomes was a major challenge. The gap issue[1] (number 5-5 in Taylor, 2008) that is focused on methodologies for approximating dose–response relationships warrants attention, as it is likely that DRI efforts in the future will face the same challenges.
- With the exception of the inclusion of osteoporosis within the bone health measures, the existing data precluded the use of a chronic disease such as cancer or heart disease as an indicator for DRI development. However, had it been possible, this DRI process would have benefited from guidelines specifying what, if any, differences may apply to using chronic disease endpoints versus other types of endpoints for DRI development (gap issue number 4-4[2] in Taylor, 2008).
- In the committee's judgment, sufficient new data were available to allow the development of EARs and RDAs, and it was no longer necessary to make use of AI estimates for calcium and vitamin D, except for infants. The AI is useful in that it allows the specification of some type of a reference value for use in public health settings—which is better than the absence of any value. However, it presents challenges in public health applications (gap issue num-

[1]"New methodologies—many from other fields of study—are emerging and can be useful for examining and approximating dose–response relationships when available data are limited. These should be more closely examined and incorporated into the DRI process as appropriate" (Taylor, 2008).

[2]"There is considerable interest—as well as more than 10 years of experience—surrounding the inclusion of chronic disease indicators within DRI development. A variety of perspectives were put forward. There is a need for focused discussions about how to include chronic disease indicators in the DRI process, including specific approaches for addressing their confounders, identification of appropriate biomarkers, and quantifying their effects" (Taylor, 2008).

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ber 4-2[3] in Taylor, 2008), because it is not entirely consistent with the statistical approach based on distributions of requirements that underpin the DRIs. Guidelines for the use of AIs would be helpful, both those that exist for other nutrients at this time as well as those that might be specified in the future.

POPULATION SEGMENTS AND CONDITIONS OF INTEREST

Adiposity

As highlighted in Chapter 3, excess adiposity or obesity—defined as a body mass index (BMI) measure of 30 mg/m^2 or higher—is associated with lower serum 25OHD concentrations (and higher parathyroid hormone levels) than found in non-obese counterparts. This would appear to be due to sequestration of 25OHD by adipose tissue, given that supplementation of obese and lean persons with vitamin D appears to result in no significant difference in response between the two groups (Jones, 2008). Moreover, a few studies of modest weight loss have found circulating 25OHD levels to increase despite no increased intake of vitamin D from diet or sun exposure (Riedt et al., 2005; Reinehr et al., 2007; Zittermann et al., 2009; Tzotzas et al., 2010), suggesting release from adipose stores with adipose depletion. Further, neither season nor ethnicity influences these biochemical parameters (Alemzadeh et al., 2008).

An important concern is whether the lower serum 25OHD levels associated with obesity have meaningful consequences for the DRI indicator of bone health. Evidence for effects of obesity on bone density is mixed. The combined influence of increased weight-bearing activity and endogenous synthesis of estrogen due to outcomes of increased adiposity has long been associated with higher bone density (Reid, 2008). In a population-based study in Finland of perimenopausal and early postmenopausal women, Pesonen et al. (2005) found that increased body weight was a strong predictor of high bone density. Likewise, Morin and Leslie (2009), in a retrospective cohort study, found a strong correlation between higher BMI category and high bone density in postmenopausal women.

Although these and other studies have suggested that total body mass contributes to bone density and would appear to support the role of increased weight-bearing activity as a factor positively influencing bone density (Prentice et al., 1991; Khosla et al., 1996; Wortsman et al., 2000; Finkelstein et al., 2002, 2008), more recent studies lead to further ques-

[3]"There is broad interest in addressing the AIs as a component of the DRI values, but no clear path has emerged in terms of clarifying, adapting or eliminating AIs. Nor is there agreement about directions to be taken in the future for AI development" (Taylor, 2008).

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tions. The distribution of body fat may influence bone mass, such that excess intra-abdominal fat could adversely affect bone remodeling and even contribute to greater fracture risk (Premaor et al., 2010; Sukumar et al., 2011). One possibility is that intra-abdominal adipose tissue is more biologically active than subcutaneous fat, secreting cytokines and adipokines that negatively affect osteoblast and osteoclast activity (Kawai and Rosen, 2010). Moreover, both lean and fat mass contribute to weight-bearing effects. Because obesity is accompanied by increases in both lean mass and fat mass, at least in younger individuals, it is difficult to attribute the effect on bone density to fat mass as opposed to lean mass. Further, body composition changes with age, even in the obese; in turn, there may be less lean body mass in older individuals.

This complicates the ability to clarify how adiposity may affect bone health. As noted, some studies have suggested that adiposity or increased fat mass itself may be a factor in the development rather than the prevention of osteoporosis, particularly in the elderly. Zhao et al. (2007) observed that when the effect of mechanical loading from high body weight on bone density was statistically controlled, fat mass was inversely correlated with bone mineral content. Further investigation by Zhao et al. (2008) suggested that molecular signaling pathways involved in osteoblast differentiation may contribute to the previously identified effect of increased adiposity on decreased bone mineral content, although a mechanism has not been elucidated. However, this science is just emerging and it is premature to speculate on its significance or relevance to bone health and bone density.

At this time, there is the possibility that obesity, at least in older persons, may not be beneficial for bone health and may be demonstrated to be a risk factor, not an advantage, for decreased bone density and, in turn, reduced bone health. There is no evidence that increases in calcium or vitamin D nutriture beyond the requirements specified for non-obese persons can affect this purported outcome.

Persons Living at Upper Latitudes in North America

The question of the impact of latitude on vitamin D nutriture is often a topic of concern or, at least, interest. The issue, however, is set in the context of the inability to specify a safe dose of sunlight that could contribute to vitamin D synthesis while also avoiding the risk of skin cancer. There are also the recognized challenges associated with quantifying the contributions from sun exposure coupled with the limited information on the role of stored vitamin D during seasonal changes. The prevailing assumption about the effect of latitude is that ultraviolet B (UVB) penetration decreases with increasing latitude (i.e., distance from the equator) and

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this, in turn, causes persons living at higher latitudes in North America to experience little or no UVB exposure, making them at risk for vitamin D deficiency. This assumption may not be entirely accurate. However, the question of latitude may work in tandem with other factors, discussed below, such as limited sun exposure overall or cultural and dietary practices. This section focuses only on the issue of latitude per se.

The relationship between UVB penetration and latitude is complex and not merely a function of distance from the equator. Other factors that come into play include the reduced atmosphere at the poles (about 50 percent less than at the equator), more cloud cover at the equator than at the poles, differences in ozone cover, and the duration of sunlight in summer versus winter. Geophysical surveys have indicated that UVB penetration over 24 hours during the summer months at Canadian north latitudes equals or exceeds UVB penetration at the equator (Lubin et al., 1998), suggesting that persons living in the northern latitudes are not necessarily receiving notably less total sunlight during the year. Rather, it suggests that there may be considerable opportunity during the spring, summer, and fall months in the far north for humans to form vitamin D and store it in liver and fat. Likewise, animals living in the same region that are consumed as part of the traditional diet are also rich sources of vitamin D (Keiver et al., 1988; Kenny et al., 2004; Brunborg et al., 2006; Kuhnlein et al., 2006).

These factors help to explain why latitude alone does not appear to predict serum 25OHD concentrations in humans. In a Finnish study, healthy subjects living above the Arctic Circle (latitude 66°N) did not have lower serum 25OHD levels than subjects living in southern Finland; in fact, the group living above the Arctic Circle had higher levels. Both groups achieved mean serum 25OHD levels above 90 nmol/L during the summer, whereas the mean serum 25OHD level at the winter nadir was 56 nmol/L in the south and 68 nmol/L in those living above the Arctic Circle (Lamberg-Allardt et al., 1983).

Persons Experiencing Reduced Vitamin D Synthesis from Sun Exposure

The DRIs for vitamin D established in this report are based on the assumption of minimal sun exposure. Therefore, they are regarded as adequate for persons who may be experiencing a reduced synthesis of vitamin D from sun exposure. Assuming that some population groups may be consuming less than the current DRI values for vitamin D, the question is to what extent are these persons at risk for vitamin D deficiency, or, conversely, to what extent can inadvertent sun exposure be expected to compensate for lower intakes for these persons?

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Dark Skin

As described in Chapter 3, skin pigmentation—due to melanin in the epidermal layer—can reduce the amount of vitamin D synthesized by the human body. The amount of UVB required for changes in serum 25OHD levels is partly related to the degree of skin pigmentation. Further, a number of reports through the years have indicated consistently lower serum 25OHD levels in persons identified as black compared with those identified as white (Specker et al., 1985; Harkness and Cromer, 2005; Stein et al., 2006; Armas et al., 2007; Basile et al., 2007; Bodnar et al., 2007). Looker et al. (2008), using the National Health and Nutrition Examination Surveys (NHANES) 2000 to 2004, reported lower serum 25OHD levels for non-Hispanic blacks compared with Mexican Americans and whites. Mexican Americans had serum 25OHD concentrations that were intermediate between those of non-Hispanic blacks and whites.

The question is whether the consistently lower levels of serum 25OHD for persons with dark skin pigmentation have significant health consequences. Based on the data of Looker et al. (2008), non-Hispanic blacks in the NHANES had an average serum 25OHD concentration of 40.14 nmol/L (± 0.88 nmol/L [standard error of the mean]). Given that 40 nmol/L may be reflective of an acceptable median level for serum 25OHD in serum based on this committee's work, it is difficult to suggest that this average serum 25OHD level is indicative of widespread deficiency, although such conclusions cannot be based solely on mean values. However, at least for those of African American ancestry, there are corollary data to suggest that rates of osteoporosis and bone disease are not higher among African Americans; in fact, African Americans have reduced rates of fracture and osteoporosis compared with whites (see Chapter 4). There are no data in this regard for other ethnic groups with dark skin, such as South Asians, so firm conclusions about their risk related to bone health cannot be drawn. Furthermore, it is possible that risk may be introduced or modulated by an array of variables, including cultural and ethnic practices.

Given the unknowns, dark-skinned immigrant groups who now reside in North America may present a concern, as described below. There is also a concern for dark-skinned infants and children whose overall diet may be low in calcium and who may have low serum 25OHD levels, especially if exclusively breast-fed and not otherwise supplemented (see below). The vitamin D and calcium issues related specifically to African Americans have been described earlier in Chapter 4.

South Asian and Middle Eastern immigrant groups South Asians (e.g., Indians, Pakistanis, Sri Lankans) are now residing in greater numbers in North America, and are reported to be at increased risk for vitamin D–

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deficiency. This group has a significant presence in Canada and is growing in number (Statistics Canada, 2010). A recent study by Wu et al. (2009) measured vitamin D intakes and serum 25OHD levels in three different ethnic groups in southern Ontario and found that levels were significantly lower in South Asians than in Eastern Asian or European groups. Over the past few decades, there have been sporadic reports of vitamin D–deficiency rickets in Canadians, almost always in breast-fed, dark-skinned Canadians of African or Asian descent, but the total number of cases, even in a major metropolitan area like Toronto, is small (17 over a 5-year period from 1988 to 1993) (Binet and Kooh, 1996). Similar to the situation in African Americans (see Chapter 3), the lower serum 25OHD levels observed are not associated with significant rises in the rates of bone disease (osteomalacia or rickets) in the Canadian South Asian cohort. In other South Asian communities living at relatively high latitudes (> 50°N) in Europe (e.g., Scotland), there have been reports of rickets and osteomalacia dating back to the early 1970s (Ford et al., 1976; Goel et al., 1976) and suggestions that vitamin D deficiency might also be associated with higher rates of tuberculosis (Yesudian et al., 2008). Although ensuring that the DRIs are met for these groups should reduce the risk for deficiency states to the extent that their conditions mimic those from minimal sun exposure, it is considered advisable to exercise vigilance for this growing group.

Some immigrant populations or religious groups adhere to cultural practices regarding clothing that can greatly reduce exposure to sun light and exacerbate the effects of low intake of vitamin D. There is the suggestion that at least 20 percent of the body's surface must be exposed to UVB for serum 25OHD levels to increase (Specker et al., 1985; Hollis, 2005). Whether such sun exposure is a wise public health practice for any group is not the issue, only that there is a need for awareness when such cultural practices limit sun exposure.

Dark-skinned, exclusively breast-fed infants In 2000, a report was published concerning rickets among nine children from various areas of the United States (Shah et al., 2000). Eight children were described as African American, and one child was described as Hispanic. All patients were primarily breast-fed for more than 11 months, with minimal intake of dairy products and without vitamin D supplementation. Breast milk, is of course, not a source of vitamin D for infants. This report had been preceded by a 1979 report from Bachrach et al. (1979), who noted 24 cases of vitamin D–deficiency rickets in black, breast-fed infants who were otherwise healthy and had no underlying malabsorptive or renal diseases, but whose parents belonged to groups that subscribed to dietary restrictions and clothing habits that minimized their exposure to sunlight. Later, a 2001 report described a black infant who was breast-fed until 10 months of age and then weaned to a soy food beverage that was not fortified with vitamin D

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or calcium (Carvalho et al., 2001). The infant developed normally until about 9 months of age when the child's height and weight became severely arrested. In 2003, DeLucia et al. (2003) commented on 43 children with nutritional rickets reported from 1986 through 2002 and located in the New Haven, Connecticut area. Approximately 86 percent were of African American, Hispanic, or Middle Eastern descent. More than 93 percent of the children had been breast-fed. In this case, the authors implicated both low calcium intake as well as marginal vitamin D nutriture in rickets.

A recent 2-year survey of Canadian pediatricians found the incidence of rickets in their patients to be 2.9 per 100,000; the mean age at diagnosis was 1.4 years (range of 2 weeks to 6.3 years). Ninety-four percent of the children with rickets had been breast-fed. Additional risk factors included dark skin, living in the far north, born of mother who took no vitamin supplements, limited sun exposure, emigrated from a region where vitamin D deficiency is endemic, and delayed initiation of solid foods (Ward et al., 2007).

Vitamin D supplementation of partially or fully breast-fed infants should begin in the first week of life and provide approximately 400 IU/day, as breast milk is not a source of this nutrient for infants, and sun exposure to compensate for this cannot be adequately described but neither can it be recommended given the concerns for skin cancer. It is important to be especially vigilant regarding supplementation in the case of exclusively breast-fed, dark-skinned infants, as they appear to be at higher risk than lighter-skinned infants.

Use of Sunscreen

Sunscreen absorbs ultraviolet light and prevents it from reaching the skin. It has been reported that sunscreen with a sun protection factor (SPF) of 8 based on the UVB spectrum can decrease vitamin D synthetic capacity by 95 percent, whereas sunscreen with an SPF of 15 can reduce synthetic capacity by 98 percent (Matsuoka et al., 1987). The extent and frequency of use of sunscreen are unknown, and therefore the significance of the role that sunscreen may play in reducing the opportunity to synthesize vitamin D is unclear. Increases in serum 25OHD levels seen in summer months in national surveys conducted in both the United States and Canada would suggest either that sunscreen is not used consistently by the population as a whole or that the actual decrease in serum 25OHD level due to appropriate use of sunscreen has been overstated. Although inconsistent with advice provided by the American Academy of Dermatology[4] and the National

[4]Available online at http://www.aad.org/media/background/news/Releases/American_Academy_of_Dermatology_Issues_Updated_Pos/.(accessed July 28, 2010).

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Council on Skin Cancer Prevention[5] for skin cancer protection, given the carcinogenic potential of UVB light, one report indicated that there is adequate vitamin D production when exposure of hands, face, arms and legs to sunlight is for an amount of time equal to about 25 percent of what it would take to develop a "mild sunburn"; after this extent of exposure, a sunscreen should be applied to prevent damage (Holick, 2003). However, this is in contrast to a recent report on mathematical models of observational data regarding the impact of seasonal sun exposure (Diffey, 2010). The effect of the use of sunscreen, as with other factors that may limit exposure, warrants vigilance. However, its use should not constitute a concern, given that the DRI values assume minimal sun exposure.

Indoor Environments and Institutionalized Older Persons

Increased urbanization and the normative condition among North Americans to work and recreate indoors cannot be quantified or addressed in terms of increased risk for vitamin D deficiency. The newly established DRI values assume minimal sun exposure, and therefore vitamin D intake need not be increased above this level for normal persons living in urban settings and spending time primarily indoors.

However, data for institutionalized, frail older persons suggest a propensity for lower serum 25OHD levels generally. Causation, however, is uncertain. It is likely that many factors contribute, such as their restriction to primarily indoor environments often coupled with inadequate total intake overall. Further, aging skin is known to be less effective in synthesizing vitamin D in part because of a decrease in skin provitamin D (7-dehydrocholesterol) levels and in part because of alterations in skin morphology (MacLaughlin and Holick, 1985). The EAR and RDA values have taken this group into consideration to the extent possible and allowed by the data. Given the unknowns, however, monitoring institutionalized elderly people for vitamin D (and calcium) nutriture is appropriate. Supplementation, however, should not be random and without cause, because excess intakes of these nutrients may have adverse consequences for this frail sub-population.

Alternative Diets or Changes in Dietary Patterns

Dairy and Animal Product Exclusion: Lactose Intolerance, Cow's Milk Food Allergy, Ovo-Vegetarianism, and Veganism

Exclusion of dairy products occurs therapeutically in those with lactose intolerance or cow's milk food allergy, and voluntarily in those who are

[5]Available online at http://www.skincancerprevention.org/ (accessed July 28, 2010).

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vegans or non-lacto vegetarians. As noted in the recent National Institutes of Health (NIH) Consensus Statement on Lactose Intolerance (Brannon et al., 2010; Suchy et al., 2010), exclusion of dairy products, all of which are rich sources of calcium and some of which are fortified with vitamin D (e.g., fluid milks, some yogurts, and limited other dairy products [Yetley, 2008]), can be a risk factor for inadequate intakes of calcium and vitamin D. This is also true for vegans (Craig, 2009) and likely others who systematically exclude dairy foods as well as other animal products from their diets. However, as pointed out by the American Dietetic Association (American Dietetic Association and Dieticians of Canada, 2003; Craig and Mangels, 2009) as well as the Dietitians of Canada (American Dietetic Association and Dietitians of Canada, 2003), appropriately planned vegetarian diets, including total vegetarian or vegan diets, are healthful and nutritionally adequate.

The North American prevalence of lactose intolerance, a clinical syndrome characterized by diarrhea, bloating and/or flatulence following consumption of lactose, is challenging to determine because the parameters surrounding lactose intolerance, lactose malabsorption, and lactase non-persistence are not well defined, and frequent self-diagnosis occurs (Brannon et al., 2010; Suchy et al., 2010). The prevalence of cow's milk allergy reported in a systematic evidence review (Rona et al., 2007) was 0.6 to 0.9 percent by skin test, specific immunoglobulin E measurement, or food challenge test; this is lower than the self-reported prevalence of 3 percent. Similarly to lactose intolerance, individuals may perceive that they have cow's milk food allergy when they do not. With respect to vegetarians, in 2006, approximately 1.4 percent of U.S. adults and nearly 1 percent of children and adolescents 8 to 18 years of age self-reported that they were vegans, and 2.3 to 3 percent reported themselves to be vegetarians.[6] In a 2002 survey, about 4 percent of Canadian adults reported being vegetarians (American Dietetic Association and Dietitians of Canada, 2003). Although there are few data to document the consequences of poorly planned diets that exclude dairy or animal products—it is noted that Craig (2009) reported a 30 percent increased risk of fracture for vegans—it is best to assume that persons who have chosen or must follow such diets should make special efforts to ensure nutritional adequacy.

Strategies for ensuring adequate intakes of calcium and vitamin D vary depending on the reason for dietary exclusion. Using an Agency for Healthcare Research and Quality systematic evidence review as a basis (Shaukat et al., 2010; Wilt et al., 2010), an NIH Consensus Panel found that individuals with lactose intolerance or lactose malabsorption are able to tolerate up to 12 g of lactose, the equivalent of one cup of milk, in a

[6]Available online at http://www.vrg.org/journal/vj2006issue4/vj2006issue4poll.htm (accessed July 28, 2010).

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single dose and may be able to tolerate larger amounts if consumed in smaller doses spread over the day and with other foods. Larger amounts of reduced-lactose dairy products such as certain yogurts and fluid milks as well as virtually unrestricted amounts of reduced-fat hard cheeses with very low amounts of lactose may be ingested to ensure adequate intakes of calcium. For those who avoid all dairy because of allergies or personal choice, consumption of non-dairy sources of calcium, such as low-oxalate vegetables (e.g., kale, bok choy, Chinese cabbage, broccoli, and collards), calcium-containing tofu, or fortified plant-based foods, such as cereals or fruit juice are feasible strategies to ensure adequate intakes of highly bioavailable calcium (Weaver et al., 1999). Finally, supplements of calcium are also a strategy, although care should be taken not to over-supplement.

Meeting vitamin D needs is more challenging in the absence of sun exposure. Plant foods are not natural sources of vitamin D,[7] but the marketplace in the United States is increasingly offering plant-based fortified alternatives such as cereals and juices. In addition, the Canadian food supply includes margarines fortified with vitamin D and plant-based beverages that are fortified with vitamin D and calcium. Such fortified foods can be helpful in meeting the DRIs across age groups. As with calcium, a dietary supplement of vitamin D is also an option, but total intake (foods plus supplements) should not exceed the Tolerable Upper Intake Level (UL).

Changes in Dietary Patterns of Indigenous Canadian Populations

Among the indigenous Canadian populations, switching from a traditional diet that contains vitamin D–rich foods to a westernized diet may increase the likelihood of vitamin D deficiency, especially if UVB exposure is limited or avoided. This has been underscored by a survey of Inuit living in Greenland, which reported that those consuming a westernized diet had lower serum 25OHD levels than those consuming a traditional diet (32 vs. 53 nmol/L in summer, 29 vs. 41 nmol/L in winter) (Rejnmark et al., 2004). As noted above, there is ample opportunity during the spring, summer, and fall months in the far north for animals that commonly comprise the traditional diet of indigenous groups to form vitamin D and store it in liver and fat. In turn, the blubber and liver of various arctic marine mammals (e.g., seal, narwhal, beluga, walrus) and fish (e.g., char, cisco, lake trout, loche, sculpin, whitefish) are sources of vitamin D for those who consume a traditional diet (Keiver et al., 1988; Kenny et al., 2004; Brunborg et al., 2006; Kuhnlein et al., 2006).

[7]Some algal supplements and mushrooms that have been processed with irradiation contain vitamin D, but not in significant amounts. Available online at http://ods.od.nih.gov/factsheets/vitamind.asp (accessed July 28, 2010).

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The Canadian Health Measures Survey does not collect data on these indigenous populations living at upper northern latitudes, and overall dietary and health data for them are limited. One recent survey (Kuhnlein et al., 2008) in northern Canada found that the intakes of vitamin D differed by ethnic group. The median vitamin D intake was 200 IU/day in both Yukon First Nations[8] and Dene/Métis. However, much higher median intakes were found in older (over age 40) Inuit who consumed a traditional diet (1,000 IU/day and 680 IU/day in men and women, respectively), whereas younger Inuit had much lower intakes (328 IU/day and 372 IU/day in men and women, respectively). This research group also surveyed indigenous women of reproductive age from various communities in the Canadian Arctic and found the mean daily intakes of vitamin D to be 456 IU/day in Inuit from Qikiqtarjuaq, 364 IU/day in Inuit from 18 other communities, and 228 IU/day in a combined data set of Dene, Métis, and Yukon First Nations. Pregnant and lactating women had higher vitamin D intakes, with the highest mean intake being 816 IU/day in lactating Inuit from Qikiqtarjuaq (Berti et al., 2008). Neither of these surveys measured serum 25OHD levels.

A 1999 survey (Smith, 1999) estimated vitamin D intakes and measured serum 25OHD levels in 121 pregnant women living in the Inuvik region of the Northwest Territories. The sample included 33 whites, 51 Inuit, and 37 First Nations people. The investigator did not report whether the First Nations and Inuit mothers were consuming a traditional or a western diet; moreover, the accuracy for the measures of the vitamin D content of traditional foods is unclear. The estimated daily mean vitamin D intake of Inuit and First Nations people was 324 IU/day with supplements (136 IU/day without) compared with 532 IU with supplements (232 IU without) to whites. At the point of delivery, the plasma levels of 25OHD were lower in the First Nations and Inuit mothers and their babies than in their white counterparts. Not quite as far north, a survey of 104 pregnant women from three First Nations communities in northern Manitoba found that their serum 25OHD levels ranged from < 15 nmol/L (undetectable) to 63 nmol/L, with mean values of 18, 21, and 24 nmol/L in each of the three communities (Smith, 1999). No information was provided in that report as to whether the women were consuming a traditional or western diet. A chart review was done of all babies born in 1993 and 1994 to determine how many had been diagnosed with rickets, and a high prevalence was found. Despite similar serum 25OHD levels in all three communities,

[8]First Nation: A term that came into common usage in the 1970s to replace the word "Indian." Among its uses, the term "First Nations peoples" refers to the Indian peoples in Canada, both Status and non-Status. Definitions available online at http://www.ainc-inac.gc.ca/ap/tln-eng.asp (accessed July 28, 2010).

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there was a marked difference in the prevalence of rickets: 85/1,000 and 55/1,000 in two communities, but none in the third. No clear explanation for the differing prevalence was obtained by the investigator (Smith, 1999).

Taken as a whole, the limited data surrounding indigenous Canadian populations suggest a basis for concern regarding vitamin D nutriture, most notably in the likelihood that typical diets are changing from traditional foods to more westernized foods. Although the assumption of minimal sun exposure underpinning the DRI values may not entirely align with this group of people who may experience considerable sun exposure in the summer, ensuring that the diet meets the DRI values should provide assurances that risk of vitamin D deficiency has been greatly reduced.

Use of Calcium Supplements

The forms and nature of calcium supplements have been discussed in Chapter 2, and their possible role in kidney stone formation as well as the emerging data regarding possible adverse cardiovascular effects have been outlined in Chapter 6. The mechanisms for differential effects of food sources and supplement forms of calcium on kidney stone formation are complex and may relate to the timing of calcium administration. Approximately 80 percent of kidney stones contain calcium combined with oxalate or, less often, phosphate (Park and Pearle, 2007). Calcium in food or in supplements taken with food is believed to bind to dietary oxalate in the digestive tract, reducing the absorption and subsequent urinary excretion of oxalate and thus risk for kidney stones (urinary oxalate may be more critical than urinary calcium with respect to calcium oxalate crystallization) (Curhan et al., 1997). When calcium supplements are not taken with food, dietary oxalate is absorbed unopposed and thus is more available for stone formation. Although dairy foods, which are the major source of calcium in much of North America, have been suggested to contain an unidentified protective compound not found in supplements (Curhan et al., 1997), this possibility has not been well studied. Obtaining sufficient calcium via dietary sources is the preferred strategy—and it remains uncertain as to whether taking calcium supplements with food may reduce the likelihood of stone formation associated with supplement use. Head-to-head comparisons of different calcium supplement formulations with respect to risk for kidney stone formation are also lacking. In any case, given the desirability of not surpassing the UL for calcium intake and given that even those not meeting their requirement for calcium are nonetheless consuming some calcium from dietary sources that range from breads to dairy products, care must be taken in selecting a calcium supplement that when combined with dietary intake does not result in a total intake above the UL. The UL for a sizable proportion of the population, including groups that commonly

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consume calcium supplements, is 2,000 mg/day, which is relatively close to the EAR and RDA values. For these more vulnerable groups, supplements containing amounts less than the RDA may be appropriate given that their diet is likely to contain at least some calcium. Further, until better information is available to clarify the possible link between supplement use and kidney stone formation, taking calcium supplements with foods is advisable.

Moreover, in the case of persons prone to developing kidney stones who cannot get adequate calcium from diet (e.g., due to lactose intolerance), there is limited evidence from small, short-term trials suggesting that supplemental calcium in moderate doses may not increase risk for stone recurrence (Levine et al., 1994; Williams et al., 2001; Lewandowski and Rodgers, 2004). Again, taking supplements with food is desirable.

The ULs are defined for the healthy, general population. Nonetheless, gray areas are acknowledged to exist between healthy people and those with medical conditions; for some persons in these gray areas a calcium intake as high as the UL may no longer be considered without any risk. The effect of calcium intake in situations of hypercalciuria is not fully understood, but conditions leading to hypercalciuria (which may be exacerbated by adding extra vitamin D to an already high calcium intake) may warrant a more cautious approach to ULs for calcium in the future. In older adults experiencing illness or decline, hypercalciuria may develop. For pregnant women experiencing absorptive hypercalciuria and therefore at higher risk of renal stone formation, keeping calcium intake below the UL may also be most appropriate. Similarly, as lactation drives bone resorption, urinary calcium excretion decreases, the ionized serum calcium concentration rises slightly, intravascular volume is contracted and occasionally women become hypercalcemic. Under these and similar conditions, ensuring a calcium intake below the UL may be most appropriate. Greater surveillance of urinary calcium excretion in future studies may shed more light on the relationship between higher levels of total calcium intake and risk of hypercalciuria or hypercalcemia under special conditions.

Oral Contraceptive Use

The use of ethinyl estradiol oral contraceptives (OCs) has been hypothesized to reduce bone resorption and preserve bone density in premenopausal and postmenopausal women. This concept was based on clinical and observational evidence that ethinyl estrogen–based hormone replacement therapy reduced risk for osteoporosis in postmenopausal women (Zittermann, 2000). A non-systematic review of clinical trials carried out before 1994 indicated that the evidence at that time largely supported positive effects of OCs on bone density in postmenopausal women, although a number of trials in the review showed no effects (DeCherney,

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1996). Among clinical trials and observational evidence examining the effects of OCs on bone density from the past two decades, results have been mixed and, when considered in total, are inconclusive. A systematic review of 75 studies of varied design, including 11 randomized controlled trials, examined outcomes of OC use and bone density in healthy premenopausal, amenorrheic premenopausal, anorexic premenopausal, and perimenopausal women (Liu and Lebrun, 2006). A meta-analysis was not done; however, the review found good evidence for a positive effect of OCs on bone density in perimenopausal women, fair evidence for an effect in amenorrheic premenopausal women, and limited evidence for an effect in anorexic and healthy premenopausal women.

Observational studies published since Liu and Lebrun (2006) also suggest mixed results from studies on OC use and bone density that may be related to the population group studied. A small study on OC use and bone density and bone size in a young white female cohort found that OC use had a significant negative effect on bone density at the spine and heel and resulted in a non-significant decrease in hip bone density (Ruffing et al., 2007). Similarly, Hartard et al. (2007), in a cross–sectional analysis of young white women taking OCs, also suggested a negative effect of OCs on bone density. Women who had ever used OCs had significantly lower bone densities at the tibial shaft and femoral neck compared with those who had never used OCs. In premenopausal and postmenopausal women no significant difference was found between OCs users and never users in another cross–sectional study of the effects of OCs on bone density and bone markers (Allali et al., 2009).

Randomized trials of estrogen treatment with and without vitamin D and calcium supplementation suggest a positive effect on bone density in postmenopausal women. Recker et al. (1999) tested vitamin D and calcium supplementation with and without low-dose hormone replacement therapy for effectiveness in maintaining bone density in postmenopausal women more than 65 years of age. Although this study did not differentiate between hormone replacement therapy alone and therapy combined with vitamin D and calcium supplementation, it did suggest an effect of increasing bone density and bone markers in older women who received the combination therapy compared with those who received vitamin D and calcium supplementation alone. A randomized, double-blind, placebo-controlled trial of OC therapy either alone or combined with calcitriol therapy found a significant increase in bone density and reduction in bone resorption at the hip compared with OC therapy alone in postmenopausal women (ages 65 to 77 years) who had normal bone density for their age (Gallagher et al., 2001). Another prospective randomized trial in postmenopausal women (ages 53 to 79 years) treated with hormone replacement therapy alone or with calcitriol also found a significant increase in bone density, at multiple

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sites and total body, for the combined therapy compared with hormone replacement alone (Gutteridge et al., 2003).

Given the variability in all the study outcomes reviewed by the committee and the unresolved question of the effect of age and endogenous estrogen status on the ability of OCs to preserve bone density or prevent bone resorption, specific recommendations to address the impact of OCs with or without vitamin D and calcium supplementation for both premenopausal and postmenopausal women cannot be offered at this time.

Premature Infants

Premature infants are a clinical population and thus outside the scope of this committee's task, which is focused on the normal, healthy population. However, because premature infants are a highly vulnerable group and do raise special concerns relative to calcium and vitamin D nutriture, this group is discussed here briefly.

The minerals in human milk, especially calcium and phosphorus, do not fully meet the needs of rapidly growing premature infants who rely primarily on passive intestinal absorption of calcium, therefore "this and other factors place premature infants at high risk for nutritional rickets" (Abrams, 2005). "The recent addition of various forms of mineral salts and/or mineral fortifiers to human milk and the use of specialized preterm infant formulas with high calcium content have been reported to enhance the amount of calcium and other minerals retained from the diet, to increase the bone mineral content of the infants and to decrease the incidence of osteopenia and frank rickets in preterm infants (Schanler et al., 1988; Schanler and Abrams, 1995; Schanler, 1998)... The bioavailability of the calcium in these fortifiers may be a key aspect of their adequacy. Using a commercially available human milk fortifier, Schanler and Abrams (1995) reported that net calcium retention was 104 ± 36 mg/kg body weight per day in premature infants, a value approximating the *in utero* accretion rate during the third trimester. These retention values are well above those achieved using earlier human milk fortifiers (Schanler et al., 1988)" (Abrams, 2005).

"Of interest is that calcium absorption from both fortified human milk and specialized preterm formula averages 50 to 65 percent in many studies (Abrams et al., 1991; Bronner et al., 1992). This constancy of absorptive fraction in premature infants suggests that much of the calcium absorption by premature infants and newborn full-term infants is not vitamin D dependent…" (Abrams, 2005), which is the conclusion of a review of more than 100 balance studies by Bronner et al. (1992).

How much vitamin D is needed by premature infants is more difficult to determine. Unfortunately, there are no studies using modern isotope

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TABLE 8-1 Drugs and Their Effect on Vitamin D Metabolism

Drug Name/Category	25OHD	Calcitriol	24,25-Dihydroxyvitamin D
Aluminum	Not changed	Increase/decrease	—
Anticonvulsants (phenobarbital, Dilantin, Tegretol)	Decrease	Not changed	Decrease
Antituberculosis	Decrease	Decrease	—
Bisphosphonates	Not changed	Increase/decrease/ not changed	Increase
Cimetidine	Decrease	Not changed	Not changed
Corticosteroids	Decrease/ not changed	Decrease/not changed	Not changed
Ethanol	Increase	Decrease	—
Heparin	Not changed	Decrease	Not changed
Hypolipidemic agents	Decrease/ not changed	Not changed	—
Immunosuppressives	Not changed	Not changed	—
Ketoconazole	Not changed	Decrease	Decrease
Lithium	Not changed	Not changed	—
Rifabutin (anti-HIV)	Decrease	Not changed	—
Thiazides	Increase	Decrease	Increase

NOTE: — indicates that no information has been reported; HIV = human immunodeficiency virus.
SOURCES: Hahn et al. (1972); Favus et al. (1973); Avioli (1975); Compston and Thompson (1977); Compston and Horton (1978); Bell et al. (1979); Alfrey et al. (1980); Palmer et al. (1980); Adams et al. (1981); Williams et al. (1985); Feldman (1986); Lalor et al. (1986); Lawson-Matthew et al. (1988); Dobs et al. (1991); Katz et al. (1994); Bolland et al. (2008).

techniques of the effects of vitamin D on calcium absorption in premature infants, nor could such studies be possible practically or ethically. One study with oral vitamin D intakes as low as 160 IU/day (Koo et al., 1995) and multiple studies with intakes of 200 to 400 IU/day (Cooke et al., 1990; Pittard et al., 1991; Backstrom et al., 1999a) "demonstrated adequate serum 25OHD concentrations and clinical outcomes with oral vitamin D intakes as low as 160 IU/day (Koo et al., 1995). In addition, studies have generally failed to show any clinical benefit to increasing vitamin D intake above 400 IU/day in preterm infants (Backstrom et al., 1999b)" (Abrams, 2005).

Routine measurement of serum 25OHD levels in premature infants is not supported by currently available clinical research. No studies have re-

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lated serum 25OHD level in these infants to specific clinical outcomes, and extremely few data suggest a dose–response relationship between serum 25OHD levels and other outcomes. A normal level at different gestational ages or postnatal ages is not available for 25OHD in serum based on endpoints such as calcium absorption or bone mineral content. However, in the presence of a likely impairment of 25-hydroxylation, such as might be present in an infant with cholestasis, measurement of serum 25OHD level might be considered, especially to ensure a level at or above 50 nmol/L (20 ng/mL). "The effects of other formula components on mineral absorption have also been considered. A study using a triple lumen perfusion technique demonstrated that calcium absorption was greater using a solution that included a glucose polymer rather than lactose (Stathos et al., 1996). As glucose polymers are widely used in preterm formulas, this effect may be clinically important. Altering the fat blend of infant formula to more closely resemble that of human milk may also enhance mineral absorption in premature infants (Carnielli et al., 1995; Lucas et al., 1997)" (Abrams, 2005).

Interactions Between Vitamin D and Prescription Drugs

Although clinical practice and related guidelines are outside this committee's purview, it is useful to acknowledge that measures of the various forms of vitamin D can be affected by prescription drugs and related medications. A brief listing of key interactions can be found in Table 8-1.

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9

Information Gaps and Research Needs

The purpose of this report is to review available data and establish science-based reference values for calcium and vitamin D, known as Dietary Reference Intakes (DRIs). The approach used has been that of risk assessment, as described in Chapter 1. This final chapter outlines information gaps and research needs identified by the committee in carrying out its charge. These gaps and research needs are also organized according to the risk assessment framework. The listings are not comprehensive, but offer the committee's perspective on the major topic areas in need of attention. These needs are targeted to academic and medical researchers, national policy makers, the public health community, industry groups, and other relevant stakeholders and funding institutions. They provide a basis for organizing and prioritizing research efforts.

The general nature of the information gaps relevant to DRI development for calcium and vitamin D are outlined in Figure 9-1.

Although the uncertainties surrounding the DRIs have been described in this report and the scientific judgments made are documented, evidence from future research designed to overcome the limitations encountered by this committee can improve the ability to determine reference values in the future. Although the committee's discussions form the basis for the identification of these research needs, other sources of research needs were noted, for example the National Institutes of Health Roundtable on Vitamin D Research Needs (Brannon et al., 2008) and the report from the Tufts Medical Center Evidence-based Practice Center (Chung et al., 2010).

Table 9-1 presents the identified research needs, which are then outlined.

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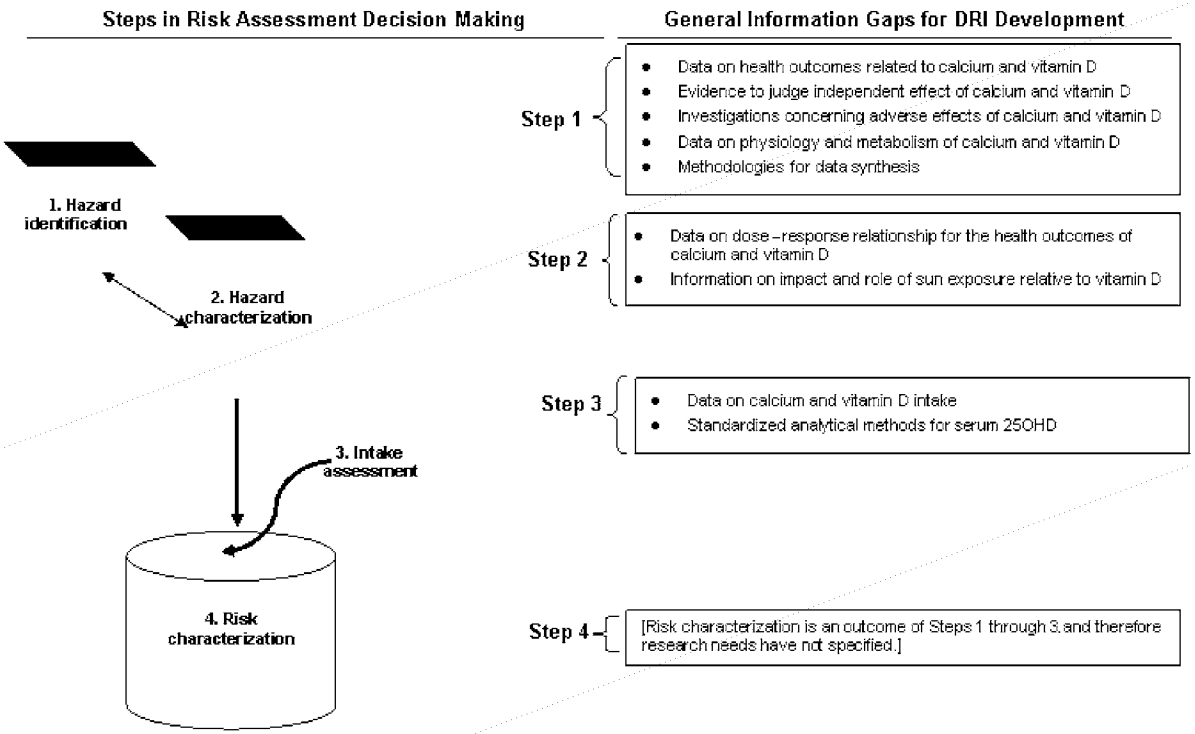


FIGURE 9-1 General nature of information gaps within the evidence base for calcium and vitamin D DRI development as related to risk assessment steps.
NOTE: 25OHD = serum 25-hydroxyvitamin D.
SOURCE: Modified from WHO, 2006.

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TABLE 9-1 Vitamin D and Calcium Research Needs Organized by Risk Assessment Steps

Research Topic	Research Questions and Identified Needs
Step 1: "Hazard Identification" or Indicator Review and Selection	
Health Outcomes and Related Conditions	1. Clarify threshold effects of calcium and vitamin D on skeletal health outcomes by life stage and for different racial/ethnic groups. 2. Elucidate inter-relationship between calcium and vitamin D, and specify independent effect(s) of each. 3. Explore causal role for vitamin D in non-skeletal health. 4. Determine the appropriateness of serum 25-hydroxyvitamin D (25OHD) as a biomarker of effect. 5. Elucidate the effect of genetic variation, including that among racial/ethnic groups, and epigenetic regulation of vitamin D on developmental outcomes.
Adverse Effects, Toxicity, and Safety	1. Develop innovative methodologies to provide for identification and assessment of adverse effects of excess calcium and vitamin D. 2. Elucidate adverse effects of long-term, high-dose calcium and vitamin D. 3. Further explore nature of vitamin D toxicity.
Basic Physiology and Molecular Pathways	1. Examine the influence of calcium and phosphate on the regulation of vitamin D activation and catabolism through parathyroid hormone and fibroblast-like growth factor-23 (FGF23). 2. Clarify 25OHD distribution in body pools including storage and mobilization from adipose tissue. 3. Evaluate the nature and significance of extra-renal production of calcitriol for health outcomes. 4. Clarify the extent to which differences exist between vitamin D_2 and vitamin D_3.
Synthesizing Evidence and Research Methodology	1. Explore enhanced methodologies for data synthesis. 2. Identify approaches to weight better potential health outcomes.
Step 2: "Hazard Characterization" or Intake-Response Assessment and Specification of DRIs	
Dose–Response Relationship	1. Conduct studies to identify specific health outcomes in relation to graded and fully measured intakes of calcium and of vitamin D. 2. Clarify the influence of age, body weight, and body composition on 25OHD levels in response to intake/exposure.

Continued

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TABLE 9-1 Continued

Research Topic	Research Questions and Identified Needs
Sun Exposure	1. Investigate whether a minimal-risk ultraviolet B (UVB) radiation exposure relative to skin cancer exists that also enables vitamin D production. 2. Clarify how physiological factors such as skin pigmentation, genetics age, body weight, and body composition influence vitamin D synthesis. 3. Clarify how environmental factors such as sunscreen use affect vitamin D synthesis.
	Step 3: Intake Assessment
Intake Assessment	1. Enhance dietary assessment methods for calcium and vitamin D intake, and methods for the measurement of calcium and vitamin D in foods and supplements. 2. Investigate food and supplement sources of calcium and vitamin D for bioequivalence, bioavailability, and safety. 3. Improve the standardization of assay for serum 25OHD.

STEP 1: "HAZARD IDENTIFICATION" OR INDICATOR REVIEW AND SELECTION

The committee found an overall lack of causal evidence from intervention studies for the task of identifying health outcome indicators. This was especially true for non-skeletal outcomes for vitamin D, but this was also true for skeletal outcomes, particularly in certain life stage groups. Data related to calcium were sparse for children and younger adults. Most vitamin D studies were conducted using older persons or postmenopausal women. Some available data suggested the possibility of ethnic differences in bone health, but this suggestion could not be further clarified. Very few studies explored the independent effects of calcium and vitamin D. Only limited data were available on adverse health effects. These information gaps, coupled with challenges in synthesizing disparate evidence for either calcium or vitamin D or their combination, presented challenges to DRI development. Further, lack of clarity concerning the physiology and metabolism of vitamin D was problematic as was the ability to judge the effects of vitamin D as a nutrient given its role as a prohormone.

Research Needs Related to Health Outcomes and Related Conditions

1. **Clarify threshold effects of calcium and vitamin D on skeletal health outcomes by life stage and for different racial/ethnic**

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groups. Although there is a solid body of evidence related to bone health and the role of calcium and vitamin D, many data gaps remain for younger age groups and for the effect under menopausal conditions. The issue of "calcium economy" among certain groups and ethnic differences in vitamin D utilization require attention.

2. **Elucidate inter-relationship between calcium and vitamin D, and specify independent effect(s) of each.** There is a need for research protocols that examine the effects of vitamin D and calcium separately rather than as a combined administration, and which better clarify the nature of the inter-relationship. Without such data, the ability to identify requirements for calcium and for vitamin D is challenging.
3. **Explore causal role for vitamin D in non-skeletal health outcomes.** Investigation of causal relationships between vitamin D nutriture and potential non-skeletal health outcomes should undergo further research. These may include but are not limited to (no particular order): immune function and anti-inflammatory effects (especially related to obesity); total and site-specific cancers; cardiovascular disease; and diabetes. More data on the role of calcium and vitamin D, and their metabolism, during pregnancy and lactation is needed.
4. **Determine appropriateness of serum 25-hydroxyvitamin D (25OHD) as a biomarker of effect.** The ability to use the relatively accessible measure of serum 25OHD as a biomarker or surrogate is limited by a number of factors including not only its role as a prohormone, but also its variability, which is due to a number of non-nutritional factors. A better understanding of its relationship to specific health outcome would be beneficial, enhancing both the quality and quantity of research available. The measure should be studied for this purpose and also should be subject to a formal validation process.
5. **Elucidate the effect of genetic variation, including that among racial/ethnic groups, and epigenetic regulation of vitamin D on developmental outcomes.** This is an emerging field of study, which will likely prove relevant to DRI development. Studies in this area may contribute notably to an understanding of population differences related to chronic disease risk.

Research Needs Related to Adverse Effects, Toxicity, and Safety

1. **Develop innovative methodologies to provide for identification and assessment of adverse effects of excess calcium and vitamin D.** The ability to study adverse effects of calcium and vitamin D is

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often limited due to ethical concerns. Creative approaches using an array of methodologies developed in other fields need to be adapted for nutritional use and incorporated into the approach for studying adverse effects of nutrients in vitro and in vivo using relevant animal models.

2. **Elucidate adverse effects of long-term, high-dose calcium and vitamin D.** The question of nutrient safety should not be a secondary aspect of study design nor can the failure to detect adverse effects as part of a study not designed for that purpose be considered an adequate assessment of safety. Dedicated studies are needed to assess adverse health effects related to long-term, high dose (although not necessarily "toxic") levels of calcium and vitamin D.
3. **Further explore the nature of vitamin D toxicity.** Although toxicity is not the most appropriate goal for setting ULs, a better understanding of the timing, doses, and mechanisms associated with vitamin D toxicity (hypervitaminosis D) would be beneficial to understanding the impact of vitamin D on the human body. Of particular import is information about the metabolic fate and dynamics of high doses of vitamin D. The identification and use of animal models (particularly large animal models) would be especially helpful. Also needed is an understanding of how weight loss in obese individuals might affect vitamin D status and adverse outcomes (e.g., bariatric surgery patients).

Research Needs Related to Basic Physiology and Molecular Pathways

1. **Examine the influence of calcium and phosphate on the regulation of vitamin D activation and catabolism through parathyroid hormone and fibroblast-like growth factor 23 (FGF23).** Identify pathways that regulate vitamin D activation and catabolism through parathyroid hormone and FGF23 in order to understand the influence of calcium and phosphate intake on vitamin D regulation.
2. **Clarify 25OHD distribution in body pools including storage and mobilization from adipose tissue.** Understanding the distribution, storage, and mobilization of 25OHD in body pools would enhance the understanding regarding relationships among exposure to vitamin D from intake or endogenous synthesis, circulation serum levels of 25OHD, and health outcomes. The role of storage compartments and factors important to the mobilization of vitamin D is noticeably lacking.
3. **Evaluate the nature and significance of extra-renal production of calcitriol for health outcomes.** Determining the significance of extra-renal production of calcitriol for health outcomes is essential

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to understand whether local production of calcitriol has an impact on health outcomes. In turn, the relevance of vitamin D nutriture and serum 25OHD for such an effect should be established.

4. **Clarify the extent to which differences exist between vitamin D_2 and vitamin D_3.** Physiological responses as well as potential for differences in safety risks for the two forms of the nutrient should be further explored.

Synthesizing Evidence and Research Methodology

1. **Explore enhanced methodologies for data synthesis.** Alternative methods for synthesizing evidence from different study types and multiple parameters that consider uncertainties (including measurement error) include teleoanalysis, confidence profile predictive meta-analysis, and generalized multi-parameter evidence synthesis. In the case of calcium and vitamin D, such approaches should facilitate quantitative estimates of effect size and dose–response relationships as needed for DRI development.
2. **Identify approaches to weight better potential health outcomes.** In order to ensure the most objective and comprehensive systematic evidence reviews in the future, approaches to better weight potential health outcomes are needed.

STEP 2: "HAZARD CHARACTERIZATION" OR INTAKE-RESPONSE ASSESSMENT AND SPECIFICATION OF DIETARY REFERENCE INTAKES

The committee encountered major challenges in determining the dose–response relationships for calcium and vitamin D. Sun exposure introduced further uncertainties regarding vitamin D.

Research Related to Dose–Response Relationships

1. **Conduct studies to identify specific health outcomes in relation to graded and fully measured intakes of calcium and vitamin D.** Too few studies are specifically designed to study the effects of graded doses of calcium or vitamin D on health outcomes, both overall and as part of the same study using the same subjects and outcome measures. Further, many studies in the calcium and vitamin D area are confounded by the failure to specify or measure and thereby take into account "background" intakes of the nutrient being studied when dose–response is being explored.

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2. **Clarify the influence of age, body weight, and body composition on serum 25OHD levels in response to intake/exposure.** Information about how factors such as age, body weight, and body composition affect the variability in serum 25OHD response to intake or exposure would assist in the process of establishing requirements for vitamin D. Such information is also important to ascertaining the measure's utility as a biomarker of effect and in making judgments about excess intake of the vitamin.

Research Needs Related to Sun Exposure

1. **Investigate whether a minimal-risk ultraviolet B (UVB) radiation exposure relative to skin cancer exists that also enables vitamin D production.** Whether a minimal or threshold UVB exposure level is possible to both enable subcutaneous vitamin D synthesis and avoid risk of skin cancer needs to be examined. Research should include assessment of the risk for skin cancer compared with the benefit of endogenous synthesis of vitamin D, particularly for at-risk populations.
2. **Clarify how physiological factors such as skin pigmentation, genetics, age, body weight, and body composition influence vitamin D synthesis.** Understanding how subcutaneous synthesis of vitamin D is affected by physiological factors and the impact of these factors on maintenance of serum 25OHD levels within normal physiologic ranges is important to integrating information about dietary intake and interpretation of serum 25OHD levels.
3. **Clarify how environmental factors such as sunscreen use affect vitamin D synthesis.** The impact of factors that affect endogenous vitamin D production, and notably the appropriate use of sunscreen for reducing cancer risk, needs to be determined to ascertain an appropriate risk-benefit profile for protected sun exposure as well as better elucidation of the role of sun exposure in determining vitamin D nutriture.

STEP 3: INTAKE ASSESSMENT

Although great strides have been made recently in providing intake data on calcium and notably on vitamin D, more data as well as a consistent approach to data reporting would be helpful. The committee encountered challenges in identifying standardized and consistent data on vitamin D intakes across general populations in the United States and Canada, particularly for population subgroups who may be at risk for inadequate or

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excessive intake. In addition, reliable data on the practice and impact of discretionary fortification on the part of food manufacturers is lacking.

1. **Enhance dietary assessment methods and comparability for calcium and vitamin D intake, and methods for the measurement of calcium and vitamin D in foods and supplements.** Methods related to dietary assessment have come far in recent years, and research in this area should continue. DRI development as it pertains to the North American population would benefit from targeted efforts to strive for comparability between the U.S. and Canadian surveys.
2. **Investigate food and supplement sources of calcium and vitamin D for bioequivalence, bioavailability, and safety.** The ability to assess whether different fortification delivery systems and food production methods affect the factors such as bioavailability or safety for both calcium and vitamin D is an important component of dietary intake assessment. Information on the practice of discretionary fortification by food manufacturers is needed.
3. **Improve the standardization of the assay for serum 25OHD.** Currently, different assays for the determination of serum 25OHD levels are in use, and they provide disparate results. In turn, reported measures are confounded by the need to understand the assay used and research reports contain results that are not readily compared. The role of standard reference materials and interlaboratory collaboration is an important aspect of overcoming the challenges that the assay methodologies present.

RELATED RESEARCH NEED

Clinical practice was outside the scope of this committee convened to develop DRIs, which was tasked primarily with describing a distribution of requirements and upper levels of intake. However, as noted in Chapter 8, the cut-point levels of serum 25OHD intended to specify deficiency and sufficiency for the purposes of interpreting laboratory analyses and for use in clinical practice have been subject to a wide variation in specification without a systematic, evidence-based consensus development process. The importance of this specification to both the well-being of the North American population and to ensuring that the population is confident in their health and nutriture results in the committee calling attention to this research need. Its broad impact requires that it be addressed by a coalition of stakeholders under the auspices of a science-based organization such as the National Institutes of Health in conjunction with equivalent science-based organizations in Canada.

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CONCLUDING REMARKS

The committee found that the greatest information gaps, and thus the most critical research needs, are related to the so-called hazard identification and hazard characterization steps in which the relationship between the nutrient and health outcomes are established. These needs for calcium and vitamin D DRI development relate to further exploring and describing both skeletal as well as non-skeletal health outcomes, long-term adverse effects of high levels of intake, and data to clarify the dose–response to intake. In the case of vitamin D, understanding the impact of sun exposure presents many challenges. Specific to the selected indicator (i.e., bone health), there is a need for more and better data related to the relatively unstudied life stage groups of children and young adults and the differences among racial/ethnic groups. Furthermore, the committee found a pressing public health need for development of consensus, science-based guidelines to establish cut-point levels for vitamin D deficiency and insufficiency.

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A

Acronyms, Abbreviations, and Glossary

ACRONYMS AND ABBREVIATIONS

25-hydroxyvitamin D
In this report, 25OHD (also referred to as calcidiol or calcifediol); indicates no distinction between D_2 and D_3 forms. When relevant, forms distinguished as $25OHD_2$ and $25OHD_3$.

1,25-dihydroxyvitamin D
In this report, calcitriol. Ercalcitriol refers to 1,25-dihydroxyvitamin D_2, but in this report, the term "calcitriol" will be used for both.

24,25-dihydroxyvitamin D
In this report, $24,25(OH)_2D$.

AHRQ	Agency for Healthcare Research and Quality
AI	Adequate Intake
ALTM	All-laboratory trimmed mean
AMDR	Acceptable Macronutrient Distribution Range
ATBC	Alpha-Tocopherol Beta-Carotene Cancer Prevention Study
BDI	Beck Depression Inventory
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BV	Bone volume

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CCHS	Canadian Community Health Survey
CDC	Centers for Disease Control and Prevention
CG	Control group
CHMS	Canadian Health Measures Survey
CI	Confidence interval
CNF	Canadian Nutrient File
CPBA	Competitive protein binding assay
CVD	Cardiovascular disease
CYP	Cytochrome P450
DBP	Vitamin D binding protein
DEQAS	Vitamin D External Quality Assurance Scheme
DNA	Deoxyribonucleic acid
DRI	Dietary Reference Intake
DXA	Dual-energy X-ray absorptiometry
EAR	Estimated Average Requirement
EPIC	European Prospective Investigation into Cancer and Nutrition
EPIDOS	Epidémiologie de l'Ostéoporose study
FGF23	Fibroblast-like growth factor-23
FN	Femoral neck
GC	Gas chromatography
GFR	Glomerular filtration rate
HPFS	Health Professionals Follow-up Study
HR	Hazard ratio
IBD	Inflammatory bowel disease
IFN	Interferon
Ig	Immunoglobulin
IG	Intervention group
IHD	Ischemic heart disease
IL	Interleukin
IOM	Institute of Medicine
iPTH	Intact parathyroid hormone
IU	International Unit
K-MMSE	Mini-Mental State Examination for Koreans

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LC	Liquid chromatography
LOAEL	Lowest-observed-adverse-effect level
LS	Lumbar spine
LSM	Least squares mean
MAS	Milk-alkali syndrome
MMSE	Mini-Mental State Examination
mo	Month(s)
mRNA	Messenger ribonucleic acid
MrOS	Osteoporotic Fractures in Men Study
MS	Mass spectrometry; Multiple sclerosis
MS/MS	Tandem mass spectrometry
NA	Not applicable
NCa	Normocalcemic
NCHS	National Center for Health Statistics
NCI	National Cancer Institute
ND	Not determined
NHANES	National Health and Nutrition Examination Survey
NHS	Nurses' Health Study
NIH	National Institutes of Health
NIST	National Institute of Standards and Technology
NOAEL	No-observed-adverse-effect level
NOD	Nonobese diabetic
NR	Not reported
NS	Not significant
OA	Osteoarthritis
OC	Oral contraceptive
OP	Osteoporosis
OR	Odds ratio
OV	Osteoid volume
PLCO	Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial
PM	Postmenopausal
POMS	Profile of Mood States
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone–related protein
RA	Rheumatoid arthritis
RANK	Receptor activator for nuclear factor κ B

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RCT	Randomized controlled trial
RDA	Recommended Dietary Allowance
RECORD	Randomised Evaluation of Calcium and/Or vitamin D trial
RIA	Radioimmunoassay
RNI	Recommended Nutrient Intake
RR	Relative risk
SD	Standard deviation
SE	Standard error
SEM	Standard error of the mean
SLE	Systemic lupus erythematosus
SPA	Single-photon absorptiometry
SPF	Sun protection factor
SRM	Standard Reference Material
TB	Tuberculosis; Total body
Th	T helper
TH	Total hip
Tr	Trochanter
TRPV6	Transient receptor potential cation channel, vanilloid family member 6
Tx	Treatment
UK	United Kingdom
UL	Tolerable Upper Intake Level
U.S.	United States
USDA	U.S. Department of Agriculture
UV	Ultraviolet
UVB	Ultraviolet B
VDDR	Vitamin D–dependent rickets
VDR	Vitamin D receptor
VDRE	Vitamin D–responsive element
VEGF	Vascular endothelial growth factor
WHI	Women's Health Initiative
WWEIA	What We Eat in America
wk	Week(s)
y	Year(s)

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GLOSSARY

Achlorhydria
A lack of hydrochloric acid in the digestive juices in the stomach.

Adenoma
A benign epithelial tumor of glandular origin.

Adequate Intake
The recommended average daily intake level of a nutrient based on observed or experimentally determined approximations or estimates of intakes that are assumed to be adequate for a group (or groups) of apparently healthy people; used when the Recommended Dietary Allowance cannot be determined.

Adipokines
Cytokines, growth factors, and other proteins produced and secreted by adipose tissue.

Adipose tissue
A connective tissue consisting chiefly of fat cells surrounded by reticular fibers and arranged in lobular groups or along the course of one of the smaller blood vessels.

Amenorrhea
Abnormal suppression or absence of menstruation.

Anorexia
The symptom of poor appetite whatever the cause.

Anorexia nervosa
A psychophysiological disorder usually occurring in teenage women that is characterized by fear of becoming obese, a distorted self-image, a persistent aversion to food, and severe weight loss, and that is often marked by hyperactivity, self-induced vomiting, amenorrhea, and other physiological changes.

Antigen
Any substance that stimulates an immune response in the body.

Antirachitic
Cures or prevents rickets.

Asthma
A chronic inflammatory disease of the airways.

Autism
A complex developmental disability that typically appears during the first few years of life; is the result of a neurological disorder that affects the normal functioning of the brain, impacting development in the areas of social interaction and communication skills.

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Biomarker
: A biochemical, physiological, behavioral, or other alteration that can be measured in the body or its products that influences, predicts, or is associated with an established or possible outcome, health impairment, or disease.

Body mass index
: An indirect measure of body fat calculated as the ratio of a person's body weight to the square of a person's height:
BMI (kg/m^2) = weight (kilograms)/height (meters)^2
BMI (lb/in^2) = weight (pounds)/height (inches)$^2 \times 703$

Bone mineral content
: The hardness of bone results from its mineral content in the organic matrix.

Bone mineral density
: A measure of bone density that reflects the strength of bones as represented by calcium content.

Calcification
: Impregnation with calcium or calcium salts; hardening, as of tissue, by such impregnation.

Calcinosis
: The abnormal deposition of calcium salts in a part or tissue of the body.

Calcitonin
: A peptide hormone, produced by the thyroid gland in humans, that acts to lower plasma calcium and phosphate levels without augmenting calcium accretion.

Calcitriol
: Another name for 1,25-dihydroxyvitamin D.

Calcium
: A mineral found mainly in the hard part of bones, where it is stored; it is essential for healthy bones and is important for muscle contraction, heart action, nervous system maintenance, and normal blood clotting.

Calciuria
: The presence of calcium in the urine.

Cancer
: A malignant and invasive growth or tumor.

Cardiovascular disease
: Any abnormal condition characterized by dysfunction of the heart and blood vessels; includes atherosclerosis (especially coronary heart disease), cerebrovascular disease, and hypertension.

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Chondrocyte
A connective tissue cell that occupies a lacuna within the cartilage matrix.

Chylomicron
One of the microscopic particles of fat occurring in chyle (a digestive fluid) and in the blood, especially after a meal high in fat.

Computed tomography
Tomography used in diagnostic studies of internal bodily structures, in which computer analysis of a series of cross–sectional scans made along a single axis of a bodily structure or tissue is used to construct a three-dimensional image of that structure.

Creatinine
One of the nonprotein constituents of blood, a breakdown product of creatinine (protein used to make adenosine triphosphate). Increased quantities of serum creatinine are found in advanced stages of renal disease.

Crohn's disease
A chronic inflammatory disease of the intestines that primarily causes ulcerations (breaks in the lining) of the small and large intestines, but can affect the digestive system anywhere from the mouth to the anus.

Cut-point
A specified quantitative measure used to demarcate the presence or absence of a health-related condition; often used in interpreting measures obtained from analysis of blood (example: blood measures below "x" ng/mL indicate a deficiency state for Nutrient Y).

Cytochrome
Any of a class of iron-containing proteins important to cell respiration as catalysts of oxidation–reduction reactions.

Depression
A condition of general emotional dejection and withdrawal; sadness greater and more prolonged than that warranted by any objective reason.

Dermis
The sensitive connective tissue layer of the skin located below the epidermis, containing nerve endings, sweat and sebaceous glands, and blood and lymph vessels.

Diabetes mellitus
A group of metabolic diseases characterized by high blood sugar (glucose) levels that result from defects in insulin secretion or action, or both.

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Diabetes, type 1
An autoimmune disease that occurs when T cells attack and decimate the β-cells in the pancreas that are needed to produce insulin, so that the pancreas makes too little insulin (or no insulin); there is a genetic predisposition to type 1 diabetes, and the disease tends to occur in childhood, adolescence, or early adulthood (before age 30), but it may have its clinical onset at any age.

Diabetes, type 2
Disease in which the β-cells of the pancreas produce insulin but the body is unable to use it effectively because the cells of the body are resistant to the action of insulin; also known as insulin-resistant diabetes, non-insulin-dependent diabetes, and adult-onset diabetes.

Dietary Reference Intake
A set of four distinct nutrient-based reference values that replaced the former Recommended Dietary Allowance in the United States. These include Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), Adequate Intake (AI), and Tolerable Upper Intake Level (UL).

Dose–response assessment
Determination of the relationship between nutrient intake (dose) and some criterion of either adequacy or adverse effect.

Dual-energy X-ray absorptiometry
Means of measuring bone density with two X-ray beams with differing energy levels aimed at an individual's bones.

Emesis
The act or process of vomiting.

Endocrine
Pertaining to hormones and the glands that make and secrete them into the bloodstream through which they travel to affect distant organs.

Epidermis
The nonvascular outer protective layer of the skin, covering the dermis.

Ergosterol
A plant sterol that is converted into vitamin D by ultraviolet radiation.

Estimated Average Requirement
The average daily nutrient intake level that is estimated to meet the requirements of half of the healthy individuals in a particular life stage and gender group.

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Estradiol
: The most potent naturally occurring estrogen.

Etiology
: Causes and origins of disease.

Fibroblast
: A cell ubiquitous in connective tissue that makes and secretes collagen.

Glucocorticoid
: Any of a group of steroid-like compounds, such as hydrocortisone, that are produced by the adrenal cortex, are involved in carbohydrate, protein, and fat metabolism, and are used as anti-inflammatory agents.

Hematocrit
: The percentage by volume of packed red blood cells in a given sample of blood after centrifugation.

Homeostasis
: A property of cells, tissues, and organisms that allows the maintenance and regulation of the stability and constancy needed to function properly.

Hormone
: A substance, usually a peptide or a steroid, produced by one tissue and conveyed in the bloodstream to another to effect physiological activity, such as growth or metabolism.

Hydroxyapatite
: The principal bone salt that provides the compressional strength of vertebrate bone.

Hypercalcemia
: A higher than normal level of calcium in the blood.

Hypercalciuria
: Excess calcium in the urine.

Hyperglycemia
: A high blood sugar; an elevated level specifically of the sugar glucose in the blood.

Hypertension/hypertensive
: Systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg.

Hypophosphatemia
: Abnormally low concentrations of phosphates in the blood.

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Inflammatory bowel disease
: Any of several incurable and debilitating diseases of the gastrointestinal tract characterized by inflammation and obstruction of parts of the intestine.

Influenza
: An acute, commonly epidemic disease occurring in several forms, caused by numerous rapidly mutating viral strains and characterized by respiratory symptoms and general prostration.

Ligand
: An ion, a molecule, or a molecular group that binds to another chemical entity to form a larger complex.

LOAEL
: The lowest intake (or experimental dose) of a nutrient at which an adverse effect has been identified.

Lumisterol
: A naturally occurring compound that is part of the vitamin D family of steroid compounds.

Macrophage
: A type of white blood cell that ingests foreign material.

Menopause
: The state of an absence of menstrual periods for 12 months.

Metabolic syndrome
: Also called insulin resistance syndrome and Metabolic Syndrome X. A group of conditions that increase risk of heart disease, diabetes, and stroke. The five conditions are high blood pressure, high blood sugar levels, high levels of circulating triglycerides, low levels of circulating high-density lipoprotein, and excess fat in the abdominal area.

Microsome
: A small particle in the cytoplasm of a cell, typically consisting of fragmented endoplasmic reticulum to which ribosomes are attached.

Milk-alkali syndrome
: Caused by the ingestion of large amounts of calcium and absorbable alkali with resulting hypercalcemia; if untreated, can lead to metastatic calcification and renal failure.

Morbidity
: Illness or disease.

Mortality
: A fatal outcome; death.

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Multiple sclerosis
A disease in which the nerves of the central nervous system (brain and spinal cord) degenerate.

Natriuresis
Excretion of excessive amounts of sodium in the urine.

Neoplasm
A new, often uncontrolled growth of abnormal tissue; tumor.

Nephrocalcinosis
Renal lithiasis characterized by diffusely scattered foci of calcification in the kidneys.

Nephrolithiasis
Calculi in the kidneys.

NOAEL
The highest intake (or experimental dose) of a nutrient at which no adverse effect has been observed.

Nutrient
A substance (such as a chemical element or inorganic compound) that an organism needs to live and grow; a substance used in an organism's metabolism that must be taken in from its environment.

Nutriture
A state of nutrition in the body.

Osteoblast
A cell from which bone develops.

Osteoclast
A large multinucleate cell found in growing bone that resorbs bony tissue, as in the formation of canals and cavities.

Osteocyte
A branched cell imbedded in the matrix of bone tissue.

Osteogenesis
Formation and development of bony tissue.

Osteoid
Resembling bone; the bone matrix, especially before calcification.

Osteomalacia
The softening of bone, the depletion of calcium from bone; may be caused by poor dietary intake or poor absorption of calcium and other minerals needed to harden bones and can be a characteristic feature of vitamin D deficiency in adults.

Osteopenia
A condition of bone in which decreased calcification, decreased density, or reduced mass occurs.

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Osteoporosis
: A condition characterized by a decrease in bone density (a decrease in bone strength that results in fragile bones); leads to abnormally porous bone that is compressible, like a sponge.

Parathyroid gland
: A gland that regulates calcium, located behind the thyroid gland in the neck, which secretes parathyroid hormone.

Parathyroid hormone
: A hormone that is made by the parathyroid gland and that is critical to calcium and phosphorus balance.

Perimenopause
: The interval in which a women's body begins its transition into menopause.

Periosteal
: Pertaining to the periosteum, the membrane covering the bones.

Phosphate
: A form of phosphoric acid; calcium phosphate makes bones and teeth hard.

Polyuria
: The excessive passage of urine, resulting in profuse urination and urinary frequency.

Preeclampsia
: A toxic condition developing in late pregnancy characterized by a sudden rise in blood pressure, generalized edema, proteinuria, severe headache, and visual disturbances that may result in eclampsia (convulsive or coma state) if untreated.

Previtamin D_3
: A short-lived intermediate form arising from exposure of provitamin D_3 (7-dehydrocholesterol) in the skin to UVB irradiation. Body heat quickly changes previtamin D_3 into vitamin D_3.

Prohormone
: An intraglandular precursor of a hormone.

Provitamin D_3 (7-dehydrocholesterol)
: A provitamin present in the skin of humans as well as the milk of mammals that becomes vitamin D_3 when exposed to ultraviolet light.

Recommended Dietary Allowance
: The average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97.5 percent) healthy individuals in a particular life stage and gender group.

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Rheumatoid arthritis
An autoimmune disease that causes chronic inflammation of the joints.

Rickets
A disorder caused by a deficiency of vitamin D, calcium, or phosphate, which leads to softening and weakening of the bones and is seen most commonly in children 6 to 24 months of age.

Sarcoidosis
A disease that results from a specific type of inflammation of tissues of the body that can appear in almost any body organ, often starting in the lungs or lymph nodes.

Scleroderma
A pathological thickening and hardening of the skin caused by swelling and thickening of fibrous tissue.

Systemic lupus erythematosus
A chronic, autoimmune, inflammatory disease of connective tissue that causes fever, weakness, fatigue, joint pains, and skin lesions on the face, neck, or arms.

Tachysterol
An isomer of ergosterol that forms vitamin D_2 when irradiated with ultraviolet light.

Tolerable Upper Intake Level
The highest average daily nutrient intake level that is likely to pose no risk of adverse effects to almost all individuals in the general population. As intake increases above the Tolerable Upper Intake Level, the potential risk of adverse effects may increase.

Transgenic
Having genetic material (deoxyribonucleic acid) from another species.

Tuberculosis
A highly contagious infection caused by the bacterium called *Mycobacterium tuberculosis.*

Ultraviolet
Pertaining to electromagnetic radiation having wavelengths in the range of approximately 5 to 400 nm; shorter than visible light, but longer than X-rays.

Ultraviolet B
Medium wavelength (280 to 320 nm) ultraviolet rays from the sun; help synthesis of vitamin D_3; the "burning" rays in the ultraviolet spectrum.

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Vasodilatation
: Relaxation or widening of the blood vessels; leads to a lowered blood pressure.

Vitamin D
: Also referred to as calciferol; comprises a group of fat-soluble secosterols. The two major forms are vitamin D_2 and vitamin D_3 (both vitamin D_2 and vitamin D_3 can be synthesized commercially and may be found in dietary supplements or fortified foods; they differ only in their side chain structure).

Vitamin D_2
: Also referred to as ergocalciferol; originates from plants and is found in the human diet.

Vitamin D_3
: Also referred to as cholecalciferol; is synthesized in the skin of humans from 7-dehydrocholesterol and is also consumed in the diet via the intake of animal-based foods.

Vitamin D–resistant rickets
: An inherited form of rickets characterized by high concentrations of phosphate in the blood due to defective renal tubular reabsorption of phosphate and subnormal absorption of dietary calcium.

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B

Issues and Interests Identified by Study Sponsors

1. For a reference value related to adequate intakes, particular consideration should be given to the selection of indicators of adequacy for the various age, gender, and life stage groups that will allow for the determination of the type of reference value of highest relevance to the needs of the sponsors. Such needs are most readily met in the case of vitamin D and calcium by the establishment of an Estimated Average Requirement[1] (EAR). The EAR is useful because it is the best type of reference value for assessing the adequacy of estimated nutrient intakes of groups and for planning intakes for groups. It is also the most useful type of reference value when planning and assessing total diets. These are necessary and primary applications by the study sponsors.

2. For reference values related to excessive intakes, Tolerable Upper Intake Levels (UL) for the various age, gender, and life stage groups are needed. Efforts should be made to examine if a critical adverse effect can be selected, which will allow for the determination of a Benchmark Intake[2] (aka "Benchmark Dose").

3. In determining the reference values for vitamin D, confounding factors are important considerations, notably those that affect the DRI population groups such as latitude, sun exposure, skin pigmentation, vi-

[1]Estimated Average Requirement: The average daily nutrient intake level that is estimated to meet the requirements of half of the healthy individuals in a particular life stage and gender group (*Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*, IOM [2006]).

[2]Benchmark Intake: The intake of a substance that is expected to result in a prespecified level of effect. (*A Model for Establishing Upper Levels of Intake for Nutrients and Related Substances*, [WHO/FAO]).

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tamin D stores, and obesity will be considered. In addition, specification as to whether the EAR and UL are related to lean body mass or to energy intake would be useful, as data allow.

4. The target population of interest for the reference values are the people residing in the United States and Canada, including those whose needs for or sensitivity to vitamin D or calcium may be affected by particular conditions such as obesity or oral contraceptive use; those with highly pigmented skin; those with risk factors for chronic disease; and those with chronic or other diseases that do not alter their requirements for or sensitivity to vitamin D or calcium. For vitamin D, the target population may also include subgroups within the general population whose requirements for vitamin D intakes may need to be considered within the context of limited endogenous synthesis or differences in metabolic handling of vitamin D (e.g., limited sun exposure because of latitude, clothing, institutionalization, dark skin pigmentation; older persons with reduced capacity for dermal synthesis; racial/ethnic differences in metabolic handling of these nutrients). In deriving the reference values, it is useful if the relevance of study populations found in the literature is considered relative to the target population. It is also important to identify as data allow the special populations whose nutrient requirements or sensitivities differ from the general population as described above for whom DRI values are derived (e.g., diseased persons, persons using drugs known to alter the nutrient requirements or safety profiles).

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C

Methods and Results from the AHRQ-Ottawa Evidence-Based Report on Effectiveness and Safety of Vitamin D in Relation to Bone Health

The purpose of this systematic evidence-based review, referred to as AHRQ-Ottawa,[1] requested by the Office of Dietary Supplements, National Institutes of Health and conducted by the University of Ottawa Evidence-based Practice Center (UO-EPC) was to review and synthesize the published literature on five key questions.

1. Are specific circulating concentrations of 25 hydroxyvitamin D (25[OH]D) associated with bone health outcomes in:
   A. Children: rickets, bone mineral density (BMD), bone mineral content (BMC), fractures, or parathyroid hormone (PTH)?
   B. Women of reproductive age (including pregnant and lactating women): BMD, calcaneal ultrasound, fractures, PTH?
   C. Elderly men and postmenopausal women: BMD, fractures, falls?
2. Do food fortification, sun exposure, and/or vitamin D supplementation affect circulating concentrations of 25(OH)D?
3. What is the evidence regarding the effect of supplemental doses of vitamin D on bone mineral density and fracture or fall risk and does this vary with age groups, ethnicity, body mass index, or geography?

[1] Cranney, A., T. Horsley, S. O'Donnell, H. A. Weiler, L. Puil, D. S. Ooi, S. A. Atkinson, L. M. Ward, D. Moher, D. A. Hanley, M. Fang, F. Yazdi, C. Garritty, M. Sampson, N. Barrowman, A. Tsertsvadze and V. Mamaladze. 2007. Effectiveness and Safety of Vitamin D in Relation to Bone Health. Evidence Report/Technology Assessment No. 158. (Prepared by the University of Ottawa Evidence-based Practice Center (UO-EPC) under Contract No. 290-02-0021.) AHRQ Publication No. 07-E013. Rockville, MD: Agency for Healthcare Research and Quality.

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4. Is there a level of sunlight exposure that is sufficient to maintain adequate vitamin D levels but does not increase the risk of non-melanoma or melanoma skin cancer?
5. Does intake of vitamin D above current reference intakes lead to toxicities (e.g., hypercalcemia, hypercalciuria, and calcification of soft tissue or major organs)?

The review focused on electronic searches of the medical literature to identify publications addressing the aforementioned questions. Out of 9,150 citations, 112 RCTs, 19 prospective cohorts, 30 case–control studies, and 6 before-after studies were systematically reviewed, and each was rated on quality and used to assess the strength of evidence for each outcome.

The methods and results chapters of the AHRQ-Ottawa evidence review are reprinted below. The report in its entirety, including appendices and evidence tables, can be accessed and viewed at http://www.ahrq.gov/clinic/tp/vitadtp.htm#Report.

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D

Methods and Results from the AHRQ-Tufts Evidence-Based Report on Vitamin D and Calcium

The purpose of this systematic evidence-based review, referred to as AHRQ-Tufts,[1] requested by the Office of Dietary Supplements/National Institutes of Health, the Public Health Agency of Canada, Health Canada, and the Food and Drug Administration and conducted by the Tufts Evidence-based Practice Center (EPC), was to answer key scientific questions on how dietary vitamin D and calcium intake effect health outcomes. The key questions addressed in the AHRQ-Tufts reports are as follows:

Key Question 1. What is the effect of vitamin D, calcium, or combined vitamin D and calcium intakes on clinical outcomes, including growth, cardiovascular diseases, body weight outcomes, cancer, immune function, pregnancy or birth outcomes, mortality, fracture, renal outcomes, and soft tissue calcification?

Key Question 2. What is the effect of vitamin D, calcium, or combined vitamin D and calcium intakes on surrogate or intermediate outcomes, such as hypertension, blood pressure, and bone mineral density?

[1] Chung, M., E. M. Balk, M. Brendel, S. Ip, J. Lau, J. Lee, A. Lichtenstein, K. Patel, G. Raman, A. Tatsioni, T. Terasawa and T. A. Trikalinos. 2009. Vitamin D and Calcium: A Systematic Review of Health Outcomes. Evidence Report No. 183. (Prepared by the Tufts Evidence-based Practice Center under Contract No. HHSA 290-2007-10055-I.) AHRQ Publication No. 09-E015. Rockville, MD: Agency for Healthcare Research and Quality.

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Key Question 3. What is the association between serum 25(OH)D concentrations or calcium balance and clinical outcomes?

Key Question 4. What is the effect of vitamin D or combined vitamin D and calcium intakes on serum 25(OH)D concentrations?

Key Question 5. What is the association between serum 25(OH)D concentrations and surrogate or intermediate outcomes?

The review focused on electronic searches of the medical literature (1969–April 2009) to identify publications addressing the aforementioned questions. One hundred and sixty-five primary articles and 11 systematic reviews that incorporated more than 200 additional primary articles were systematically reviewed, and each was rated on quality and used to assess the strength of evidence for each outcome.

The methods and results chapters of the AHRQ-Tufts evidence review are reprinted below. The report in its entirety, including appendices and evidence tables, can be accessed and viewed at http://www.ahrq.gov/clinic/tp/vitadcaltp.htm.

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E

Literature Search Strategy

In order to review the most relevant scientific literature available, the committee and staff regularly conducted thorough searches of several online bibliographic data bases, including Medline, Science Direct, and WorldCat/First Search. General searches on vitamin D, calcium, and health outcomes were first conducted to identify primary literature. Using the results of the primary search, key search terms were developed and secondary searches were then conducted. Search terms were also chosen based on relevance to the report outline and topics included in the previous IOM report (IOM, 1997). Although initial searches were general, subsequent searches focused on retrieving studies that were not covered by the evidence-based reviews conducted by AHRQ-Ottawa (Cranney et al., 2007) and AHRQ-Tufts (Chung et al., 2009). Similar to the methodology used by the AHRQ reports searches were limited to English. As the study progressed, focused searches were conducted as needed and general searches were carried out to identify newly published articles. See Box E-1 for an example of how searches were conducted.

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TABLE E-1 Sample Search History for Literature Published After AHRQ-Tufts

Number	Searches	Results
1	exp Vitamin D/	34296
2	(25-hydroxy vit D or plasma vit D or 25OHD or 25-OHD)	956
3	(25OHD3 or "25(OH)D3" or 25-OHD3 or "25-(OH)D3").tw.	1205
4	("25(OH)D" or "25-(OH)D" or "25-OH-D").tw.	1696
5	25-hydroxycholecalciferol.tw.	877
6	25-hydroxyergocalciferol.tw.	27
7	calcidiol.tw.	227
8	Calcifediol/	2443
9	(vit adj (d or d2 or d3)).mp.	244
10	Ergocalciferols/	2050
11	Ergocalciferol$.tw.	322
12	Cholecalciferol/	4499
13	Cholecalciferol$.tw.	1172
14	calciferol.tw.	363
15	or/1-14	35253
16	exp Calcium/	218026
17	exp Calcium Carbonate/ or exp Calcium Citrate/ or exp Calcium Phosphates/ or exp Calcium Malate/	24264
18	exp Calcium, Dietary/ Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1950 to Present> (7533) EBM Reviews—Cochrane Central Register of Controlled Trials <3rd Quarter 2009> (515) Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1950 to Present> (7533) EBM Reviews—Cochrane Central Register of Controlled Trials <3rd Quarter 2009> (515)	8048
19	calcium.tw.	250569
20	or/16-19	373131
21	15 or 20	392115
22	21 and (200905* or 200906* or 200907* or 200908*).ed.	3840
23	limit 22 to english language [Limit not valid in CCTR; records were retained]	3544
24	limit 23 to humans [Limit not valid in CCTR; records were retained]	1906

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TABLE E-1 Continued

Number	Searches	Results
25	limit 24 to (addresses or bibliography or biography or comment or congresses or consensus development conference or consensus development conference, nih or dictionary or directory or duplicate publication or editorial or in vitro or interview or lectures or letter or news or newspaper article or "review") [Limit not valid in CCTR; records were retained]	443
26	24 not 25	1463
27	randomized controlled trial.pt.	537352
28	controlled clinical trial.pt.	156124
29	randomized controlled trials/	62621
30	Random Allocation/	85834
31	Double-blind Method/	187602
32	Single-Blind Method/	21457
33	clinical trial.pt.	729105
34	Clinical Trials.mp. or exp Clinical Trials/	268451
35	exp Clinical Trial/	587643
36	(clinic$ adj25 trial$).tw.	218792
37	((singl$ or doubl$ or trebl$ or tripl$) adj (mask$ or blind$)).tw.	214271
38	Placebos/	46595
39	placebo$.tw.	225224
40	random$.tw.	736338
41	trial$.tw.	569938
42	(latin adj square).tw.	3500
43	Comparative Study.tw.	55680
44	exp Evaluation studies/	140739
45	Follow-Up Studies/	421249
46	Prospective Studies/	316292
47	(control$ or prospectiv$ or volunteer$).tw.	2441089
48	Cross-Over Studies/ Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1950 to Present> (24638) EBM Reviews—Cochrane Central Register of Controlled Trials <3rd Quarter 2009> (18713) Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1950 to Present> (24638) EBM Reviews—Cochrane Central Register of Controlled Trials <3rd Quarter 2009> (18713)	43351

continued

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TABLE E-1 Continued

Number	Searches	Results
49	or/27-48	3820192
50	49 and 26	551
51	21	392115
52	limit 51 to english language [Limit not valid in CCTR; records were retained]	351480
53	limit 52 to yr="2009—2010" Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1950 to Present> (8085) EBM Reviews—Cochrane Central Register of Controlled Trials <3rd Quarter 2009> (89) Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1950 to Present> (8085) EBM Reviews—Cochrane Central Register of Controlled Trials <3rd Quarter 2009> (89)	8174
54	limit 53 to humans [Limit not valid in CCTR; records were retained] Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1950 to Present> (2956) EBM Reviews—Cochrane Central Register of Controlled Trials <3rd Quarter 2009> (89) Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1950 to Present> (2956) EBM Reviews—Cochrane Central Register of Controlled Trials <3rd Quarter 2009> (89)	3045
55	remove duplicates from 54 Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1950 to Present> (2751) EBM Reviews—Cochrane Central Register of Controlled Trials <3rd Quarter 2009> (3) Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1950 to Present> (2751) EBM Reviews—Cochrane Central Register of Controlled Trials <3rd Quarter 2009> (3)	2754

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REFERENCES

Chung M., E. M. Balk, M. Brendel, S. Ip, J. Lau, J. Lee, A. Lichtenstein, K. Patel, G. Raman, A. Tatsioni, T. Terasawa and T. A. Trikalinos. 2009. Vitamin D and Calcium: A Systematic Review of Health Outcomes. Evidence Report No. 183. (Prepared by the Tufts Evidence-based Practice Center under Contract No. HHSA 290-2007-10055-I.) AHRQ Publication No. 09-E015. Rockville, MD: Agency for Healthcare Research and Quality.

Cranney A., T. Horsley, S. O'Donnell, H. A. Weiler, L. Puil, D. S. Ooi, S. A. Atkinson, L. M. Ward, D. Moher, D. A. Hanley, M. Fang, F. Yazdi, C. Garritty, M. Sampson, N. Barrowman, A. Tsertsvadze and V. Mamaladze. 2007. Effectiveness and Safety of Vitamin D in Relation to Bone Health. Evidence Report/Technology Assessment No. 158. (Prepared by the University of Ottawa Evidence-based Practice Center (UO-EPC) under Contract No. 290-02-0021.) AHRQ Publication No. 07-E013. Rockville, MD: Agency for Healthcare Research and Quality.

IOM (Institute of Medicine). 1997. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington, DC: National Academy Press.

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F

Evidence Maps

As part of the committee's approach to the evaluation of data, evidence maps were developed to assist the committee in organizing its review of the available evidence for each indicator of interest. No effort was made to "fine tune" these maps, but rather they were an initial tool used by the committee to quickly summarize the nature of the available data. The evidence maps below show the evidence from mechanistic and animal data, observational studies, and randomized controlled trials (RCTs). In its evaluation of RCT evidence, the committee considered the study design and whether an indicator was pre-specified as a primary or secondary outcome. Overall, the committee evaluated individual studies and when needed, took methodological differences into account when categorizing a study for inclusion in the evidence maps. However the committee acknowledges the variability in the quality of studies within and across categories, and relied on its expert judgment and a detailed evaluation of each study when drawing conclusions about the relevance and application of an indicator to further consideration in the DRI process. Chapter 4 provides detailed text on each of the key studies that were considered by the committee for the selection of an indicator.

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TABLE F-1 Evidence Map for Cancer/Neoplasms

Indicator	Mechanistic Data	Animal Data	Observational Studies	Randomized Trials: Primary Outcome	Randomized Trials: Secondary or Non–Pre-specified Outcomes[a]
Calcium					
All cancers	✓	✓	✓	✓[b]	✓[b]
Breast cancer	—	—	✓	—	—
Colorectal cancer	✓	✓	✓	✓[b]	✓[b]
Colorectal adenoma	✓	✓	✓	✓	—
Prostate cancer	—	✓	✓	—	—
Vitamin D					
All cancers	✓	✓	✓	—	✓[b]
Breast cancer	—	—	✓	—	✓[b]
Colorectal cancer	✓	✓	✓	—	✓[b]
Colorectal adenoma	✓	✓	✓	—	✓[b]
Prostate cancer	—	—	✓	—	—

NOTE: ✓ = evidence is published; — = no available evidence.
[a]Secondary outcomes often were not pre-specified by the investigators.
[b]Limited data.

TABLE F-2 Evidence Map for Cardiovascular Diseases (CVD)/ Hypertension

Indicator	Mechanistic Data	Animal Data	Observational Studies	Randomized Trials: Primary Outcome	Randomized Trials: Secondary or Non–Pre-specified Outcome[a]
Calcium	✓	✓	✓	—	✓[b]
Vitamin D	✓	✓	✓	—	✓[b]

NOTE: ✓ = evidence is published; — = no available evidence.
[a]Secondary outcomes often were not pre-specified by the investigators.
[b]Limited data.

TABLE F-3 Evidence Map for Diabetes (Type 2) and Metabolic Syndrome

Indicator	Mechanistic Data	Animal Data	Observational Studies	Randomized Trials: Primary Outcome	Randomized Trials: Secondary or Non–Pre–specified Outcomes[a]
Vitamin D	✓	✓	✓	✓	✓

NOTE: ✓ = evidence is published; — = no available evidence.
[a]Secondary outcomes often were not pre-specified by the investigators.

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TABLE F-4 Evidence Map for Falls and Physical Performance

				Randomized Trials	
Indicator	Mechanistic Data	Animal Data	Observational Studies	Primary Outcome	Secondary or Non–Pre-specified Outcomes[a]
Calcium	✓	✓	✓	✓	✓
Vitamin D	✓	✓	✓	✓	✓

NOTE: ✓ = evidence is published; — = no available evidence.
[a]Secondary outcomes often were not pre-specified by the investigators.

TABLE F-5 Evidence Map for Immune Function

				Randomized Trials	
Indicator	Mechanistic Data	Animal Data	Observational Studies	Primary Outcome	Secondary or Non–Pre-specified Outcomes[a]
Vitamin D					
Asthma	✓	✓	✓	—	—
Diabetes (Type 1)	✓	✓	✓	—	—
Irritable bowel and crohn's disease	✓	✓	✓	—	—
Multiple sclerosis	✓	✓	✓	—	—
Rheumatoid arthritis	✓	—	✓	—	—
Systemic lupus erythematosus	✓	✓	✓	—	—
Tuberculosis	✓	✓	✓	✓	✓
Influenza/ Upper respiratory infections	✓	✓	✓	✓	—

NOTE: ✓ = evidence is published; — = no available evidence.
[a]Secondary outcomes often were not pre-specified by the investigators.

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TABLE F-6 Evidence Map for Neuropsychological Functioning

				Randomized Trials	
Indicator	Mechanistic Data	Animal Data	Observational Studies	Primary Outcome	Secondary or Non–Pre-specified Outcomes[a]
Vitamin D					
Autism	✓	✓	✓	—	—
Cognitive Function	✓	✓	✓	—	—
Depression	✓	—	✓	✓	—

NOTE: ✓ = evidence is published; — = no available evidence.
[a]Secondary outcomes often were not prespecified by the investigators.

TABLE F-7 Evidence Map for Preeclampsia of Pregnancy

				Randomized Trials	
Indicator	Mechanistic Data	Animal Data	Observational Studies	Primary Outcome	Secondary or Non–Pre-specified Outcomes[a]
Calcium	—	—	✓	✓	✓
Vitamin D	—	—	✓	—	—

NOTE: ✓ = evidence is published; — = no available evidence.
[a]Secondary outcomes often were not pre-specified by the investigators.

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TABLE F-8 Evidence Map for Bone Health

Indicator	Mechanistic Data	Animal Data	Observational Studies	Randomized Trials: Primary Outcome	Randomized Trials: Secondary or Non–Pre-specified Outcomes[a]
Calcium absorption	✓	✓	✓	✓	✓
Calcium balance	✓	✓	✓	✓	✓
BMD/BMC	✓	✓	✓	✓	✓
Fracture risk	✓	✓	✓	✓	✓
Osteomalacia/ rickets	✓	✓	✓	✓	✓

Intermediate	Mechanistic Data	Animal Data	Observational Studies	Randomized Trials: Primary Outcome	Randomized Trials: Secondary or Non–Pre-specified Outcomes[a]
25OHD	✓	✓	✓	✓	✓
PTH	✓	✓	✓	✓	✓

NOTE: Data may reflect studies designed to study combined calcium and vitamin D administration, calcium alone, or vitamin D alone. ✓ = evidence is published; — = no available evidence.

[a]Secondary outcomes often were not pre-specified by the investigators.

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G

Cases Studies of Vitamin D Toxicity

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TABLE G-1 Case Studies of Vitamin D Toxicity

Study	Patient/Population	Preparation; Dose	Duration
Children			
Djamil and Tu-Tunji. 1931. Lancet letter to the editor	2-yr-old male	Vigantol (irradiated ergosterol); 3 tsp	1 d
1947. BMJ letter to editor	Not specified	Cod liver oil	
Ross. 1952. Journal of Pediatrics :815-822	4 infants ages 8-14 mo	Irradited ergosterol containing an estimated 30,000–40,000 IU vitamin	Daily for 8-12 mo
Jacqz et al. 1985	Infants with hypercalcemia (2 cases with vitamin D toxicity) Case 1: 3 mo old Case 2: 7 mo old	Vitamin D and calcium supplementation 300 μg D_3	
Besbas et al. 1989. Turkish J Pediatrics 31:239-244	Case 1: 3 mo old	Vitamin D: 45,000 IU/d	45 d
	Case 2: 4 mo old	Vitamin D: 60,000 IU/d	30 d
Dent. 1964. BMJ letter to editor	6 yr old	Vitamin D (Calciferol Tablets B.P.): 1.25 mg. (~50,000 IU)/d	9 mo
Counts et al. 1975. Ann Internal Med 82:196-200	4-yr-old male	Vitamin D_2 (Drisdol): 50,000 up to 100,000 IU/d	2 mo following bilateral nephrectomy
DeWind. 1960. Arch Dis Child 36:373-380	5.5 yr old	Vitamin D: 100,000 IU + cod liver oil-2 T + multivitamin	daily × 2–3 mo; and continued intake of tx vitamin D for 1 yr after hospitalization
Barrueto et al. 2005. Pediatrics 116:e453-e456	2-yr-old male	Vitamin D (ergocalciferol): 2,400,000 IU	4 d

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Serum Calcium	Serum 25(OH)D	Symptoms/Health Effects
		Edema and albuminuria
		Response from editor: A toxic dose of more than 200,000 units would only be achieved with ingestion of 2.65 L cod liver oil/d
18–19 mg/dL		All presented with anorexia, weight loss, weakness; 2 infants recovered within 6–9 mo following removal of vitamin D; 2 infants died: autopsy showed fibrotic changes in vascular tissue, calcification of other tissues was noted, particularly lung
		Both cases presented with anorexia, diarrhea, and vomiting
10.5 mg/dL 10.5 mg/dL	129 ng/ml 126 ng/ml	
19.5 mg/dL 17.6 mg/dL		Calcium phosphate crystals in urine; bilateral medullary nephrocalcinosis; vomiting and lethargy; both pts recovered without incident
		Extreme thirst, hypercalcemia, symptoms of diabetes insipidus
17.2 mg/dL	635 ng/ml	Leg pain, cessation of growth resulting from bone resorption; serum calcium, accompanied by nausea and vomiting. Tx with Ca-free dialysate failed to reduce serum Ca; prednisolone for 7 d; calcitonin tx stabilized serum Ca
17 mg/dL		Nausea and non-tender lumps over both tibias; X-rays showed alternating patterns of increased and decreased bone density. Loss of bone density and tissue calcification continued despite removal of vitamin D and the pt died
14.4 mg/dL	470 ng/ml	Constipation and colic; persistent hypertension; no renal, cardiac, neurological symptoms noted. Acute toxicity treated with furosemide, calcitonin, and hydrocortisol

continued

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TABLE G-1 Continued

Study	Patient/Population	Preparation; Dose	Duration
Adults			
Puig. 1998. Ann Internal Med 128(7):601-602	66-yr-old female	Vitamin D: 200 IU + 1,000 mg calcium/twice daily	3 yr
Rizzoli et al. 1994. Bone 15:193-198	7 adults ages 55–84	Vitamin D_3: 30,000–60,000 IU/d	3 weeks to 7.5 yr
Davies and Adams. 1976. The Lancet	Case 1: 59-yr-old female post-thyroidectomy for 40 yr	Vitamin D: 50,000–100,000 IU/d	>30 yr
	Case 2: 71-yr-old female with Paget's disease	150,000 IU/d	7 yr
	Case 3: 51-yr-old female	100,000 IU/d	10 yr
1950. BMJ letter to editor		Vitamin D_2: 100,000 IU/d	3 weeks
Streck et al. 1979. Arch Intern Med 139:974-977	49-yr-old female post-thyroidectomy	Vitamin D: 100,000 units/d; plus high calcium diet	3.8 yr
Sterling and Rupp. 1967. Acta Endocrinologica 54:380-384	69-yr-old male with carcinoma of the larynx	Vitamin D (Calciferol): 100,000 units/d	3 weeks
Aub. 1951. Amer Prac 2(11):976-981	59-yr-old female	Vitamin D: 150,000 units/d	6–8 weeks
Vieth et al. 2002. Lancet 359:672	29- and 63-yr-old related males	Vitamin D poisoning: 12.6 mg D_3/g crystalline sugar (~1,700,000 IU/d)	7 mo

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Serum Calcium	Serum 25(OH)D	Symptoms/Health Effects
4.04 mmol/L (16.2 mg/dL)	696 nmol/L (278.8 ng/ml)	Anemia and dehydration; toxicity treated with milk-free diet
3.30 mmol/L (mean) (13.2 mg/dL) (range = 2.52–4.59 mmol/L) (10.8–18.4 mg/dL)	710 nmol/L (mean) (284.5 ng/ml) (range = 221–1692 nmol/L) (88.5–677.9 ng/ml)	Asthenia, anorexia, nausea, polydipsia, polyuria; hypercalciuria; PTH levels were low normal. Discontinuation of vitamin D normalized calcemia in 3 d and calcidiol levels in 3 mo; bisphosphonate was used to inhibit bone resorption
3.1 mmol/L (12.4 mg/dL		Pts reported nausea, vomiting; case 3 had extensive arterial and ligamentous calcification; tx with corticosteroids and withdrawal of vitamin D
4.5 mmol/L (18 mg/dL)	450 nmol/L (180.3 ng/ml)	
3.75 mmol/L (15 mg/dL)	400 nmol/L (160.3 ng/ml)	
		Pt reported feeling well. Response from editor: Feeling well occurs early in toxicity. Toxic dose varies from 200,000–400,000 IU daily for 10 d.
12.8 mg/dL; (Urinary calcium: 493–600 mg/24 hr)	283 ng/mL	Tx with prednisone resolved hypercalcemia via inhibition of bone resorption of calcium
3.8–5.1 mEq/L (15.2–20.4 mg/dL)		Nausea, anorexia, polyuria that progressed to dehydration and coma. Removal of vitamin D and tx with corticosteroids resolved elevated calcium and CV abnormality
14.3 mg/dL		Weight loss, memory loss; evidence of renal damage and corneal calcification. Tx not discussed
3.82 mmol/L (15.3 mg/dL)	1,555 nmol/L (623 ng/ml)	Anorexia, fever, chills, vomiting, increased thirst; 5 kg weight loss; conjunctivitis, acute renal failure, PTH <1 pmol/L. Tx with IV hydrocortisone, sodium phosphate, and pamidronic acid; both patients survived.

continued

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TABLE G-1 Continued

Study	Patient/Population	Preparation; Dose	Duration
Lilienfeld-Toal et al. 1978. Klin Wschr 56:715-717	70 yr old	Vitamin D_3: 15 mg/d	3 weeks
Selby et al. (1995)	6 patients (most were hypoparathyroid)	2.5–5.0 mg/d, (80,000 IU to 200,000 IU D_2)/d	2–13 yr
Irnell (1969) Acta Med Scand. 185:147-152, 1969)	34-yr old	270,000 IU/d 45,000 IU/d	10 d 6 yr
Accidental or Industrial Poisoning			
Scanlon et al. 1995. Am J Public Health 85:1418-1422	234 survey respondents	Milk over-fortified with vitamin D at 70–600X concentration; (>50 IU/100 g)	Intake range: (oz/d) < 5.5 5.5–11.0 11.1–19.6 ≥ 19.7
Blank et al. 1995. Am J Public Health 85:656-659	Hospital discharge, lab, and health dept data from cases of hypervitaminosis D	Milk over-fortified with vitamin D + other risk factors, i.e., use supplements; sun sensitivity, history of cancer	~3 yr
Jacobus et al. 1992. New Engl J Med 326:1173-1177	8 individuals ages 8 mo to 82 yr consumed milk excessively fortified with vitamin D	Milk over-fortified with cholecalciferol at concentrations of 396,400 and 376,800 IU/ml	Variable exposure
Thomson and Johnson. 1986. Postgrad Med J 62:1025-1028	7 family members; 3 adults and 4 children ages 1.5 to 14 yr	Unknown food source containing excessively high vitamin D	Single exposure
Pettifor et al. 1995. Ann Intern Med 122:511-513	10 family members and 1 servant; age range 8-69 yr ingested oil containing a veterinary vitamin D concentrate	Cholecalciferol concentrate in peanut oil = 2 million U/g	Unknown exposure

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Serum Calcium	Serum 25(OH)D	Symptoms/Health Effects
6.1 mval/L	498 nmol/L (200 ng/ml)	Fatigue and psychotic symptoms; no evidence of 2° osteoporosis was found. Tx with vitamin D was interrupted; the increased body pool of calcium returned to normal when serum vitamin D levels decreased to 200 ng/ml
3.26 mmol/L (mean) (13.04 mg/dL)	842 nmol/L (mean) (337.3 ng/ml)	Admitted for hypercalcemia; renal failure
6.6 mEq/L 8.5–9.6 mEq/L		Patient exhibited symptoms of toxicity (tiredness, vomiting, diarrhrea, polyuria, weight loss, muscular weakness, headache) at 45,000 IU/d
mean (mg/dL) 2.4 2.3 2.4 2.4	mean (ng/ml) 32.8 39.5 41.3 44.7	Linear regression model showed a 1 oz increase in milk intake was associated with 1.39 ng/ml increase in serum 25(OH)D. No association was found between milk intake and elevated serum calcium; there was an association with elevated serum 25(OH) D and urinary calcium
13.1 mg/dL (mean for 35 cases)	224 ng/ml (mean for 35 cases)	Consumption of milk from sources other than the over-fortified milk was not associated with hypervitaminosis D
7 of 8 had hypercalcemia; 1 had hypercalcuria with normocalcemia	Mean for all cases: 731 ± 434 nmol/L (293 ± 174 ng/ml)	Vitamin D_3 concentrate in milk that was up to 580 times in excess resulted in elevated serum vitamin D_3, but not D_2 in consumers. All consumers of the milk had elevated 25(OH)D levels and most had hypercalcemia
2.72– 4.08 nmol/L (10.9–16.3 mg/ dL)	832–1,287 nmol/L (333.0– 515.6 ng/ml)	Serum calcium levels returned to normal within 24 d but 25(OH)D levels remained elevated for 1 yr; 1,25 (OH)D was not significantly elevated in the adults
3.46–4.61 nmol/L (13.8–18.4 mg/ dL)	847–1,652 nmol/L (339.3– 661.9 ng/ml)	Cholecalciferol poisoning did not elevate total 1-25 $(OH)_2D$ in 8 and only marginally in 3 of intoxicated patients; but did elevate free 1-25 $(OH)_2D$ in all

continued

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TABLE G-1 Continued

Study	Patient/Population	Preparation; Dose	Duration
Hodges. 1985. British Med J 290:748-749.	32-yr-old male working with crystalline vitamin D in a laboratory setting	Unknown exposure	Intermittent exposure: 32 d in 1981; 11 d in 1982; 22 d in 1983
Klontz. 2007. New Engl J Med 357:308-309	58-yr-old female diagnosed with diabetes and rheumatoid arthritis	Vitamin D_3 overdose in a supplement; 186,906 IU/6 capsules	~2 mo
Down et al. 1979. Postgrad Med J 55:897-902	3 family members; 2 adults ages 24 yr and 1 infant aged 11 mo	Cholecalciferol concentrate in nut oil = 5 million IU/ml	Single exposure
Chiricone et al. 2003. J Nephrol 15:917-921	Case reports:	Multivitamin preparation per injection; 100,000 IU vitamin D/vial	
	62-yr-old male		3 vials/d per 20 d/3 mo: total exposure estimate = 18,000,000 IU
	55-yr-old female		3 vials/d per 20 d/1.5 mo total exposure estimate = 9,000,000 IU

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Serum Calcium	Serum 25(OH)D	Symptoms/Health Effects
3.5–3.7 mmol/L (~14 mg/dL)	496 ng/ml	Polydypsia, polyuria, anorexia, nausea; tx with IV saline, furosemide; hydrocortisone
3.75 mmol/L (15 mg/dL)	1,171 nmol/L (469.2 ng/ml)	Fatigue, constipation, back pain, forgetfulness, nausea, vomiting; tx with IV saline, furosemide, and pamidronate
3.95 mmol/L (15.8 mg/dL)	58–60 IU/ml (145–150 ng/ml)	Both adults developed renal failure. The female aborted a 10-week fetus at 3 weeks post-diagnosis for hypervitaminosis D. Plasma vitamin D levels were 60 IU/ml 5 weeks post-diagnosis; nephrocalcinosis persisted in the adult male but neither had long-term renal impairment
(mean for adults at 5 weeks post-exposure)	(5 weeks post-exposure)	
15.3 mg/dL	>150 ng/ml	Renal colic, confusion, lethargy, and weakness; reported passing small stones; tx with IV saline, furosemide, glucocorticoids
11.3 mg/dL	>150 ng/ml	

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H

Estimated Intakes of Calcium and Vitamin D from National Surveys

UNITED STATES

In order to assist the committee, staff from the National Cancer Institute (NCI), National Institutes of Health (NIH), provided more detailed information regarding calcium and vitamin D intakes among the U.S. population than is available from Bailey et al. (2010).[1] The data tables provided are included in this appendix (Tables H-1 through H-4). The data are based on the 2005–2006 reports from the National Health and Nutrition Examination Survey (NHANES), specifically What We Eat in America,[2] but incorporate (1) information on supplement use to allow the estimation of a total intake; (2) a shrinkage estimator approach rather than a Monte Carlo approach to obtain usual intake percentiles; and (3) supplement use as a covariate. These data form the basis for some of the figures in Chapter 7, as specified in the figure legends.

CANADA

Intake data for calcium and vitamin D from the Canadian Community Health Survey (CCHS), Cycle 2.2, Nutrition are shown in Tables H-5 and

[1]Bailey, R. L., K. W. Dodd, J. A. Goldman, J. J. Gahche, J. T. Dwyer, A. J. Moshfegh, C. T. Sempos and M. F. Picciano. 2010. Estimation of Total Usual Calcium and Vitamin D Intake in the United States. Journal of Nutrition 140(4): 817-22.

[2]Available online at http://www.ars.usda.gov/Services/docs.htm?docid=13793 (accessed August 13, 2010).

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TABLE H-1 Estimated Calcium Intake (mg/day) in the United States from Food Sources Only, NHANES 2003–2006

DRI Sex/Age Group		Mean	Percentile						
			1st	5th	25th	50th	75th	95th	99th
M 1–3	Est.	998.4	372.5	501.1	733.2	968.0	1213.6	1604.7	2102.1
	[S.E.]	[28.8]	[35.2]	[25.3]	[21.6]	[35.2]	[49.3]	[79.1]	[237.1]
M 4–8	Est.	1058.6	413.3	597.4	854.6	1044.8	1207.4	1583.3	1961.0
	[S.E.]	[29.7]	[52.3]	[35.8]	[32.4]	[39.0]	[46.7]	[51.7]	[112.5]
M 9–13	Est.	1074.8	472.2	587.7	864.2	1055.1	1267.6	1600.0	1933.2
	[S.E.]	[31.7]	[62.9]	[64.8]	[35.7]	[45.0]	[39.7]	[78.9]	[166.8]
M 14–18	Est.	1269.3	423.4	585.6	884.2	1168.7	1564.5	2346.0	2980.8
	[S.E.]	[38.3]	[69.3]	[59.3]	[35.0]	[39.2]	[54.6]	[157.9]	[211.8]
M 19–30	Est.	1210.9	459.0	582.4	868.9	1126.5	1490.1	2109.3	2576.8
	[S.E.]	[33.6]	[30.0]	[42.6]	[31.5]	[33.6]	[55.4]	[112.7]	[184.5]
M 31–50	Est.	1116.2	410.3	558.1	821.1	1051.9	1354.2	1886.1	2278.6
	[S.E.]	[24.9]	[69.2]	[31.6]	[25.5]	[30.0]	[40.2]	[80.4]	[118.1]
M 51–70	Est.	952.1	314.3	443.0	686.3	882.6	1148.2	1704.4	2272.5
	[S.E.]	[20.0]	[25.0]	[24.0]	[18.2]	[18.2]	[30.9]	[60.3]	[182.1]
M 71+	Est.	871.9	323.7	407.3	640.2	833.3	1059.6	1422.1	1860.7
	[S.E.]	[25.6]	[18.9]	[25.0]	[20.7]	[21.1]	[38.4]	[77.5]	[185.0]
F 1–3	Est.	986.0	381.6	541.5	758.0	936.3	1130.2	1545.1	1821.6
	[S.E.]	[28.1]	[35.2]	[27.3]	[28.2]	[26.3]	[38.5]	[89.1]	[130.9]
F 4–8	Est.	951.3	408.7	515.9	739.2	898.4	1105.9	1527.3	1891.3
	[S.E.]	[26.9]	[32.1]	[39.8]	[31.7]	[30.2]	[43.9]	[56.9]	[137.9]
F 9–13	Est.	966.9	384.6	496.1	743.0	938.2	1161.8	1564.1	1992.2
	[S.E.]	[44.9]	[63.0]	[52.3]	[49.5]	[42.5]	[59.5]	[136.4]	[319.7]
F 14–18	Est.	875.8	301.0	428.0	657.5	825.5	1047.8	1458.3	1909.1
	[S.E.]	[25.3]	[29.4]	[37.6]	[27.4]	[33.0]	[43.0]	[78.1]	[191.4]
F 19–30	Est.	838.5	314.3	437.2	640.2	791.9	1011.3	1329.8	1643.1
	[S.E.]	[25.7]	[52.4]	[37.9]	[24.2]	[24.8]	[33.8]	[54.9]	[228.8]
F 31–50	Est.	866.7	311.4	387.7	621.9	797.4	1045.2	1514.1	2083.6
	[S.E.]	[20.6]	[14.9]	[20.7]	[15.6]	[19.0]	[26.7]	[74.9]	[198.5]
F 51–70	Est.	788.4	280.2	407.5	587.1	755.4	942.0	1352.5	1664.7
	[S.E.]	[22.4]	[24.7]	[16.1]	[16.9]	[22.9]	[29.8]	[54.3]	[49.3]
F 71+	Est.	749.7	286.7	344.5	536	706.5	895.3	1336.7	1709.5
	[S.E.]	[18.1]	[27.1]	[16.9]	[18.6]	[16.5]	[26.1]	[46.0]	[175.1]

SOURCE: Personal communication, K. Dodd, NIH/NCI, November 17, 2009.

H-6. These values have been obtained from the publicly available reports[3] and form the basis for some of the figures in Chapter 7, as specified in the figure legends.

[3]Available online at http://www.statcan.gc.ca/cgi-bin/imdb/p2SV.pl?Function=getSurvey&SDDS=3226&lang=en&db=imdb&adm=8&dis=2#a2 (access August 13, 2010).

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TABLE H-2 Estimated Calcium Intake (mg/day) in the United States from Food and Dietary Supplements, NHANES 2003–2006

DRI Sex/Age Group		Mean	Percentile						
			1st	5th	25th	50th	75th	95th	99th
M 1–3	Est.	1008.4	372.5	514.3	740.4	969.6	1217.7	1604.7	2102.1
	[S.E.]	[28.4]	[35.2]	[29.2]	[23.7]	[30.0]	[53.7]	[89.6]	[237.1]
M 4–8	Est.	1087.2	459.7	636.3	874.6	1070.4	1248.1	1633.3	2044.0
	[S.E.]	[31.1]	[53.4]	[40.3]	[30.0]	[33.8]	[52.7]	[60.3]	[163.2]
M 9–13	Est.	1092.8	472.2	613.0	876.6	1070.3	1278.7	1664.9	2124.5
	[S.E.]	[32.9]	[62.9]	[65.7]	[34.9]	[47.4]	[37.8]	[74.0]	[125.0]
M 14–18	Est.	1296.9	423.4	592.0	904.9	1210.2	1584.1	2439.9	3057.7
	[S.E.]	[41.1]	[60.5]	[62.3]	[39.8]	[42.4]	[54.2]	[148.9]	[219.4]
M 19–30	Est.	1259.5	459.0	595.0	890.0	1165.8	1545.1	2159.2	2686.9
	[S.E.]	[34.0]	[30.3]	[40.0]	[35.7]	[41.1]	[52.4]	[161.6]	[175.6]
M 31–50	Est.	1220.2	440.2	573.8	874.0	1139.5	1487.4	2164.7	2706.3
	[S.E.]	[27.4]	[79.1]	[29.4]	[28.0]	[28.9]	[47.7]	[66.9]	[220.5]
M 51–70	Est.	1092.0	349.6	463.5	737.3	992.9	1321.4	2021.7	2743.1
	[S.E.]	[21.4]	[24.2]	[21.0]	[19.5]	[20.5]	[30.9]	[128.2]	[205.1]
M 71+	Est.	1087.0	338.5	455.4	740.3	966.8	1282.4	2014.7	3059.8
	[S.E.]	[28.6]	[27.4]	[20.2]	[30.3]	[28.9]	[36.9]	[71.1]	[412.4]
F 1–3	Est.	977.3	397.2	545.0	774.8	946.5	1135.7	1547.5	1824.6
	[S.E.]	[28.1]	[37.7]	[23.7]	[32.2]	[31.0]	[32.9]	[86.4]	[124.7]
F 4–8	Est.	974.1	435.1	533.5	756.1	912.7	1144.4	1541	1891.3
	[S.E.]	[27.1]	[32.3]	[35.0]	[31.5]	[27.8]	[46.5]	[46.1]	[123.2]
F 9–13	Est.	988.4	389.3	498.4	749.1	942.1	1200.3	1587.9	2086.3
	[S.E.]	[47.1]	[60.8]	[52.6]	[49.9]	[39.1]	[60.8]	[122.1]	[280.4]
F 14–18	Est.	917.8	301.0	430.5	662.7	867.3	1100.3	1607.7	2008.3
	[S.E.]	[30]	[29.4]	[36.7]	[29.1]	[41.4]	[55.5]	[117.9]	[105.2]
F 19–30	Est.	945.4	314.3	437.2	660.1	863.2	1094.6	1671.3	2550.8
	[S.E.]	[29.8]	[52.4]	[38.6]	[30.6]	[21.7]	[51]	[137.7]	[349.1]
F 31–50	Est.	1055.4	326.0	441.6	690.6	933.7	1274.7	2099.2	2661.7
	[S.E.]	[28.3]	[15.9]	[22.0]	[20.9]	[25.6]	[56.5]	[151.7]	[128.0]
F 51–70	Est.	1186.5	280.2	439.7	709.3	1043.6	1487.0	2364.0	3697.9
	[S.E.]	[37.3]	[35.7]	[23.1]	[34.4]	[43.6]	[56.2]	[109.8]	[792.6]
F 71+	Est.	1139.4	290.2	385.1	663.5	982.9	1474.9	2297.7	3071.5
	[S.E.]	[24.9]	[22.1]	[31.5]	[18.8]	[35.9]	[46.7]	[148.5]	[203.2]

SOURCE: Personal communication, K. Dodd, NIH/NCI, November 17, 2009.

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TABLE H-3a Estimated Vitamin D Intake (IU/day) in the United States from Food Sources Only, NHANES 2005–2006

DRI Sex/ Age Group		Mean	Percentile						
			1st	5th	25th	50th	75th	95th	99th
M 1–3	Est.	288	48	88	176	280	396	500	632
	[S.E.]	[8]	[12]	[16]	[16]	[16]	[24]	[24]	[84]
M 4–8	Est.	256	64	92	180	236	312	440	508
	[S.E.]	[12]	[12]	[16]	[16]	[8]	[8]	[20]	[96]
M 9–13	Est.	224	56	68	156	216	284	424	572
	[S.E.]	[8]	[28]	[20]	[16]	[12]	[16]	[48]	[88]
M 14–18	Est.	240	28	48	104	196	320	628	824
	[S.E.]	[16]	[4]	[12]	[8]	[16]	[16]	[56]	[104]
M 19–30	Est.	200	28	48	96	168	280	440	556
	[S.E.]	[12]	[8]	[12]	[12]	[12]	[16]	[44]	[80]
M 31–50	Est.	216	36	56	120	176	280	508	700
	[S.E.]	[12]	[4]	[8]	[8]	[16]	[16]	[40]	[60]
M 51–70	Est.	204	36	60	120	176	272	416	608
	[S.E.]	[12]	[12]	[8]	[12]	[8]	[28]	[64]	[128]
M 71+	Est.	224	56	80	144	208	280	396	604
	[S.E.]	[16]	[8]	[12]	[12]	[16]	[24]	[64]	[144]
F 1–3	Est.	272	48	100	176	260	348	500	700
	[S.E.]	[16]	[20]	[16]	[12]	[16]	[20]	[64]	[48]
F 4–8	Est.	216	60	84	140	208	280	388	448
	[S.E.]	[12]	[16]	[12]	[16]	[16]	[16]	[32]	[32]
F 9–13	Est.	208	28	64	124	196	272	416	612
	[S.E.]	[24]	[24]	[28]	[24]	[20]	[28]	[76]	[196]
F 14–18	Est.	148	28	44	76	132	188	380	492
	[S.E.]	[8]	[8]	[12]	[12]	[12]	[12]	[64]	[60]
F 19–30	Est.	140	16	28	80	116	188	308	472
	[S.E.]	[12]	[8]	[12]	[12]	[12]	[16]	[20]	[100]
F 31–50	Est.	176	24	32	84	136	232	412	652
	[S.E.]	[12]	[4]	[4]	[8]	[12]	[12]	[76]	[64]
F 51–70	Est.	156	36	48	88	140	192	332	452
	[S.E.]	[16]	[8]	[8]	[8]	[12]	[24]	[40]	[184]
F 71+	Est.	176	20	44	100	160	236	352	524
	[S.E.]	[8]	[8]	[8]	[12]	[8]	[12]	[24]	[92]

SOURCE: Personal communication, K. Dodd, NIH/NCI, November 17, 2009.

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TABLE H-3b Estimated Vitamin D Intake (μg/day) in the United States from Food Sources Only, NHANES 2005–2006

DRI Sex/ Age Group		Mean	Percentile						
			1st	5th	25th	50th	75th	95th	99th
M 1–3	Est.	7.20	1.20	2.20	4.40	7.00	9.90	12.50	15.80
	[S.E.]	[0.20]	[0.30]	[0.40]	[0.40]	[0.40]	[0.60]	[0.60]	[2.10]
M 4–8	Est.	6.40	1.60	2.30	4.50	5.90	7.80	11.00	12.70
	[S.E.]	[0.30]	[0.30]	[0.40]	[0.40]	[0.20]	[0.20]	[0.50]	[2.40]
M 9–13	Est.	5.60	1.40	1.70	3.90	5.40	7.10	10.60	14.30
	[S.E.]	[0.20]	[0.70]	[0.50]	[0.40]	[0.30]	[0.40]	[1.20]	[2.20]
M 14–18	Est.	6.00	0.70	1.20	2.60	4.90	8.00	15.70	20.60
	[S.E.]	[0.40]	[0.10]	[0.30]	[0.20]	[0.40]	[0.40]	[1.40]	[2.60]
M 19–30	Est.	5.00	0.70	1.20	2.40	4.20	7.00	11.00	13.90
	[S.E.]	[0.30]	[0.20]	[0.30]	[0.30]	[0.30]	[0.40]	[1.10]	[2.00]
M 31–50	Est.	5.40	0.90	1.40	3.00	4.40	7.00	12.70	17.50
	[S.E.]	[0.30]	[0.10]	[0.20]	[0.20]	[0.40]	[0.40]	[1.00]	[1.50]
M 51–70	Est.	5.10	0.90	1.50	3.00	4.40	6.80	10.40	15.20
	[S.E.]	[0.30]	[0.30]	[0.20]	[0.30]	[0.20]	[0.70]	[1.60]	[3.20]
M 71+	Est.	5.60	1.40	2.00	3.60	5.20	7.00	9.90	15.10
	[S.E.]	[0.40]	[0.20]	[0.30]	[0.30]	[0.40]	[0.60]	[1.60]	[3.60]
F 1–3	Est.	6.80	1.20	2.50	4.40	6.50	8.70	12.50	17.50
	[S.E.]	[0.40]	[0.50]	[0.40]	[0.30]	[0.40]	[0.50]	[1.60]	[1.20]
F 4–8	Est.	5.40	1.50	2.10	3.50	5.20	7.00	9.70	11.20
	[S.E.]	[0.30]	[0.40]	[0.30]	[0.40]	[0.40]	[0.40]	[0.80]	[0.80]
F 9–13	Est.	5.20	0.70	1.60	3.10	4.90	6.80	10.40	15.30
	[S.E.]	[0.60]	[0.60]	[0.70]	[0.60]	[0.50]	[0.70]	[1.90]	[4.90]
F 14–18	Est.	3.70	0.70	1.10	1.90	3.30	4.70	9.50	12.30
	[S.E.]	[0.20]	[0.20]	[0.30]	[0.30]	[0.30]	[0.30]	[1.60]	[1.50]
F 19–30	Est.	3.50	0.40	0.70	2.00	2.90	4.70	7.70	11.80
	[S.E.]	[0.30]	[0.20]	[0.30]	[0.30]	[0.30]	[0.40]	[0.50]	[2.50]
F 31–50	Est.	4.40	0.60	0.80	2.10	3.40	5.80	10.30	16.30
	[S.E.]	[0.30]	[0.10]	[0.10]	[0.20]	[0.30]	[0.30]	[1.90]	[1.60]
F 51–70	Est.	3.90	0.90	1.20	2.20	3.50	4.80	8.30	11.30
	[S.E.]	[0.40]	[0.20]	[0.20]	[0.20]	[0.30]	[0.60]	[1.00]	[4.60]
F 71+	Est.	4.40	0.50	1.10	2.50	4.00	5.90	8.80	13.10
	[S.E.]	[0.20]	[0.20]	[0.20]	[0.30]	[0.20]	[0.30]	[0.60]	[2.30]

SOURCE: Personal communication, K. Dodd, NIH/NCI, November 17, 2009.

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TABLE H-4a Estimated Vitamin D Intake (IU/day) in the United States from Food and Dietary Supplements, NHANES 2005–2006

DRI Sex/ Age Group		Mean	Percentile						
			1st	5th	25th	50th	75th	95th	99th
M 1–3	Est.	364	48	108	220	332	484	804	884
	[S.E.]	[16]	[16]	[20]	[16]	[20]	[28]	[80]	[48]
M 4–8	Est.	372	68	124	224	312	488	776	996
	[S.E.]	[16]	[32]	[20]	[12]	[28]	[36]	[24]	[128]
M 9–13	Est.	300	56	92	172	244	332	600	856
	[S.E.]	[28]	[12]	[20]	[16]	[12]	[52]	[40]	[2236]
M 14–18	Est.	276	32	48	112	232	368	704	972
	[S.E.]	[20]	[4]	[12]	[12]	[24]	[40]	[72]	[100]
M 19–30	Est.	264	28	52	100	192	368	712	1024
	[S.E.]	[16]	[8]	[12]	[12]	[20]	[24]	[76]	[156]
M 31–50	Est.	316	40	72	144	256	448	724	1000
	[S.E.]	[12]	[4]	[8]	[12]	[24]	[24]	[24]	[124]
M 51–70	Est.	352	44	64	136	260	528	780	988
	[S.E.]	[16]	[12]	[8]	[12]	[20]	[24]	[96]	[352]
M 71+	Est.	428	60	108	196	336	596	844	1552
	[S.E.]	[28]	[16]	[12]	[20]	[32]	[20]	[112]	[1800]
F 1–3	Est.	336	72	128	208	304	416	676	864
	[S.E.]	[16]	[20]	[24]	[24]	[32]	[24]	[44]	[76]
F 4–8	Est.	316	64	104	176	256	392	672	1888
	[S.E.]	[24]	[16]	[12]	[16]	[16]	[56]	[60]	[952]
F 9–13	Est.	308	36	64	132	216	356	756	1024
	[S.E.]	[40]	[24]	[28]	[28]	[24]	[76]	[160]	[2268]
F 14–18	Est.	200	28	44	84	144	252	568	696
	[S.E.]	[20]	[8]	[12]	[20]	[12]	[40]	[44]	[72]
F 19–30	Est.	232	16	32	84	156	308	640	996
	[S.E.]	[12]	[8]	[12]	[12]	[16]	[20]	[72]	[140]
F 31–50	Est.	308	24	36	104	232	472	784	984
	[S.E.]	[20]	[4]	[4]	[12]	[20]	[36]	[60]	[88]
F 51–70	Est.	404	36	64	136	308	560	936	2908
	[S.E.]	[40]	[8]	[12]	[16]	[36]	[28]	[88]	[1544]
F 71+	Est.	400	32	80	148	356	572	940	1296
	[S.E.]	[20]	[12]	[8]	[12]	[36]	[32]	[28]	[128]

SOURCE: Personal communication, K. Dodd, NIH/NCI, November 17, 2009.

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TABLE H-4b Estimated Vitamin D Intake (μg/day) in the United States from Food and Dietary Supplements, NHANES 2005–2006

DRI Sex/ Age Group		Mean	Percentile						
			1st	5th	25th	50th	75th	95th	99th
M 1–3	Est.	9.10	1.20	2.70	5.50	8.30	12.10	20.10	22.10
	[S.E.]	[0.40]	[0.40]	[0.50]	[0.40]	[0.50]	[0.70]	[2.00]	[1.20]
M 4–8	Est.	9.30	1.70	3.10	5.60	7.80	12.20	19.40	24.90
	[S.E.]	[0.40]	[0.80]	[0.50]	[0.30]	[0.70]	[0.90]	[0.60]	[3.20]
M 9–13	Est.	7.50	1.40	2.30	4.30	6.10	8.30	15.00	21.40
	[S.E.]	[0.70]	[0.30]	[0.50]	[0.40]	[0.30]	[1.30]	[1.00]	[55.90]
M 14–18	Est.	6.90	0.80	1.20	2.80	5.80	9.20	17.60	24.30
	[S.E.]	[0.50]	[0.10]	[0.30]	[0.30]	[0.60]	[1.00]	[1.80]	[2.50]
M 19–30	Est.	6.60	0.70	1.30	2.50	4.80	9.20	17.80	25.60
	[S.E.]	[0.40]	[0.20]	[0.30]	[0.30]	[0.50]	[0.60]	[1.90]	[3.90]
M 31–50	Est.	7.90	1.00	1.80	3.60	6.40	11.20	18.10	25.00
	[S.E.]	[0.30]	[0.10]	[0.20]	[0.30]	[0.60]	[0.60]	[0.60]	[3.10]
M 51–70	Est.	8.80	1.10	1.60	3.40	6.50	13.20	19.50	24.70
	[S.E.]	[0.40]	[0.30]	[0.20]	[0.30]	[0.50]	[0.60]	[2.40]	[8.80]
M 71+	Est.	10.70	1.50	2.70	4.90	8.40	14.90	21.10	38.80
	[S.E.]	[0.70]	[0.40]	[0.30]	[0.50]	[0.80]	[0.50]	[2.80]	[45.00]
F 1–3	Est.	8.40	1.80	3.20	5.20	7.60	10.40	16.90	21.60
	[S.E.]	[0.40]	[0.50]	[0.60]	[0.60]	[0.80]	[0.60]	[1.10]	[1.90]
F 4–8	Est.	7.90	1.60	2.60	4.40	6.40	9.80	16.80	47.20
	[S.E.]	[0.60]	[0.40]	[0.30]	[0.40]	[0.40]	[1.40]	[1.50]	[23.80]
F 9–13	Est.	7.70	0.90	1.60	3.30	5.40	8.90	18.90	25.60
	[S.E.]	[1.00]	[0.60]	[0.70]	[0.70]	[0.60]	[1.90]	[4.00]	[56.70]
F 14–18	Est.	5.00	0.70	1.10	2.10	3.60	6.30	14.20	17.40
	[S.E.]	[0.50]	[0.20]	[0.30]	[0.50]	[0.30]	[1.00]	[1.10]	[1.80]
F 19–30	Est.	5.80	0.40	0.80	2.10	3.90	7.70	16.00	24.90
	[S.E.]	[0.30]	[0.20]	[0.30]	[0.30]	[0.40]	[0.50]	[1.80]	[3.50]
F 31–50	Est.	7.70	0.60	0.90	2.60	5.80	11.80	19.60	24.60
	[S.E.]	[0.50]	[0.10]	[0.10]	[0.30]	[0.50]	[0.90]	[1.50]	[2.20]
F 51–70	Est.	10.10	0.90	1.60	3.40	7.70	14.00	23.40	72.70
	[S.E.]	[1.00]	[0.20]	[0.30]	[0.40]	[0.90]	[0.70]	[2.20]	[38.60]
F 71+	Est.	10.00	0.80	2.00	3.70	8.90	14.30	23.50	32.40
	[S.E.]	[0.50]	[0.30]	[0.20]	[0.30]	[0.90]	[0.80]	[0.70]	[3.20]

SOURCE: Personal communication, K. Dodd, NIH/NCI, November 17, 2009.

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TABLE H-5 Estimated Calcium Intake (mg/day) in Canada from Food Sources Only

DRI Sex/ Age Group		Mean	Percentile						
			5th	10th	25th	50th	75th	90th	95th
Both 1–3	Est.	1051	552	650	826	1041	1292	1567	1753
	[S.E.]	[18]	[23]	[23]	[22]	[22]	[27]	[37]	[44]
Both 4–8	Est.	1036	585	666	814	1003	1228	1472	1635
	[S.E.]	[16]	[21]	[19]	[17]	[18]	[26]	[39]	[49]
M 9–13	Est.	1219	620	718	906	1164	1482	1827	2066
	[S.E.]	[27]	[27]	[27]	[27]	[31]	[41]	[57]	[75]
M 14–18	Est.	1300	670	785	1002	1288	1633	2001	2249
	[S.E.]	[28]	[38]	[37]	[35]	[35]	[43]	[60]	[75]
M 19–30	Est.	1107	516	606	784	1029	1340	1691	1934
	[S.E.]	[35]	[31]	[32]	[33]	[38]	[54]	[81]	[104]
M 31–50	Est.	938	440	518	680	893	1156	1458	1675
	[S.E.]	[17]	[24]	[24]	[24]	[24]	[31]	[47]	[62]
M 51–70	Est.	832	390	457	588	776	1025	1304	1498
	[S.E.]	[17]	[18]	[18]	[18]	[20]	[27]	[41]	[54]
M > 70	Est.	762	336	398	523	702	932	1193	1377
	[S.E.]	[33]	[20]	[21]	[23]	[29]	[40]	[57]	[72]
M 19+	Est.	931	413	489	647	868	1151	1475	1708
	[S.E.]	[13]	[11]	[12]	[13]	[15]	[20]	[30]	[39]
F 9–13	Est.	993	515	596	749	950	1188	1440	1611
	[S.E.]	[24]	[23]	[22]	[23]	[26]	[33]	[46]	[58]
F 14–18	Est.	917	420	500	660	888	1166	1459	1659
	[S.E.]	[21]	[21]	[22]	[23]	[25]	[33]	[51]	[68]
F 19–30	Est.	867	407	479	622	820	1063	1323	1498
	[S.E.]	[27]	[25]	[25]	[26]	[28]	[36]	[51]	[63]
F 31–50	Est.	827	389	457	599	785	1027	1287	1477
	[S.E.]	[19]	[21]	[22]	[22]	[23]	[28]	[40]	[53]
F 51–70	Est.	740	344	410	534	702	910	1138	1302
	[S.E.]	[13]	[14]	[14]	[14]	[15]	[19]	[27]	[36]
F > 70	Est.	690	341	397	509	661	849	1060	1211
	[S.E.]	[17]	[17]	[18]	[20]	[22]	[26]	[34]	[42]
F 19+	Est.	793	373	440	572	752	982	1234	1413
	[S.E.]	[10]	[10]	[10]	[10]	[12]	[15]	[21]	[28]

SOURCE: Statistics Canada, CCHS, Cycle 2.2, Nutrition (2004).

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TABLE H-6a Estimated Vitamin D Intake in Canada (IU/day) from Food Sources Only

DRI Sex/ Age Group		Mean	Percentile						
			5th	10th	25th	50th	75th	90th	95th
Both 1–3	Est.	260	84	116	176	252	340	436	500
	[S.E.]	[4]	[8]	[8]	[8]	[8]	[8]	[12]	[16]
Both 4–8	Est.	240	100	124	168	224	296	376	432
	[S.E.]	[4]	[4]	[4]	[4]	[4]	[8]	[12]	[16]
M 9–13	Est.	280	124	148	196	264	352	444	512
	[S.E.]	[8]	[8]	[8]	[8]	[8]	[12]	[16]	[20]
M 14–18	Est.	304	108	140	200	288	400	544	648
	[S.E.]	[8]	[8]	[8]	[8]	[12]	[12]	[24]	[32]
M 19–30	Est.	236	88	108	148	208	288	388	464
	[S.E.]	[8]	[8]	[8]	[8]	[12]	[16]	[28]	[40]
M 31–50	Est.	232	92	112	148	204	288	396	476
	[S.E.]	[8]	[8]	[8]	[8]	[8]	[12]	[24]	[36]
M 51–70	Est.	284	92	112	160	236	360	540	692
	[S.E.]	[20]	[8]	[8]	[12]	[16]	[28]	[48]	[72]
M > 70	Est.	252	88	108	148	212	308	440	552
	[S.E.]	[16]	[8]	[8]	[8]	[16]	[20]	[32]	[44]
M 19+	Est.	248	88	108	148	212	312	444	552
	[S.E.]	[8]	[4]	[4]	[4]	[8]	[12]	[20]	[28]
F 9–13	Est.	228	88	112	152	208	280	368	428
	[S.E.]	[8]	[8]	[8]	[8]	[8]	[8]	[16]	[20]
F 14–18	Est.	200	60	80	120	176	256	356	428
	[S.E.]	[8]	[4]	[8]	[8]	[8]	[12]	[16]	[28]
F 19–30	Est.	188	68	88	120	168	232	312	372
	[S.E.]	[8]	[8]	[8]	[8]	[8]	[12]	[16]	[24]
F 31–50	Est.	208	76	92	128	180	264	384	480
	[S.E.]	[12]	[8]	[8]	[8]	[12]	[20]	[40]	[60]
F 51–70	Est.	200	68	84	120	176	268	392	492
	[S.E.]	[12]	[8]	[8]	[8]	[12]	[16]	[36]	[52]
F > 70	Est.	212	80	100	136	188	272	376	460
	[S.E.]	[12]	[8]	[8]	[8]	[12]	[16]	[24]	[36]
F 19+	Est.	200	72	88	124	176	256	368	460
	[S.E.]	[4]	[4]	[4]	[4]	[4]	[8]	[16]	[28]

SOURCE: Statistics Canada, CCHS, Cycle 2.2, Nutrition (2004).

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TABLE H-6b Estimated Vitamin D Intake in Canada (μg/day) from Food Sources Only

DRI Sex/ Age Group		Mean	Percentile						
			5th	10th	25th	50th	75th	90th	95th
Both 1–3	Est.	6.5	2.1	2.9	4.4	6.3	8.5	10.9	12.5
	[S.E.]	[0.1]	[0.2]	[0.2]	[0.2]	[0.2]	[0.2]	[0.3]	[0.4]
Both 4–8	Est.	6.0	2.5	3.1	4.2	5.6	7.4	9.4	10.8
	[S.E.]	[0.1]	[0.1]	[0.1]	[0.1]	[0.1]	[0.2]	[0.3]	[0.4]
M 9–13	Est.	7.0	3.1	3.7	4.9	6.6	8.8	11.1	12.8
	[S.E.]	[0.2]	[0.2]	[0.2]	[0.2]	[0.2]	[0.3]	[0.4]	[0.5]
M 14–18	Est.	7.6	2.7	3.5	5.0	7.2	10.0	13.6	16.2
	[S.E.]	[0.2]	[0.2]	[0.2]	[0.2]	[0.3]	[0.3]	[0.6]	[0.8]
M 19–30	Est.	5.9	2.2	2.7	3.7	5.2	7.2	9.7	11.6
	[S.E.]	[0.2]	[0.2]	[0.2]	[0.2]	[0.3]	[0.4]	[0.7]	[1.0]
M 31–50	Est.	5.8	2.3	2.8	3.7	5.1	7.2	9.9	11.9
	[S.E.]	[0.2]	[0.2]	[0.2]	[0.2]	[0.2]	[0.3]	[0.6]	[0.9]
M 51–70	Est.	7.1	2.3	2.8	4.0	5.9	9.0	13.5	17.3
	[S.E.]	[0.5]	[0.2]	[0.2]	[0.3]	[0.4]	[0.7]	[1.2]	[1.8]
M > 70	Est.	6.3	2.2	2.7	3.7	5.3	7.7	11.0	13.8
	[S.E.]	[0.4]	[0.2]	[0.2]	[0.2]	[0.4]	[0.5]	[0.8]	[1.1]
M 19+	Est.	6.2	2.2	2.7	3.7	5.3	7.8	11.1	13.8
	[S.E.]	[0.2]	[0.1]	[0.1]	[0.1]	[0.2]	[0.3]	[0.5]	[0.7]
F 9–13	Est.	5.7	2.2	2.8	3.8	5.2	7.0	9.2	10.7
	[S.E.]	[0.2]	[0.2]	[0.2]	[0.2]	[0.2]	[0.2]	[0.4]	[0.5]
F 14–18	Est.	5.0	1.5	2.0	3.0	4.4	6.4	8.9	10.7
	[S.E.]	[0.2]	[0.1]	[0.2]	[0.2]	[0.2]	[0.3]	[0.4]	[0.7]
F 19–30	Est.	4.7	1.7	2.2	3.0	4.2	5.8	7.8	9.3
	[S.E.]	[0.2]	[0.2]	[0.2]	[0.2]	[0.2]	[0.3]	[0.4]	[0.6]
F 31–50	Est.	5.2	1.9	2.3	3.2	4.5	6.6	9.6	12.0
	[S.E.]	[0.3]	[0.2]	[0.2]	[0.2]	[0.3]	[0.5]	[1.0]	[1.5]
F 51–70	Est.	5.0	1.7	2.1	3.0	4.4	6.7	9.8	12.3
	S.E.	[0.3]	[0.2]	[0.2]	[0.2]	[0.3]	[0.4]	[0.9]	[1.3]
F > 70	Est.	5.3	2.0	2.5	3.4	4.7	6.8	9.4	11.5
	S.E.	[0.3]	[0.2]	[0.2]	[0.2]	[0.3]	[0.4]	[0.6]	[0.9]
F 19+	Est.	5.0	1.8	2.2	3.1	4.4	6.4	9.2	11.5
	[S.E.]	[0.1]	[0.1]	[0.1]	[0.1]	[0.1]	[0.2]	[0.4]	[0.7]

SOURCE: Statistics Canada, CCHS, Cycle 2.2, Nutrition (2004).

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I

Proportion of the Population Above and Below 40 nmol/L Serum 25-Hydroxyvitamin D Concentrations and Cumulative Distribution of Serum 25-Hydroxyvitamin D Concentrations: United States and Canada

The data in this appendix are provided for the readers of this report and were not reviewed by the committee.

For the United States (Table I-1 and Figures I-1 through I-8), the tables and figures for serum 25-hydroxyvitamin D (25OHD) concentrations are from analyses conducted in August 2010 by the National Center for Health Statistics, U.S. Centers for Disease Control and Prevention and based on the National Health and Nutrition Examination Survey (NHANES) 2003–2006.

For Canada (Table I-2 and Figures I-9 through I-14), the tables and figures for serum 25OHD concentrations are from analyses conducted in August 2010 by Statistics Canada and based on the Canadian Health Measures Survey (CHMS), Cycle 1, 2007–2009.

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PERSONS ABOVE AND BELOW 40 NMOL/L SERUM 25-HYDROXYVITAMIN D CONCENTRATIONS FROM NATIONAL SURVEYS

TABLE I-1 United States: Prevalence of Serum 25OHD Concentrations (QC adjusted) Above and Below 40 nmol/L by Total Population and by Race/Ethnicity (ages 1 year and older) from NHANES 2003–2006

	Estimate (95% Confidence Interval)				
	Total	Non-Hispanic White	Non-Hispanic Black	Mexican American	Others
Percent < 40 nmol/L	18.8 (16.3–21.5)	10.6 (8.9–12.4)	53.6 (48.9–58.2)	27.2 (22.8–32.0)	27.2 (23.2–31.7)
Percent ≥ 40 nmol/L	81.2 (78.5–83.7)	89.5 (87.6–91.1)	46.4 (41.8–51.1)	72.9 (68.0–77.2)	72.8 (68.3–76.8)

SOURCE: NHANES 2003–2006.

TABLE I-2 Canada: Prevalence of Serum 25OHD Concentrations Above and Below 40 nmol/L in Canada by Total Population (ages 9 years and older) from CHMS, Cycle 1, 2007–2009

	Estimate (95% Confidence Interval)
Percent < 40 nmol/L	13.0 (9.9, 16.1)
Percent ≥ 40 nmol/L	87.0 (83.9, 90.1)

SOURCE: CHMS, Cycle 1, 2007–2009.

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UNITED STATES: CUMULATIVE DISTRIBUTION OF SERUM 25OHD CONCENTRATIONS BY AGE GROUP

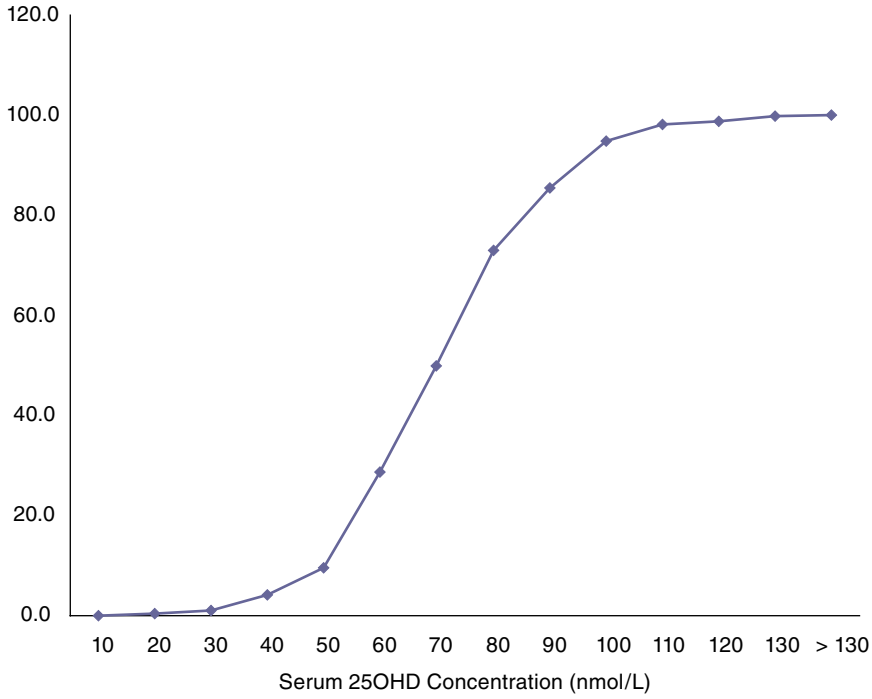


FIGURE I-1 Cumulative distribution of serum 25OHD (QC adjusted) for 1- to 3-year-olds in the United States for the 2003 to 2006 time period.
SOURCE: NHANES 2003–2006.

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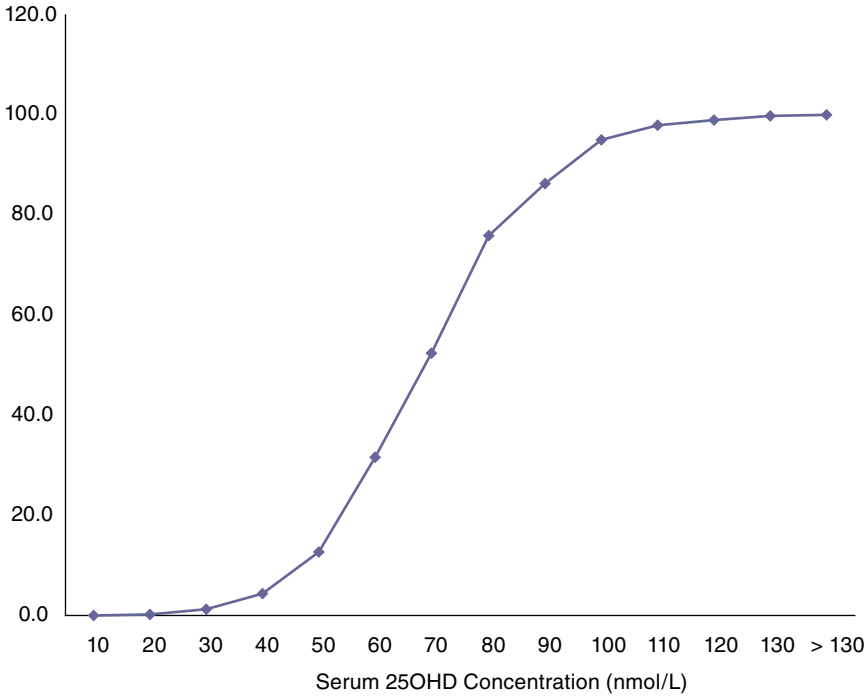


FIGURE I-2 Cumulative distribution of serum 25OHD (QC adjusted) for 4- to 8-year-olds in the United States for the 2003 to 2006 time period.
SOURCE: NHANES 2003–2006.

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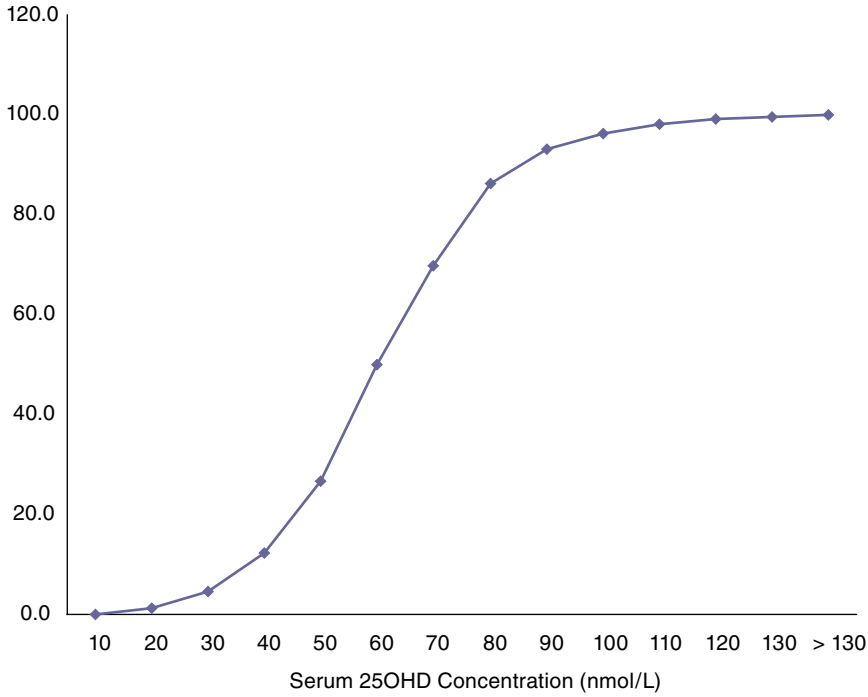


FIGURE I-3 Cumulative distribution of serum 25OHD (QC adjusted) for 9- to 13-year-olds in the United States for the 2003 and 2006 time period.
SOURCE: NHANES 2003–2006.

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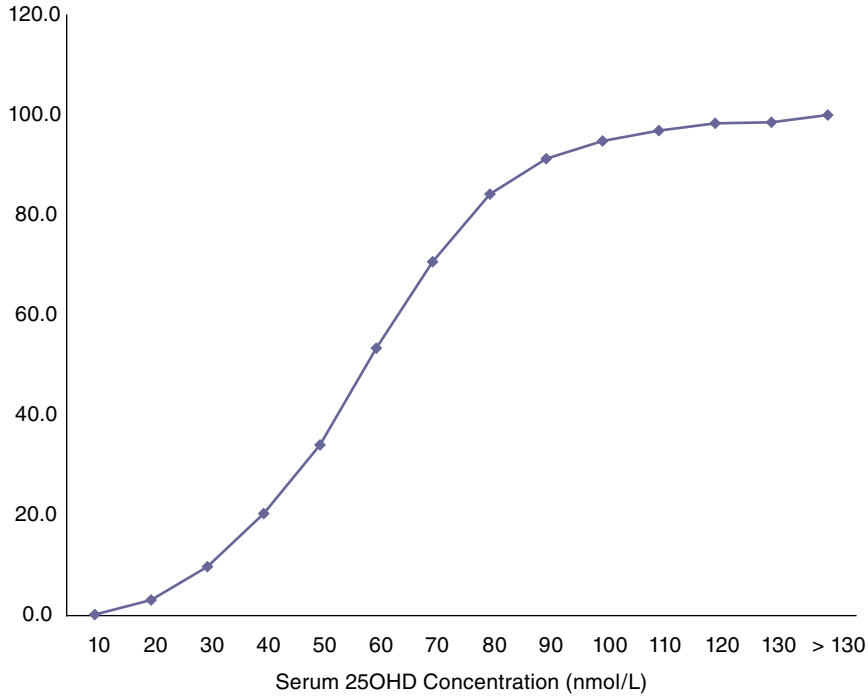


FIGURE I-4 Cumulative distribution of serum 25OHD (QC adjusted) for 14- to 18-year-olds in the United States for the 2003 to 2006 time period.
SOURCE: NHANES 2003–2006.

Vieth Ex 2029 IPR2023-00726 1004

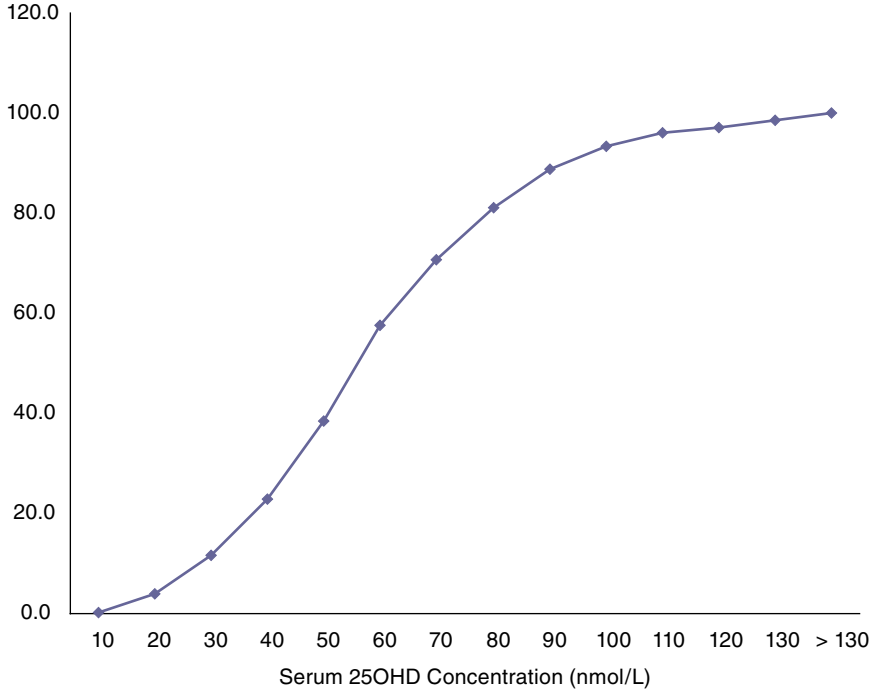


FIGURE I-5 Cumulative distribution of serum 25OHD (QC adjusted) for 19- to 30-year-olds in the United States for the 2003 to 2006 time period.
SOURCE: NHANES 2003–2006.

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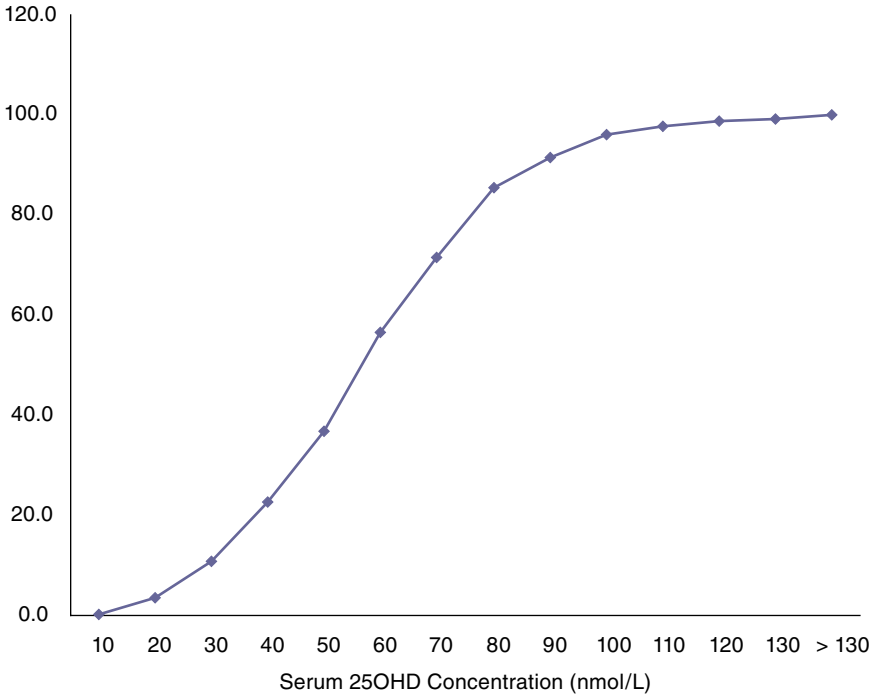


FIGURE I-6 Cumulative distribution of serum 25OHD (QC adjusted) for 31- to 50-year-olds in the United States for the 2003 to 2006 time period.
SOURCE: NHANES 2003–2006.

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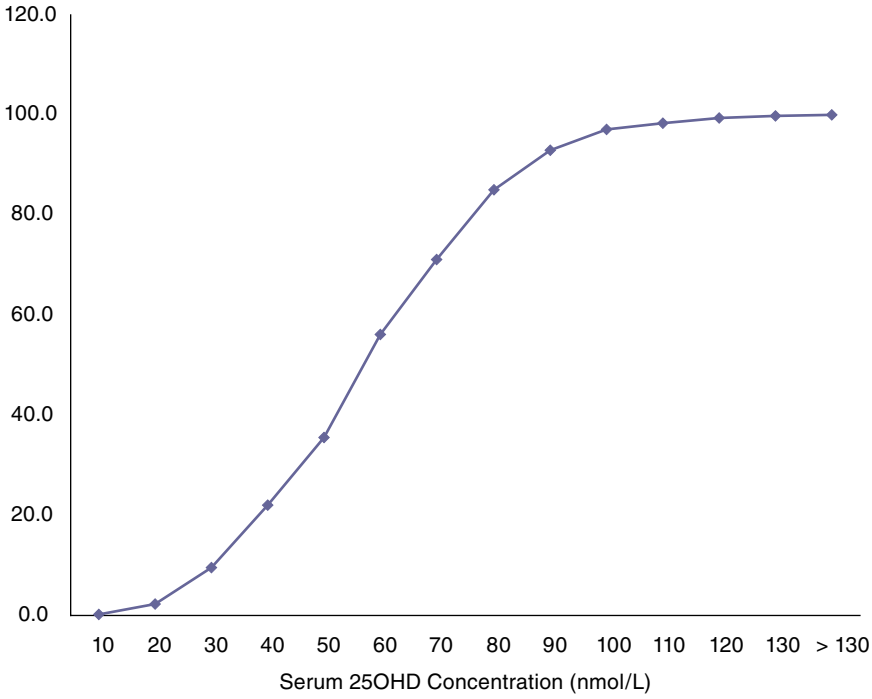


FIGURE I-7 Cumulative distribution of serum 25OHD (QC adjusted) for 51- to 70-year-olds in the United States for the 2003 to 2006 time period.
SOURCE: NHANES 2003–2006.

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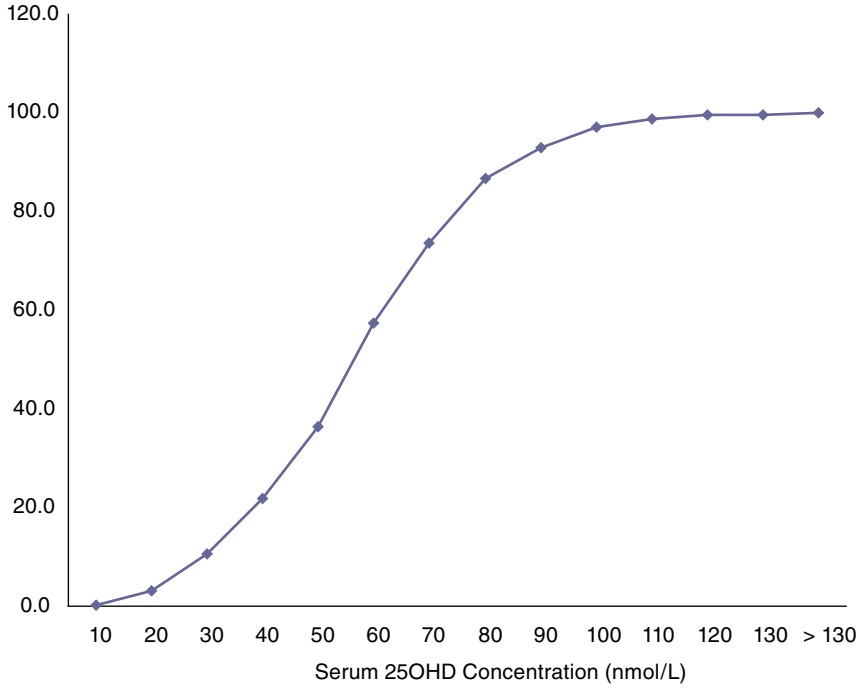


FIGURE I-8 Cumulative distribution of serum 25OHD (QC Aajusted) for > 70-year-olds in the United States for the 2003 to 2006 time period.
SOURCE: NHANES 2003–2006.

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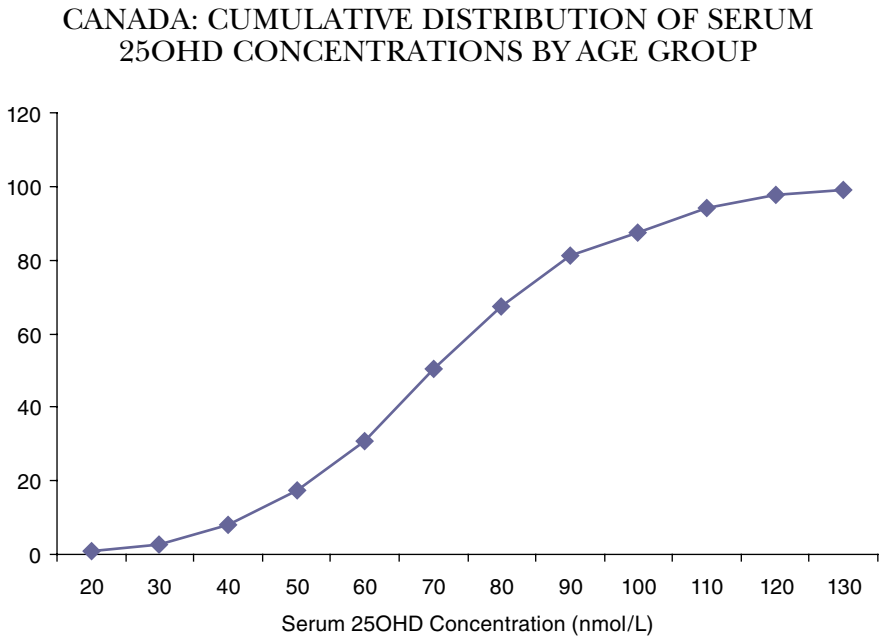


FIGURE I-9 Cumulative distribution of serum 25OHD for 9- to 13-year-olds in Canada for the 2007 to 2009 time period.
SOURCE: CHMS, Cycle 1, 2007–2009.

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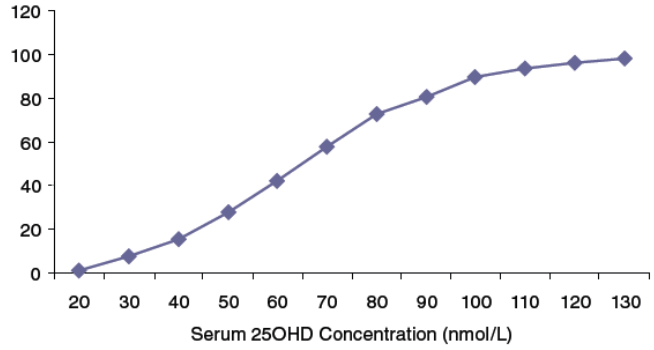


FIGURE I-10 Cumulative distribution of serum 25OHD for 14- to 18-year-olds in Canada for the 2007 to 2009 time period.
SOURCE: CHMS, Cycle 1, 2007–2009.

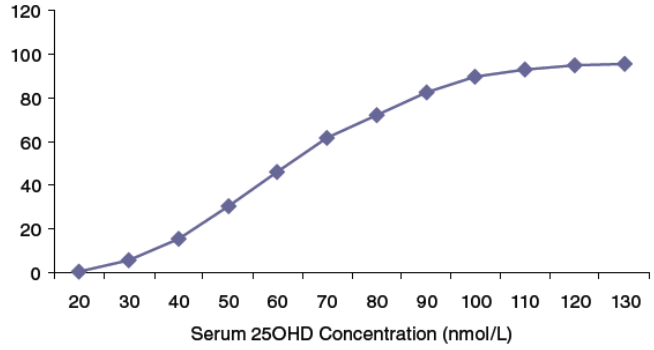


FIGURE I-11 Cumulative distribution of serum 25OHD for 19- to 30-year-olds in Canada for the 2007 to 2009 time period.
SOURCE: CHMS, Cycle 1, 2007–2009.

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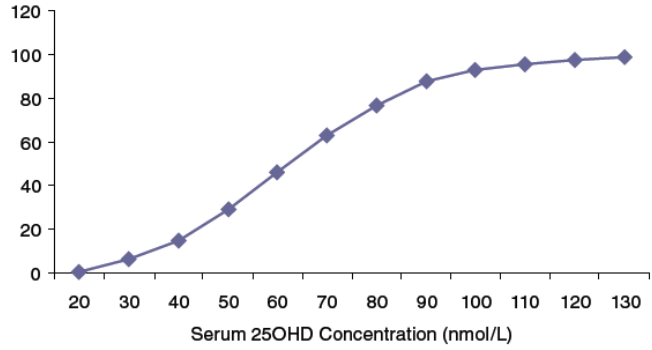


FIGURE I-12 Cumulative distribution of serum 25OHD for 31- to 50-year-olds in Canada for the 2007 to 2009 time period.
SOURCE: CHMS, Cycle 1, 2007–2009.

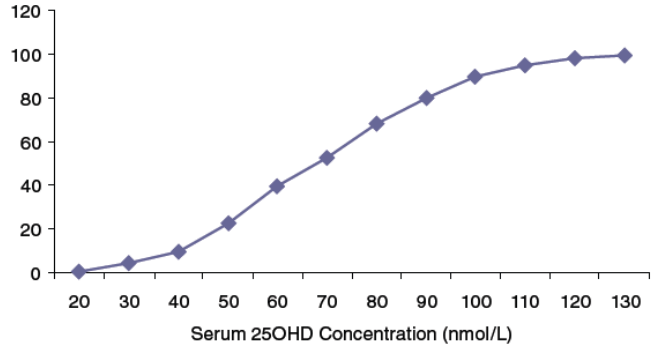


FIGURE I-13 Cumulative distribution of serum 25OHD for 51- to 70-year-olds in Canada for the 2007 to 2009 time period.
SOURCE: CHMS, Cycle 1, 2007–2009.

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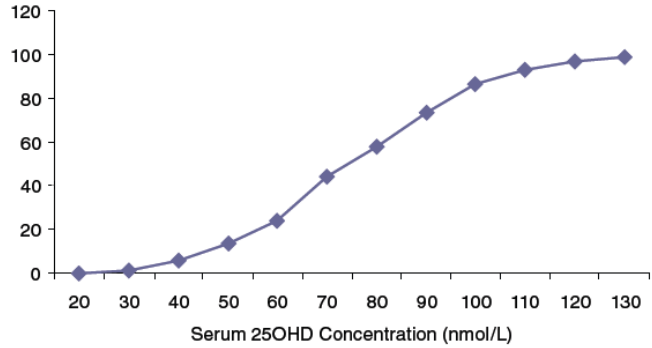


FIGURE I-14 Cumulative distribution of serum 25OHD for 71- to 79-year-olds in Canada for the 2007 to 2009 time period.
SOURCE: CHMS, Cycle 1, 2007–2009.

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Workshop Agenda and Open Session Agendas

IOM Committee to Review Dietary Reference Intakes for Vitamin D and Calcium
March 26, 2009
Room 100
500 Fifth Street NW, Washington DC
2:00 pm–4:30 pm

Open Session Agenda

2:00 pm	Welcome *Catharine Ross, Chairperson*
2:05 pm	Presentations from Study Sponsors: U.S. and Canadian Governments *David Klurfeld, U.S. Department of Agriculture/Agricultural Research Service* *Danielle Brule, Health Canada* *Kathryn McMurry, U.S. Department of Health and Human Services*
2:30 pm	—Discussion with Committee Members—
2:50 pm	Presentations on Survey Data Available to the Committee United States: *Margaret McDowell, National Center for Health Statistics*

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Canada: *Mary L'Abbe, Health Canada*

3:05 pm —Discussion with Committee Members—

3:20 pm Systematic Reviews in Nutrition/DRIs
Joseph Lau, Tufts Medical Center

3:40 pm —Discussion with Committee Members—

3:45 pm Analysis of Vitamin D in Food Control Materials and Fortified Foods
Wm. Craig Byrdwell, U.S. Department of Agriculture/Agricultural Research Service/Beltsville Human Nutrition Research Center

4:00 pm Analytical Issues for Detecting 25(OH)D in Serum
Christine Pfeiffer and Rosemary Schleicher, Centers for Disease Control and Prevention

4:15 pm —Joint Discussion with Committee Members—

4:40 pm Adjourn Open Session

Committee to Review Dietary Reference Intakes for Vitamin D and Calcium
500 Fifth Street NW, Washington DC, Room 100
August 4, 2009
8:00 am–5:00 pm

INFORMATION-GATHERING WORKSHOP AGENDA

7:30 am Registration and Check-in

8:00 am Welcome and Overview of Committee Process & Open Session
Catharine Ross, Chair

SESSION 1: Agency for Healthcare Research and Quality (ARHQ)

8:10 am Development of AHRQ Review: *Relationships of Vitamin D and Calcium Intakes to Nutrient Status Indicators and Health Outcomes* (released June 2009)
Joseph Lau, Tufts University

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8:35 am Committee Discussion with Dr. Lau and Tufts University Staff

SESSION 2: Analytical Issues: Vitamin D

9:15 am Comparison of Methods
Karen Phinney, National Institute of Standards and Technology (NIST)

9:35 am Analytical Issues: National Health and Nutrition Examination Survey (NHANES)
Clifford Johnson, National Center for Health Statistics (NCHS)

9:50 am Joint Discussion with Committee

10:15 am Break

SESSION 3: Biomarkers

10:30 am Biomarkers: General Principles for Definition and Utility as Measures of Exposure or Functional Outcome
Roberta Ness, University of Texas Health Science Center and IOM Committee on Biomarkers as Surrogate Endpoints of Chronic Disease Risk

10:50 am Committee Discussion with Dr. Ness

SESSION 4: Vascular Changes

11:00 am Vascular Changes Associated with Vitamin D and Calcium
Keith Hruska, Washington University, St. Louis

11:15 am Committee Discussion with Dr. Hruska

SESSION 5: Chronic Disease Endpoints: Observational Data versus Randomized Clinical Trials

11:35 am *Edward Giovannucci, Harvard University*
11:50 am *Barry Kramer, National Institutes of Health (NIH)*

12:05 pm Joint Discussion with Committee

12:30 pm **LUNCH**

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SESSION 6: Perspectives on Evaluating Data for Determining Reference Values for Vitamin D and Calcium

Perspectives from 1995-96 DRI Committee

1:30 pm *Stephanie Atkinson, McMaster University:* Perinatal
1:40 pm *Connie Weaver, Purdue University:* Adolescents
1:50 pm *Bess Dawson-Hughes, Tufts University:* Elderly
2:00 pm *Robert Heaney, Creighton University:* Calcium and Calcium/Vitamin D Interactions
2:10 pm *Michael Holick, Boston University:* Dietary vs. Solar Sources

2:20 pm Joint Discussion with Committee

2:45 pm Break

Perspectives from Other Vitamin D and Calcium Experts

3:00 pm *Bruce Hollis, Medical University of South Carolina:* Assay Methodologies
3:10 pm *Cedric Garland, University of California—San Diego:* Cancer
3:20 pm *Roger Bouillon, Katholieke Universiteit Leuven:* Immune Function
3:30 pm *Reinhold Vieth, University of Toronto:* Safety of Vitamin D

3:40 pm Joint Discussion with Committee

4:00 pm Break

SESSION 7: PUBLIC COMMENTS—*5 minutes each* (required pre-registration)

GrassrootsHealth *(Carole Baggerly)*
University of California—Riverside & Vitamin D Workshop *(Tony Norman)*
University of California—San Diego *(Edward Gorham)*
Sunlight, Nutrition, and Health Research Center *(William Grant)*

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St. Luke's-Roosevelt Hospital Center *(Linda Linday)*
Autoimmunity Research Foundation *(Amy Proal)*
Weill Cornell Medical College *(Paul Albert)*
The Mount Sinai Hospital and Mount Sinai School of Medicine *(Laurie Tansman)*
International Diary Foods Association *(Michelle Matto)*
National Osteoporosis Foundation *(Roberta Biegel)*
National Dairy Council *(Jill Nicholls)*
Lallemand/American Yeast *(James Kopp, Sr.)*

5:00 Workshop Adjourned

Committee to Review Dietary Reference Intakes for Vitamin D and Calcium
Informal Small-Group Data Gathering with Survey Representatives
October 22, 2009
4:30 pm
500 Fifth Street NW, Washington DC

Presentations and Discussions on U.S. and Canadian Survey Differences

PARTICIPANTS

United States

Mr. Clifford Johnson, National Center for Health Statistics, DHANES
Dr. Alanna Moshfegh, USDA Agricultural Research Service, Food Surveys
Dr. Joanne Holden, USDA Agricultural Research Service, Nutrient Data Lab

Canada (by telephone)

Dr. Steve Brooks, Bureau of Nutritional Sciences, Health Canada
Ms. Maya Villeneuve, Nutrition Research Division, Health Canada

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Committee to Review Dietary Reference Intakes for Vitamin D and Calcium
Fifth Meeting: November 19–20, 2009
The National Academy of Sciences
Auditorium
2100 C Street, NW
Washington, DC

Open Session Agenda

Thursday, November 19, 2009

Open Session—NAS Lecture Room

8:45 am	Welcome to Open Session *Catharine Ross, chair*
9:00 am	Open Session: Discussion of the Role of Vitamin D and Calcium in Kidney Function, Neophrocalcinosis, and Nephrolithiasis (Response to Targeted Questions)
I.	Epidemiology of Renal Toxicity: Vitamin D and Calcium *Gary Curhan, Harvard Medical School, Brigham & Women's Hospital*
II.	Pathophysiology of Renal Toxicity in Children *Craig Langman, Feinberg School of Medicine, Northwestern University, Chicago*
III.	Pathophysiology of Renal Toxicity in Adults *David Bushinsky, School of Medicine and Dentistry, University of Rochester*
10:00 am	Q&A with Committee Members
11:00 am	Adjourn open session

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Biographical Sketches of Committee Members

A. CATHARINE ROSS, Ph.D., is Professor and occupant of the Dorothy Foehr Huck Chair of Nutrition, Department of Nutritional Sciences, The Pennsylvania State University. Prior to her appointment at The Pennsylvania State University, she was with the Medical College of Pennsylvania. As a nutritional biochemist, Dr. Ross has studied cellular factors involved in the biosynthesis and transport of vitamin A molecules. Her focus has been on the interaction of cellular retinoid-binding proteins and enzymes that esterify retinol for transport, storage, and oxidation with the intent to link biochemical findings with nutritional studies to better understand how vitamin A homeostasis is regulated by dietary status and metabolic conditions. She also investigates the role of retinoids in immune function, principally antibody production. She currently serves as Editor-in-Chief of the Journal of Nutrition. She is past Associate Editor for both the Journal of Lipid Research and the 9th and 10th editions of Modern Nutrition in Health and Disease and has served on several other editorial boards for various scientific publications. Dr. Ross has received numerous awards including the Mead-Johnson Award from the American Institute of Nutrition and the Osborne and Mendel Award from the American Society for Nutritional Sciences; Dr. Ross is a Fellow of the American Association for the Advancement of Science. She is active within a range of professional societies including the American Association of Immunologists, Sigma Xi, and the American Physiological Society, and has served on a number of committees for the American Society for Nutrition and the Federation of the American Societies for Experimental Biology. She is also active on the National Institute of Health-National Institute of Diabetes and Digestive

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and Kidney Diseases Board of Scientific Counselors and is chair of the NIH Integrated Nutrition and Metabolic Processes Study Section. Dr. Ross is a member of the National Academy of Sciences (2003) and has served on the IOM Food and Nutrition Board (1997–2003), as a member of the Panel on Micronutrients for the Dietary Reference Intakes (1999–2001), and as a member of the Committee on Opportunities in the Nutrition of Food Sciences (1991–1993). Dr. Ross received her Ph.D. from Cornell University in biochemistry, molecular and cell biology.

STEVEN A. ABRAMS, M.D., is Professor of Pediatrics, Baylor College of Medicine, Houston. His research focus is mineral metabolism in infants as well as calcium intake and absorption in adolescents. His work includes the study of stable isotopes of iron and zinc and the overall relationship of mineral nutriture to health. His research is supported by the United States Department of Agriculture and by the NIH. Dr. Abrams, a neonatologist, is a Diplomat of the Board of Medical Examiners, the American Board of Pediatrics, and the Sub-board of Neonatal-Perinatal Medicine. He has received a number of awards including the Centrum Center for Nutrition Science Award from the American Society for Nutrition Sciences and the Norman Kretchmer Memorial Award in Nutrition and Development from the American Society for Clinical Nutrition. He is a member of an advisory panel for The Milk Processor Education Program (MilkPEP). He is an associate editor of The American Journal of Clinical Nutrition. Dr. Abrams is also a member of the American Society for Nutrition, the American Academy of Pediatrics, and the American Society for Bone and Mineral Research. Dr. Abrams has served as an IOM committee member for the Committee on the Use of Dietary Reference Intakes in Nutrition Labeling (2002–2003), the Panel on Calcium and Related Nutrients for Dietary Reference Intakes (1996–1997), and the Subcommitee on Upper Safe Reference Levels of Nutrients (1996–1997). Dr. Abrams received his medical degree from The Ohio State University College of Medicine.

JOHN F. ALOIA, M.D., is Chief Academic Officer, Department of Academic Affairs, Winthrop-University Hospital, Mineola, New York and is Professor of Medicine and Associate Dean at State University of New York at Stony Brook. Dr. Aloia's recent publications address differences in skeletal and muscle mass with aging in black and white women; optimal vitamin D status and serum parathyroid hormone in African American women; and the reference range for serum parathyroid hormone. His other research interest's center on bone metabolism and, in particular, issues related to pathogenic mechanisms responsible for the development of skeletal fragility and osteoporosis. The focus of this investigation is the influence of various regulatory factors on the skeleton. Dr. Aloia is the recipient of several

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awards and is first author on a range of peer-reviewed articles focusing on bone metabolism. He oversees an active research program designed to explore the use of drugs to treat osteoporosis and reverse low bone mineral density. Dr. Aloia receives research funding through the Empire Clinical Research Investigator Program, which awards competitive grants through the New York State Department of Health in support of physician training in the methodology, implementation, and evaluation of clinical research. The topic for this grant is the Response to Vitamin D in elderly African American Women. He has a research award from the NIH targeted to the study of vitamin D and osteoporosis prevention in elderly African American women. Previous NIH support included trials of estrogen and calcium, body composition in white and African American women, and vitamin D supplementation in African American women in midlife. Dr. Aloia is the recipient of a research grant from Merck (interaction between calcium and vitamin D intake in postmenopausal women). Dr. Aloia is also Principal Investigator at Winthrop-University Hospital for Unigene [TARSA] and Amgen Clinical Trials. Dr. Aloia participated in the NIH conference, "Vitamin D and Health in the 21st Century: An Update," held in Bethesda, Maryland, September 5–6, 2007. Dr. Aloia is a Diplomate of the National Board of Medical Examiners and is board certified in Internal Medicine and Endocrinology. He is also a member of several professional societies including the American Association of Clinical Endocrinologists, the American Association of Diabetes Educators, the American College of Physicians, the American Diabetes Association, the American Medical Association, the American Society for Bone and Mineral Research, the Endocrine Society, the International Bone and Mineral Society, and the National Osteoporosis Foundation. He received his medical degree from Creighton University Medical School, Omaha.

PATSY M. BRANNON, Ph.D., R.D., is Professor, Division of Nutritional Sciences, Cornell University where she has also served as Dean of the College of Human Ecology. Prior to moving to Cornell University, Dr. Brannon was Chair, Department of Nutrition and Food Science, University of Maryland. She has also served as Visiting Professor, Office of Dietary Supplements, NIH. Her research focus includes nutritional and metabolic regulation of gene expression, especially as relating to human development, the placenta, and exocrine pancreas. She chaired an NIH initiative to plan effective federal research related to the health effects of vitamin D; and has also co-chaired the NIH program "Vitamin D and Health in the 21st Century: An Update" as well as coordinated the vitamin D round table associated with the conference. Dr. Brannon is a member of a number of professional and scientific associations including the American Dietetics Association, the Institute of Food Technologists, and the American Association for the

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Advancement of Science. She has served on the Executive Board of the American Society for Nutrition and is a member of the technical expert panel on "The Relationships of Vitamin D and Calcium Intakes to Nutrient Status Indicators and Health Outcomes" for the Tufts Evidence-based Practice Center. Dr. Brannon has received numerous awards including the Pew Faculty Scholar in Nutrition award as well as the Centennial Laureate award from Florida State University. Dr. Brannon has published widely in the field and has more than 50 peer-reviewed journal articles to her credit. She received her Ph.D. from Cornell University in nutritional biochemistry.

STEVEN K. CLINTON, M.D., Ph.D., is Professor in the Department of Internal Medicine, Division of Medical Oncology at The Ohio State University. He is the Program Leader for the Molecular Carcinogenesis and Chemoprevention Program of the Comprehensive Cancer Center and serves the James Cancer Hospital as Director of Prostate and Genitourinary Oncology. Dr. Clinton is a faculty member of the campus wide Ohio State University Nutrition Graduate Program (OSUN) and is Co-Director of the Center for Advanced Functional Foods Research and Entrepreneurship. Dr. Clinton's research examines fundamental mechanisms underlying the development of cancer and studies novel prevention and therapeutic strategies in human clinical trails. His cancer research interests within nutritional sciences include the roles of energy intake, bioactive lipids, vitamin D, and carotenoids, and other phytochemicals. Dr. Clinton earned a Ph.D. from The University of Illinois at Urbana-Champaign in nutritional sciences followed by his medical degree at the same institution. After completing his internship and residency in internal medicine at the University of Chicago, he pursued subspecialty training in medical oncology at the Dana-Farber Cancer Institute and Harvard Medical School where he remained on the faculty prior to joining The Ohio State University in 1998. Dr. Clinton has received a number of awards, including The Emil Frei III Fellowship in Clinical Investigation, a Preventive Oncology Academic Award from the National Cancer Institute-NIH, and The Bertha Bouroncle Distinguished Faculty Teaching Award, and is a Fellow of the American Association for the Advancement of Science. He is a member of several professional organizations, including Advancing Science Serving Society, American Association for Cancer Research, American Society of Clinical Oncology, and American Society of Nephrology. Over the past three decades, Dr. Clinton's research has been supported by many organizations including the National Cancer Institute/NIH, the Department of Defense Congressionally Directed Medical Research Programs, The American Cancer Society, the American Institute for Cancer Research, the Lance Armstrong Foundation, and Development funds from the Arthur G. James Cancer Hospital.

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RAMON A. DURAZO-ARVIZU, Ph.D., is Associate Professor of Preventive Medicine and Epidemiology, Loyola University Chicago, Stritch School of Medicine. His focus is applied statistics including the analysis of time to an event data (survival analysis) and the analysis of longitudinal data. In addition, his expertise includes analysis of national data bases including the National Health Interview Survey (NHIS) and the National Health and Nutrition Examination Survey (NHANES). He has developed models to explain the relationship between body mass index and mortality in blacks and whites. His more recent survival analysis relates to vitamin D and mortality rates and related relationships concerning vitamin D and parathryroid hormone levels. Dr. Durazo-Arvizu is the author of more than 20 articles in peer-reviewed journals, has received two emerging investigative professionals awards, and is a member of the Society for the Advancement of Chicanos and Native Americans in Science, The American Statistical Association, The Royal Statistical Society, and The International Biometry Society. He received his Ph.D. from the University of Arizona in applied mathematics.

J. CHRISTOPHER GALLAGHER, M.D., is the Professor of Medicine and Chief of the Bone Metabolism Section at Creighton University Medical Center in Omaha, Nebraska. Dr. Gallagher is an endocrinologist who specializes in osteoporosis, menopause, vitamin D metabolism, and treatment. He is certified by the American Board of Internal Medicine and the English Board of Internal Medicine. He has participated in numerous clinical trials in osteoporosis and in menopausal women. His current research focus is dose ranging safety studies on vitamin D supplementation in older women and is funded by a grant from the National Institute on Aging and similar studies in younger women funded by the Department of Defense. In past research in the vitamin D area his group showed the impact of dietary factors such as calcium and caffeine on bone loss in elderly women and its interaction with the vitamin D receptor and studies showing that vitamin D metabolites can reduce falls in the elderly. Dr. Gallagher also receives clinical trial funding from Wyeth-Ayerst Laboratoris, AMGEN, and Unigene to test several prescription drugs and therapies under development. He has authored or co-authored more than 210 articles including 93 peer-reviewed journal articles, 10 book chapters, review articles, and presentations at meetings. He is a Past President of the North American Menopause Society (NAMS). He is the recipient of several awards including the Vitamin D Research Career Award from the International Vitamin D Society and the Creighton University Distinguished Career Award. Dr. Gallagher received his medical degree from Manchester University Medical School in England and his training in bone and vitamin D research at the Medical Research Council (MRC) Mineral Metabolism Unit in Leeds, England and at the Endocrine Research Unit at the Mayo Clinic.

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RICHARD L. GALLO, M.D., Ph.D., is Professor of Medicine and Pediatrics, Chief Division of Dermatology, University of California, San Diego and Chief of the Dermatology Section of the VA San Diego.. Dr. Gallo's major research interests are innate immune defense systems in skin by host defense peptides and glycosaminoglycans as well as mechanistic, diagnostic, and therapeutic implications of these molecules in human skin disease. He has written extensively on issues related to the physiology and pathology of skin immunology and is responsible for several landmark discoveries in the role of host defense peptides in human health including uncovering important functions for vitamin D in the immune system. He has been elected to the board of directors of the Association of Professor of Dermatology. In 2006 he received the Montagna Award from the Society of Investigative dermatology, and in 2007 he received the CE.R.I.E.S. Dermatology Research Award from the Centre de Recherches et d'Investigations Epidermiques et Sensorielles. Dr. Gallo is a member of the American Dermatology Association and the American Society of Clinical Investigation. He has authored or co-authored more than 125 peer-reviewed articles and has received numerous NIH research grants and research support from the Veterans Administration. Dr. Gallo received his medical degree from the University of Rochester School of Medicine and his Ph.D. from the University of Rochester in radiation biology and biophysics

GLENVILLE JONES, B.Sc., Ph.D., is Craine Professor and Head, Department of Biochemistry, Queen's University, Ontario, Canada. His research focus is vitamin D metabolism and mechanism of action. He has published more than 175 peer-reviewed journal articles related to vitamin D metabolism, vitamin D-related cytochrome P450s, and the analysis of vitamin D metabolites. He employs unique transfected cell models and knockout mouse models to study the activation or breakdown of calcitriol or retinoic acid with the long-term goal of establishing the structure and function of the cytochrome P-450 enzymes involved in the complex metabolic pathways of calcitriol or retinoic acid. His laboratory has been supported by grants from the Canadian Institute of Health Research for more than 30 years. Dr. Jones serves on the Scientific Advisory Board of the not-for-profit Vitamin D External Quality Assessment Scheme and on the Scientific Advisory Board of Cytochroma, Inc., an applied genomics and drug discovery company focused on cytochrome P450 genes and the function of the proteins encoded by those genes in order to address unmet medical needs. He holds one non-competitive grant from Cytochroma, Inc. to study calcitriol analogs and cytochrome P450 inhibitors used for the treatment of renal disease. Dr. Jones is a member of several societies including the American Society for Bone and Mineral Research, the Canadian Society for Clinical Investigation, the Canadian Society for Nutritional Sciences, and the Canadian

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Society for Endocrinology and Metabolism. He is the recipient of a Vitamin Career Achievement Award from the international Vitamin D community. He also sits on the Scientific Program Organizing Committee of the 14th Workshop on Vitamin D, is a member of the expert panel on "The Relationships of Vitamin D and Calcium Intakes to Nutrient Status Indicators and Health Outcomes" for the Tufts Evidence-based Practice Center, and is a member of the Genzyme Speaker's Bureau. Dr. Jones received his Ph.D. from Liverpool University, England.

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Summary Tables

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Tolerable Upper Intake Levels, Elements

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Dietary Reference Intakes (DRIs): Estimated Average Requirements
Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Calcium (mg/d)	CHO (g/kg/d)	Protein (g/d)	Vit A (μg/d)[a]	Vit C (mg/d)	Vit D (μg/d)	Vit E (mg/d)[b]	Thiamin (mg/d)	Riboflavin (mg/d)	Niacin (mg/d)[c]
Infants										
0–6 mo										
6–12 mo			1.0							
Children										
1–3 y	500	100	0.87	210	13	10	5	0.4	0.4	5
4–8 y	800	100	0.76	275	22	10	6	0.5	0.5	6
Males										
9–13 y	1,100	100	0.76	445	39	10	9	0.7	0.8	9
14–18 y	1,100	100	0.73	630	63	10	12	1.0	1.1	12
19–30 y	800	100	0.66	625	75	10	12	1.0	1.1	12
31–50 y	800	100	0.66	625	75	10	12	1.0	1.1	12
51–70 y	800	100	0.66	625	75	10	12	1.0	1.1	12
> 70 y	1,000	100	0.66	625	75	10	12	1.0	1.1	12
Females										
9–13 y	1,100	100	0.76	420	39	10	9	0.7	0.8	9
14–18 y	1,100	100	0.71	485	56	10	12	0.9	0.9	11
19–30 y	800	100	0.66	500	60	10	12	0.9	0.9	11
31–50 y	800	100	0.66	500	60	10	12	0.9	0.9	11
51–70 y	1,000	100	0.66	500	60	10	12	0.9	0.9	11
> 70 y	1,000	100	0.66	500	60	10	12	0.9	0.9	11
Pregnancy										
14–18 y	1,000	135	0.88	530	66	10	12	1.2	1.2	14
19–30 y	800	135	0.88	550	70	10	12	1.2	1.2	14
31–50 y	800	135	0.88	550	70	10	12	1.2	1.2	14
Lactation										
14–18 y	1,000	160	1.05	885	96	10	16	1.2	1.3	13
19–30 y	800	160	1.05	900	100	10	16	1.2	1.3	13
31–50 y	800	160	1.05	900	100	10	16	1.2	1.3	13

NOTE: An Estimated Average Requirement (EAR) is the average daily nutrient intake level estimated to meet the requirements of half of the healthy individuals in a group. EARs have not been established for vitamin K, pantothenic acid, biotin, choline, chromium, fluoride, manganese, or other nutrients not yet evaluated via the DRI process.

[a]As retinol activity equivalents (RAEs). 1 RAE = 1 μg retinol, 12 μg β-carotene, 24 μg α-carotene, or 24 μg β-cryptoxanthin. The RAE for dietary provitamin A carotenoids is two-fold greater than retinol equivalents (RE), whereas the RAE for preformed vitamin A is the same as RE.

[b]As α-tocopherol. α-tocopherol includes *RRR*-α-tocopherol, the only form of α-tocopherol that occurs naturally in foods, and the *2R*-stereoisomeric forms of α-tocopherol (*RRR*-, *RSR*-, *RRS*-, and *RSS*-α-tocopherol) that occur in fortified foods and supplements. It does not include the *2S*-stereoisomeric forms of α-tocopherol (*SRR*-, *SSR*-, *SRS*-, and *SSS*-α-tocopherol), also found in fortified foods and supplements.

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Vit B_6 (mg/d)	Folate (µg/d)[d]	Vit B_{12} (µg/d)	Copper (µg/d)	Iodine (µg/d)	Iron (mg/d)	Magnesium (mg/d)	Molybdenum (µg/d)	Phosphorus (mg/d)	Selenium (µg/d)	Zinc (mg/d)
					6.9					2.5
0.4	120	0.7	260	65	3.0	65	13	380	17	2.5
0.5	160	1.0	340	65	4.1	110	17	405	23	4.0
0.8	250	1.5	540	73	5.9	200	26	1,055	35	7.0
1.1	330	2.0	685	95	7.7	340	33	1,055	45	8.5
1.1	320	2.0	700	95	6	330	34	580	45	9.4
1.1	320	2.0	700	95	6	350	34	580	45	9.4
1.4	320	2.0	700	95	6	350	34	580	45	9.4
1.4	320	2.0	700	95	6	350	34	580	45	9.4
0.8	250	1.5	540	73	5.7	200	26	1,055	35	7.0
1.0	330	2.0	685	95	7.9	300	33	1,055	45	7.3
1.1	320	2.0	700	95	8.1	255	34	580	45	6.8
1.1	320	2.0	700	95	8.1	265	34	580	45	6.8
1.3	320	2.0	700	95	5	265	34	580	45	6.8
1.3	320	2.0	700	95	5	265	34	580	45	6.8
1.6	520	2.2	785	160	23	335	40	1,055	49	10.5
1.6	520	2.2	800	160	22	290	40	580	49	9.5
1.6	520	2.2	800	160	22	300	40	580	49	9.5
1.7	450	2.4	985	209	7	300	35	1,055	59	10.9
1.7	450	2.4	1,000	209	6.5	255	36	580	59	10.4
1.7	450	2.4	1,000	209	6.5	265	36	580	59	10.4

[c]As niacin equivalents (NE). 1 mg of niacin = 60 mg of tryptophan.

[d]As dietary folate equivalents (DFE). 1 DFE = 1 µg food folate = 0.6 µg of folic acid from fortified food or as a supplement consumed with food = 0.5 µg of a supplement taken on an empty stomach.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids* (2002/2005); and *Dietary Reference Intakes for Calcium and Vitamin D* (2011). These reports may be accessed via www.nap.edu.

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Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Vitamins

Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Vitamin A (μg/d)[a]	Vitamin C (mg/d)	Vitamin D (μg/d)[b,c]	Vitamin E (mg/d)[d]	Vitamin K (μg/d)	Thiamin (mg/d)
Infants						
0–6 mo	400*	40*	10*	4*	2.0*	0.2*
6–12 mo	500*	50*	10*	5*	2.5*	0.3*
Children						
1–3 y	**300**	**15**	**15**	**6**	30*	**0.5**
4–8 y	**400**	**25**	**15**	**7**	55*	**0.6**
Males						
9–13 y	**600**	**45**	**15**	**11**	60*	**0.9**
14–18 y	**900**	**75**	**15**	**15**	75*	**1.2**
19–30 y	**900**	**90**	**15**	**15**	120*	**1.2**
31–50 y	**900**	**90**	**15**	**15**	120*	**1.2**
51–70 y	**900**	**90**	**15**	**15**	120*	**1.2**
> 70 y	**900**	**90**	**20**	**15**	120*	**1.2**
Females						
9–13 y	**600**	**45**	**15**	**11**	60*	**0.9**
14–18 y	**700**	**65**	**15**	**15**	75*	**1.0**
19–30 y	**700**	**75**	**15**	**15**	90*	**1.1**
31–50 y	**700**	**75**	**15**	**15**	90*	**1.1**
51–70 y	**700**	**75**	**15**	**15**	90*	**1.1**
> 70 y	**700**	**75**	**20**	**15**	90*	**1.1**
Pregnancy						
14–18 y	**750**	**80**	**15**	**15**	75*	**1.4**
19–30 y	**770**	**85**	**15**	**15**	90*	**1.4**
31–50 y	**770**	**85**	**15**	**15**	90*	**1.4**
Lactation						
14–18 y	**1,200**	**115**	**15**	**19**	75*	**1.4**
19–30 y	**1,300**	**120**	**15**	**19**	90*	**1.4**
31–50 y	**1,300**	**120**	**15**	**19**	90*	**1.4**

NOTE: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97–98 percent) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breast-fed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake.

[a]As retinol activity equivalents (RAEs). 1 RAE = 1 μg retinol, 12 μg β-carotene, 24 μg α-carotene, or 24 μg β-cryptoxanthin. The RAE for dietary provitamin A carotenoids is two-fold greater than retinol equivalents (REs), whereas the RAE for preformed vitamin A is the same as RE.

[b]As cholecalciferol. 1 μg cholecalciferol = 40 IU vitamin D.

[c]Under the assumption of minimal sunlight.

[d]As α-tocopherol. α-tocopherol includes *RRR*-α-tocopherol, the only form of α-tocopherol that occurs naturally in foods, and the *2R*-stereoisomeric forms of α-tocopherol (*RRR*-, *RSR*-, *RRS*-, and *RSS*-α-tocopherol) that occur in fortified foods and supplements. It does not include the *2S*-stereoisomeric forms of α-tocopherol (*SRR*-, *SSR*-, *SRS*-, and *SSS*-α-tocopherol), also found in fortified foods and supplements.

[e]As niacin equivalents (NE). 1 mg of niacin = 60 mg of tryptophan; 0–6 months = preformed niacin (not NE).

[f]As dietary folate equivalents (DFE). 1 DFE = 1 μg food folate = 0.6 μg of folic acid from fortified food or as a supplement consumed with food = 0.5 μg of a supplement taken on an empty stomach.

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Riboflavin (mg/d)	Niacin (mg/d)[e]	Vitamin B_6 (mg/d)	Folate (μg/d)[f]	Vitamin B_{12} (μg/d)	Pantothenic Acid (mg/d)	Biotin (μg/d)	Choline (mg/d)[g]
0.3*	2*	0.1*	65*	0.4*	1.7*	5*	125*
0.4*	4*	0.3*	80*	0.5*	1.8*	6*	150*
0.5	**6**	**0.5**	**150**	**0.9**	2*	8*	200*
0.6	**8**	**0.6**	**200**	**1.2**	3*	12*	250*
0.9	**12**	**1.0**	**300**	**1.8**	4*	20*	375*
1.3	**16**	**1.3**	**400**	**2.4**	5*	25*	550*
1.3	**16**	**1.3**	**400**	**2.4**	5*	30*	550*
1.3	**16**	**1.3**	**400**	**2.4**	5*	30*	550*
1.3	**16**	**1.7**	**400**	**2.4**[h]	5*	30*	550*
1.3	**16**	**1.7**	**400**	**2.4**[h]	5*	30*	550*
0.9	**12**	**1.0**	**300**	**1.8**	4*	20*	375*
1.0	**14**	**1.2**	**400**[i]	**2.4**	5*	25*	400*
1.1	**14**	**1.3**	**400**[i]	**2.4**	5*	30*	425*
1.1	**14**	**1.3**	**400**[i]	**2.4**	5*	30*	425*
1.1	**14**	**1.5**	**400**	**2.4**[h]	5*	30*	425*
1.1	**14**	**1.5**	**400**	**2.4**[h]	5*	30*	425*
1.4	**18**	**1.9**	**600**[j]	**2.6**	6*	30*	450*
1.4	**18**	**1.9**	**600**[j]	**2.6**	6*	30*	450*
1.4	**18**	**1.9**	**600**[j]	**2.6**	6*	30*	450*
1.6	**17**	**2.0**	**500**	**2.8**	7*	35*	550*
1.6	**17**	**2.0**	**500**	**2.8**	7*	35*	550*
1.6	**17**	**2.0**	**500**	**2.8**	7*	35*	550*

[g]Although AIs have been set for choline, there are few data to assess whether a dietary supply of choline is needed at all stages of the life cycle, and it may be that the choline requirement can be met by endogenous synthesis at some of these stages.

[h]Because 10 to 30 percent of older people may malabsorb food-bound B_{12}, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with B_{12} or a supplement containing B_{12}.

[i]In view of evidence linking folate intake with neural tube defects in the fetus, it is recommended that all women capable of becoming pregnant consume 400 μg from supplements or fortified foods in addition to intake of food folate from a varied diet.

[j]It is assumed that women will continue consuming 400 μg from supplements or fortified food until their pregnancy is confirmed and they enter prenatal care, which ordinarily occurs after the end of the periconceptional period—the critical time for formation of the neural tube.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate* (2005); and *Dietary Reference Intakes for Calcium and Vitamin D* (2011). These reports may be accessed via www.nap.edu.

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Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements
Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Calcium (mg/d)	Chromium (µg/d)	Copper (µg/d)	Fluoride (mg/d)	Iodine (µg/d)	Iron (mg/d)	Magnesium (mg/d)	Manganese (mg/d)
Infants								
0–6 mo	200*	0.2*	200*	0.01*	110*	0.27*	30*	0.003*
6–12 mo	260*	5.5*	220*	0.5*	130*	**11**	75*	0.6*
Children								
1–3 y	**700**	11*	**340**	0.7*	**90**	**7**	**80**	1.2*
4–8 y	**1,000**	15*	**440**	1*	**90**	**10**	**130**	1.5*
Males								
9–13 y	**1,300**	25*	**700**	2*	**120**	**8**	**240**	1.9*
14–18 y	**1,300**	35*	**890**	3*	**150**	**11**	**410**	2.2*
19–30 y	**1,000**	35*	**900**	4*	**150**	**8**	**400**	2.3*
31–50 y	**1,000**	35*	**900**	4*	**150**	**8**	**420**	2.3*
51–70 y	**1,000**	30*	**900**	4*	**150**	**8**	**420**	2.3*
> 70 y	**1,200**	30*	**900**	4*	**150**	**8**	**420**	2.3*
Females								
9–13 y	**1,300**	21*	**700**	2*	**120**	**8**	**240**	1.6*
14–18 y	**1,300**	24*	**890**	3*	**150**	**15**	**360**	1.6*
19–30 y	**1,000**	25*	**900**	3*	**150**	**18**	**310**	1.8*
31–50 y	**1,000**	25*	**900**	3*	**150**	**18**	**320**	1.8*
51–70 y	**1,200**	20*	**900**	3*	**150**	**8**	**320**	1.8*
> 70 y	**1,200**	20*	**900**	3*	**150**	**8**	**320**	1.8*
Pregnancy								
14–18 y	**1,300**	29*	**1,000**	3*	**220**	**27**	**400**	2.0*
19–30 y	**1,000**	30*	**1,000**	3*	**220**	**27**	**350**	2.0*
31–50 y	**1,000**	30*	**1,000**	3*	**220**	**27**	**360**	2.0*
Lactation								
14–18 y	**1,300**	44*	**1,300**	3*	**290**	**10**	**360**	2.6*
19–30 y	**1,000**	45*	**1,300**	3*	**290**	**9**	**310**	2.6*
31–50 y	**1,000**	45*	**1,300**	3*	**290**	**9**	**320**	2.6*

NOTE: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97–98 percent) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breast-fed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake.

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Molybdenum (µg/d)	Phosphorus (mg/d)	Selenium (µg/d)	Zinc (mg/d)	Potassium (g/d)	Sodium (g/d)	Chloride (g/d)
2*	100*	15*	2*	0.4*	0.12*	0.18*
3*	275*	20*	**3**	0.7*	0.37*	0.57*
17	**460**	**20**	**3**	3.0*	1.0*	1.5*
22	**500**	**30**	**5**	3.8*	1.2*	1.9*
34	**1,250**	**40**	**8**	4.5*	1.5*	2.3*
43	**1,250**	**55**	**11**	4.7*	1.5*	2.3*
45	**700**	**55**	**11**	4.7*	1.5*	2.3*
45	**700**	**55**	**11**	4.7*	1.5*	2.3*
45	**700**	**55**	**11**	4.7*	1.3*	2.0*
45	**700**	**55**	**11**	4.7*	1.2*	1.8*
34	**1,250**	**40**	**8**	4.5*	1.5*	2.3*
43	**1,250**	**55**	**9**	4.7*	1.5*	2.3*
45	**700**	**55**	**8**	4.7*	1.5*	2.3*
45	**700**	**55**	**8**	4.7*	1.5*	2.3*
45	**700**	**55**	**8**	4.7*	1.3*	2.0*
45	**700**	**55**	**8**	4.7*	1.2*	1.8*
50	**1,250**	**60**	**12**	4.7*	1.5*	2.3*
50	**700**	**60**	**11**	4.7*	1.5*	2.3*
50	**700**	**60**	**11**	4.7*	1.5*	2.3*
50	**1,250**	**70**	**13**	5.1*	1.5*	2.3*
50	**700**	**70**	**12**	5.1*	1.5*	2.3*
50	**700**	**70**	**12**	5.1*	1.5*	2.3*

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate* (2005); and *Dietary Reference Intakes for Calcium and Vitamin D* (2011). These reports may be accessed via www.nap.edu.

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Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Total Water and Macronutrients
Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Total Water[a] (L/d)	Carbohydrate (g/d)	Total Fiber (g/d)	Fat (g/d)	Linoleic Acid (g/d)	α-Linolenic Acid (g/d)	Protein[b] (g/d)
Infants							
0–6 mo	0.7*	60*	ND	31*	4.4*	0.5*	9.1*
6–12 mo	0.8*	95*	ND	30*	4.6*	0.5*	**11.0**
Children							
1–3 y	1.3*	**130**	19*	ND[c]	7*	0.7*	**13**
4–8 y	1.7*	**130**	25*	ND	10*	0.9*	**19**
Males							
9–13 y	2.4*	**130**	31*	ND	12*	1.2*	**34**
14–18 y	3.3*	**130**	38*	ND	16*	1.6*	**52**
19–30 y	3.7*	**130**	38*	ND	17*	1.6*	**56**
31–50 y	3.7*	**130**	38*	ND	17*	1.6*	**56**
51–70 y	3.7*	**130**	30*	ND	14*	1.6*	**56**
> 70 y	3.7*	**130**	30*	ND	14*	1.6*	**56**
Females							
9–13 y	2.1*	**130**	26*	ND	10*	1.0*	**34**
14–18 y	2.3*	**130**	26*	ND	11*	1.1*	**46**
19–30 y	2.7*	**130**	25*	ND	12*	1.1*	**46**
31–50 y	2.7*	**130**	25*	ND	12*	1.1*	**46**
51–70 y	2.7*	**130**	21*	ND	11*	1.1*	**46**
> 70 y	2.7*	**130**	21*	ND	11*	1.1*	**46**
Pregnancy							
14–18 y	3.0*	**175**	28*	ND	13*	1.4*	**71**
19–30 y	3.0*	**175**	28*	ND	13*	1.4*	**71**
31–50 y	3.0*	**175**	28*	ND	13*	1.4*	**71**
Lactation							
14–18	3.8*	**210**	29*	ND	13*	1.3*	**71**
19–30 y	3.8*	**210**	29*	ND	13*	1.3*	**71**
31–50 y	3.8*	**210**	29*	ND	13*	1.3*	**71**

NOTE: This table (take from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDA) in **bold type** and Adequate Intakes (AI) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97–98 percent) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breast-fed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake.

[a]Total water includes all water contained in food, beverages, and drinking water.

[b]Based on g protein per kg of body weight for the reference body weight, e.g., for adults 0.8 g/kg body weight for the reference body weight.

[c]Not determined.

SOURCE: *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids* (2002/2005) and *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate* (2005). The report may be accessed via www.nap.edu.

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Dietary Reference Intakes (DRIs): Acceptable Macronutrient Distribution Ranges

Food and Nutrition Board, Institute of Medicine, National Academies

	Range (percent of energy)		
Macronutrient	Children, 1–3 y	Children, 4–18 y	Adults
Fat	30–40	25–35	20–35
n-6 polyunsaturated fatty acids[a] (linoleic acid)	5–10	5–10	5–10
n-3 polyunsaturated fatty acids[a] (α-linolenic acid)	0.6–1.2	0.6–1.2	0.6–1.2
Carbohydrate	45–65	45–65	45–65
Protein	5–20	10–30	10–35

[a]Approximately 10 percent of the total can come from longer-chain *n*-3 or *n*-6 fatty acids.
SOURCE: *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids* (2002/2005). The report may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Additional Macronutrient Recommendations

Food and Nutrition Board, Institute of Medicine, National Academies

Macronutrient	Recommendation
Dietary cholesterol	As low as possible while consuming a nutritionally adequate diet
Trans fatty acids	As low as possible while consuming a nutritionally adequate diet
Saturated fatty acids	As low as possible while consuming a nutritionally adequate diet
Added sugars[a]	Limit to no more than 25% of total energy

[a]Not a recommended intake. A daily intake of added sugars that individuals should aim for to achieve a healthful diet was not set.
SOURCE: *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids* (2002/2005). The report may be accessed via www.nap.edu.

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Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Vitamins
Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Vitamin A (μg/d)[a]	Vitamin C (mg/d)	Vitamin D (μg/d)	Vitamin E (mg/d)[b,c]	Vitamin K	Thiamin	Riboflavin
Infants							
0–6 mo	600	ND[e]	25	ND	ND	ND	ND
6–12 mo	600	ND	38	ND	ND	ND	ND
Children							
1–3 y	600	400	63	200	ND	ND	ND
4–8 y	900	650	75	300	ND	ND	ND
Males							
9–13 y	1,700	1,200	100	600	ND	ND	ND
14–18 y	2,800	1,800	100	800	ND	ND	ND
19–30 y	3,000	2,000	100	1,000	ND	ND	ND
31–50 y	3,000	2,000	100	1,000	ND	ND	ND
51–70 y	3,000	2,000	100	1,000	ND	ND	ND
> 70 y	3,000	2,000	100	1,000	ND	ND	ND
Females							
9–13 y	1,700	1,200	100	600	ND	ND	ND
14–18 y	2,800	1,800	100	800	ND	ND	ND
19–30 y	3,000	2,000	100	1,000	ND	ND	ND
31–50 y	3,000	2,000	100	1,000	ND	ND	ND
51–70 y	3,000	2,000	100	1,000	ND	ND	ND
> 70 y	3,000	2,000	100	1,000	ND	ND	ND
Pregnancy							
14–18 y	2,800	1,800	100	800	ND	ND	ND
19–30 y	3,000	2,000	100	1,000	ND	ND	ND
31–50 y	3,000	2,000	100	1,000	ND	ND	ND
Lactation							
14–18 y	2,800	1,800	100	800	ND	ND	ND
19–30 y	3,000	2,000	100	1,000	ND	ND	ND
31–50 y	3,000	2,000	100	1,000	ND	ND	ND

NOTE: A Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, and carotenoids. In the absence of a UL, extra caution may be warranted in consuming levels above recommended intakes. Members of the general population should be advised not to routinely exceed the UL. The UL is not meant to apply to individuals who are treated with the nutrient under medical supervision or to individuals with predisposing conditions that modify their sensitivity to the nutrient.

[a]As preformed vitamin A only.

[b]As α-tocopherol; applies to any form of supplemental α-tocopherol.

[c]The ULs for vitamin E, niacin, and folate apply to synthetic forms obtained from supplements, fortified foods, or a combination of the two.

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Niacin (mg/d)[c]	Vitamin B_6 (mg/d)	Folate (µg/d)[c]	Vitamin B_{12}	Pantothenic Acid	Biotin	Choline (g/d)	Carotenoids[d]
ND	ND	ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND	ND	ND
10	30	300	ND	ND	ND	1.0	ND
15	40	400	ND	ND	ND	1.0	ND
20	60	600	ND	ND	ND	2.0	ND
30	80	800	ND	ND	ND	3.0	ND
35	100	1,000	ND	ND	ND	3.5	ND
35	100	1,000	ND	ND	ND	3.5	ND
35	100	1,000	ND	ND	ND	3.5	ND
35	100	1,000	ND	ND	ND	3.5	ND
20	60	600	ND	ND	ND	2.0	ND
30	80	800	ND	ND	ND	3.0	ND
35	100	1,000	ND	ND	ND	3.5	ND
35	100	1,000	ND	ND	ND	3.5	ND
35	100	1,000	ND	ND	ND	3.5	ND
35	100	1,000	ND	ND	ND	3.5	ND
30	80	800	ND	ND	ND	3.0	ND
35	100	1,000	ND	ND	ND	3.5	ND
35	100	1,000	ND	ND	ND	3.5	ND
30	80	800	ND	ND	ND	3.0	ND
35	100	1,000	ND	ND	ND	3.5	ND
35	100	1,000	ND	ND	ND	3.5	ND

[d]β-Carotene supplements are advised only to serve as a provitamin A source for individuals at risk of vitamin A deficiency.

[c]ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamine E, Selenium, and Carotenoids* (2000); *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); and *Dietary Reference Intakes for Calcium and Vitamin D* (2011). These reports may be accessed via www.nap.edu.

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Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Elements
Food and Nutrition Board, Institute of Medicine, National Academies

Life Stage Group	Arsenic[a]	Boron (mg/d)	Calcium (mg/d)	Chromium	Copper (µg/d)	Fluoride (mg/d)	Iodine (µg/d)	Iron (mg/d)
Infants								
0–6 mo	ND[e]	ND	1,000	ND	ND	0.7	ND	40
6–12 mo	ND	ND	1,500	ND	ND	0.9	ND	40
Children								
1–3 y	ND	3	2,500	ND	1,000	1.3	200	40
4–8 y	ND	6	2,500	ND	3,000	2.2	300	40
Males								
9–13 y	ND	11	3,000	ND	5,000	10	600	40
14–18 y	ND	17	3,000	ND	8,000	10	900	45
19–30 y	ND	20	2,500	ND	10,000	10	1,100	45
31–50 y	ND	20	2,500	ND	10,000	10	1,100	45
51–70 y	ND	20	2,000	ND	10,000	10	1,100	45
> 70 y	ND	20	2,000	ND	10,000	10	1,100	45
Females								
9–13 y	ND	11	3,000	ND	5,000	10	600	40
14–18 y	ND	17	3,000	ND	8,000	10	900	45
19–30 y	ND	20	2,500	ND	10,000	10	1,100	45
31–50 y	ND	20	2,500	ND	10,000	10	1,100	45
51–70 y	ND	20	2,000	ND	10,000	10	1,100	45
> 70 y	ND	20	2,000	ND	10,000	10	1,100	45
Pregnancy								
14–18 y	ND	17	3,000	ND	8,000	10	900	45
19–30 y	ND	20	2,500	ND	10,000	10	1,100	45
61–50 y	ND	20	2,500	ND	10,000	10	1,100	45
Lactation								
14–18 y	ND	17	3,000	ND	8,000	10	900	45
19–30 y	ND	20	2,500	ND	10,000	10	1,100	45
31–50 y	ND	20	2,500	ND	10,000	10	1,100	45

NOTE: A Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, and carotenoids. In the absence of a UL, extra caution may be warranted in consuming levels above recommended intakes. Members of the general population should be advised not to routinely exceed the UL. The UL is not meant to apply to individuals who are treated with the nutrient under medical supervision or to individuals with predisposing conditions that modify their sensitivity to the nutrient.

[a]Although the UL was not determined for arsenic, there is no justification for adding arsenic to food or supplements.

[b]The ULs for magnesium represent intake from a pharmacological agent only and do not include intake from food and water.

[c]Although silicon has not been shown to cause adverse effects in humans, there is no justification for adding silicon to supplements.

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	Magnesium (mg/d)[b]	Manganese (mg/d)	Molybdenum (µg/d)	Nickel (mg/d)	Phosphorus (g/d)	Selenium (µg/d)	Silicon[c]	Vanadium (mg/d)[d]	Zinc (mg/d)	Sodium (g/d)	Chloride (g/d)
	ND	ND	ND	ND	ND	45	ND	ND	4	ND	ND
	ND	ND	ND	ND	ND	60	ND	ND	5	ND	ND
	65	2	300	0.2	3	90	ND	ND	7	1.5	2.3
	110	3	600	0.3	3	150	ND	ND	12	1.9	2.9
	350	6	1,100	0.6	4	280	ND	ND	23	2.2	3.4
	350	9	1,700	1.0	4	400	ND	ND	34	2.3	3.6
	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
	350	11	2,000	1.0	3	400	ND	1.8	40	2.3	3.6
	350	6	1,100	0.6	4	280	ND	ND	23	2.2	3.4
	350	9	1,700	1.0	4	400	ND	ND	34	2.3	3.6
	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
	350	11	2,000	1.0	4	400	ND	1.8	40	2.3	3.6
	350	11	2,000	1.0	3	400	ND	1.8	40	2.3	3.6
	350	9	1,700	1.0	3.5	400	ND	ND	34	2.3	3.6
	350	11	2,000	1.0	3.5	400	ND	ND	40	2.3	3.6
	350	11	2,000	1.0	3.5	400	ND	ND	40	2.3	3.6
	350	9	1,700	1.0	4	400	ND	ND	34	2.3	3.6
	350	11	2,000	1.0	4	400	ND	ND	40	2.3	3.6
	350	11	2,000	1.0	4	400	ND	ND	40	2.3	3.6

[d]Although vanadium in food has not been shown to cause adverse effects in humans, there is no justification for adding vanadium to food, and vanadium supplements should be used with caution. The UL is based on adverse effects in laboratory animals, and this data could be used to set a UL for adults but not children and adolescents.

[c]ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate* (2005); and *Dietary Reference Intakes for Calcium and Vitamin D* (2011). These reports may be accessed via www.nap.edu.

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Exhibit I

VITAMIN D



Skin conditions overview Indoor tanning Melanoma Skin cancer Sunscreen Vitamin D



Vitamin D stats and facts

- The American Academy of Dermatology recommends that healthy adults should obtain an adequate amount of Vitamin D from a diet that includes foods naturally rich in vitamin D and/or foods/beverages fortified with vitamin D.
- Because ultraviolet rays from the sun and tanning beds can cause skin cancer, the American Academy of Dermatology does not recommend getting vitamin D from sun exposure or indoor tanning.
    - The U.S. Department of Health and Human Services and the World Health Organization's International Agency of Research on Cancer panel have declared UV radiation from the sun and artificial sources, such as tanning beds and sun lamps, to be a known carcinogen (cancer-causing substance).[1,2]
    - Unprotected exposure to UV rays from the sun or indoor tanning devices is a known risk factor for the development of skin cancer, including melanoma, the deadliest form of skin cancer.[2-8]
- There is not a safe level of UV exposure from the sun or indoor tanning devices that allows for maximum vitamin D synthesis without increasing skin cancer risk.
- To protect against UV-induced skin cancer, the AAD recommends a comprehensive sun protection plan that

includes seeking shade, wearing protective clothing, and applying a broad-spectrum, water-resistant sunscreen with an SPF of 30 or higher to all skin not covered by clothing.[9,10]

- Vitamin D is critical for bone health, however, there is insufficient evidence to suggest its role in influencing or reducing the incidence of other diseases like certain types of cancer, neurologic disease, infectious disease, autoimmune disease, or cardiovascular disease.[15,16]
    - The National Academy of Medicine, formerly the Institute of Medicine (IOM) concluded that the evidence for associating vitamin D status with health benefits other than bone health was inconsistent, inconclusive as to causality and insufficient to inform nutritional requirement.[11-12]
- Research has also shown that individuals with darker skin tones may be at increased risk for lower levels of vitamin D.[13-14]
- Because the amount of vitamin D a person receives from the sun is inconsistent and increases the risk of skin cancer, the IOM's RDA was developed based on a person receiving minimal or no sun exposure.
- Research has shown getting too much Vitamin D can cause potential harm you.[15,17]
- If you have questions about how much Vitamin D you are getting or what your Vitamin D level should be, talk to your doctor.

**The RDA is the intake that covers the needs of 97-98% of the healthy, normal population.*[18]

Related AAD resources

- Skin Cancer Resource Center

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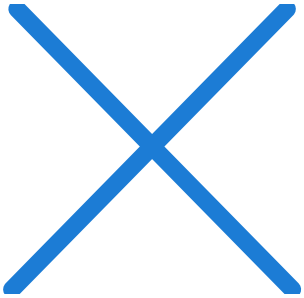
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Last updated: 2/23/23





Registration begins today at 12 pm noon (CT) for physician members, life members, and honorary members.

Go to registration and housing system

No thanks, I'll do this later

Exhibit J

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VITAMINS A, D_3 AND E IN NOVA SCOTIAN COD LIVER OILS

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There has been no production of cod liver oil in Nova Scotia for decades and in this time fish stocks and landings have been altered by the overfishing and resultant quotas and restrictions. Accordingly individual livers from cod, *Gadus morhua*, taken in three Nova Scotia fishing areas were examined for vitamins A, D_3 and E. Average contents for fish landed in Yarmouth, Lower Prospect and Canso respectively were: -Vitamin A, 1100, 420 and 850 retinol equivalents (R.E.) g^{-1} oil; vitamin D_3, 1.56, 0.88 and 0.88 $\mu g\ g^{-1}$ oil; vitamin E, 259, 286, 320 $\mu g\ g^{-1}$ oil. In terms of meeting current pharmaceutical standards for commercial oils there is a reasonable prospect of vitamin A content being at or near requirements, but vitamin D_3 would likely have to be supplemented. Vitamin E contents correlate with other oil soluble vitamin values, suggesting that the content of this oil-soluble material may be related to the age and size of fish as is the case with vitamins A and D_3.

L'huile de foie de morue n'as pas été produite en Nouvelle Ecosse depuis des décennies et en cette période les réserves et la prise de poisson ont été changés par le surpêchage et les quotas et les restrictions imposés par la suite. Les foies de morues *Gadus morhua* ont été pris de poissons attrapés dans trois régions de pêche de la Nouvelle Ecosse et ils ont été examinés pour la présence des vitamines A, D_3 et E. Les taux moyens pour des poissons pris a Yarmouth, Lower Prospect et Canso étaient respectivement : Vitamine A, 1100, 420 et 850 équivalents de retinol (E.R.) g^{-1} d'huile; vitamine D_3, 1.56, 0.88 et 0.88 $\mu g\ g^{-1}$ d'huile; vitamine E, 259, 286 et 320 $\mu g\ g^{-1}$ d'huile. Il est possible que les taux en vitamine A atteindraient les normes pharmaceutiques actuelles pour les huiles commerciales mais il faudrait probablement ajouter de la vitamine D_3. Les taux en vitamine E correspondent aux taux des autres vitamines soluble à l'huile. Ceci suggère que le taux de cette vitamine pourrait être lié à l'âge et à la taille du poisson ce qui est le cas des viamines A et D_3.

Introduction

Cod liver oil has been used for over two centuries as a food supplement (Möller, 1895; Chabré, 1936). Demand for medicinal cod liver oil produced much research and the emergence of an extensive Atlantic coast industry in the first half of the 20th century (Bailey et al., 1952). Replacement by synthetic vitamins in the 1950's caused the fish liver oil industry to die out. In recent years, there has been a resurgence of interest in pharmaceutical cod liver oil as a natural source of vitamins, thus promoting an industry which, in Nova Scotia, has not existed for at least two decades. Retail cod liver oil is imported and is usually identified on the label as "Norwegian".

Methods of vitamin analysis available to early researchers were limited to biological and chemical (colorimetric) techniques. These methods suffered from lack of precision and, in the case of colorimetry, interference without extensive clean-up procedures. Recently high performance liquid chromatography (HPLC) has been employed to determine vitamins A (Stancher and Zonta, 1984), D (Egaas and Lambertsen, 1979; Elton-Bott and Stacey, 1981; Pask-Hughes and Calam, 1982; Takeuchi et al., 1984; Stancher and Zonta, 1983) and E (Hung et al., 1980; Syvaoja et al., 1985) in fish oils.

It is apparent, from the older literature, that the fat soluble vitamin content of Atlantic cod liver oil varies greatly. The major factor influencing the liver oil potency is the spawning cycle (Drummond and Hilditch, 1930; Bailey, 1952; Cruickshank, 1962; Krassowska, 1969). The vitamin concentration in the liver oil increases during the spring-summer spawning period, when liver triglyceride is depleted, and increases in the fall-winter when lipid reserves are built up (Jangaard et al., 1967). The basic work on cod liver oil was executed over forty years ago and many things have changed. Fish populations are undoubtedly different and more accurate analytical techniques re-

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place the older colorimetric and biological methods. For these reasons, the older results for the vitamin contents of cod liver oil are not applicable to the cod being caught today.

World production of pharmaceutical fish liver oils is around 24,000 tonnes (Fisheries and Oceans, 1980). Since in Nova Scotia cod livers are currently being discarded, production of cod liver oil could supplement incomes of fishermen. With that in mind, this study was executed to update literature values of vitamin contents of Atlantic cod liver oils.

Materials and Methods

Cod Livers

Round codfish (*Gadus morhua*) were obtained from three Nova Scotian locations. The locations were Lower Prospect, Halifax Co. (n=11, July, 1982), Yarmouth (n=60, Late August, 1983) and Canso (n=7, September, 1983). The Lower Prospect and Canso fish were received from inshore boats and the Yarmouth sample from an offshore boat fishing the Browns Bank. The length and weight measurements of the cod were recorded. The individual livers were removed, placed in Mylar bags, and stored on ice. On return to the laboratory they were weighed, vacuum sealed in Mylar bags and stored at -35°C until use. Four categories were used to described appearances of the livers. They were 1-creamy white, 2-light brown, 3-dark brown, and 4-dark brown with seal worm infestation. Liver condition and general condition were calculated according to Jangaard et al. (1967).

Extraction of the Liver Oil

The individual livers were blended with anhydrous sodium sulfate and dichloromethane (1:5:3 w/w respectively). The mixture was blended for 5 minutes in a Waring Blendor, filtered under nitrogen (Whatman #1) and solvent removed under vacuum with slight heating (30°C). The oils were stored under nitrogen at -80°C until analysis.

Saponification Procedure

About 2 g oil was accurately weighted into a 50 ml leak-proof Teflon-lined screwcap centrifuge tube. To this 30 ml ethanol (90%), 1.5 ml KOH (50% w/w aq.), a few grains of EDTA (disodium ethylenediamine tetraacetate) and 1 ml sodium ascorbate solution (1.7 g ascorbic acid in 10 ml 0.1 *N* NaOH) were added. The tube was flushed with nitrogen, tightly capped, and placed in a boiling water bath. The tube was shaken by hand every 2 minutes. After exactly 15 minutes the tubes were removed and cooled under running water. One ml of a BHA (butylated hydroxy anisole) solution (1 mg ml^{-1} in hexane) was added and the unsaponifiables were extracted with peroxide free diethyl ether (1 x 100, 2 x 50 ml). The combined ether phases were washed with water (3 x 50 ml) and then dried with sodium sulfate. The solvent was evaporated at 35°/10 mm. Unsaponifiables were transferred, using ether as solvent, to a 5 ml volumetric flask. The ether was evaporated to near dryness with nitrogen. Methanol was used to bring the volume to 5 ml and undissolved cholesterol was solubilized with sonication. This solution was filtered, using a 0.45 μ Millex filter (Waters Assoc., Milford, Mass.), into a 10 ml screwcap centrifuge tube.

High Performance Liquid Chromatography

The HPLC system used included a model 6000A pump, a model 480 UV absorbance detector, a U6K injector (Waters Assoc., Milford, Mass.) and a strip-chart recorder. Chromatographic separations were carried out on normal phase silica (μ-Porasil, 30 x

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0.4 cm, 10 μ; Waters Assoc., Milford, Mass.) or reverse phase octadecane bonded silica (Hybar, 12.5 x 0.4 cm, 5 μ; Merck, Darmstadt, Germany) columns. Guard columns were hand packed with either C-18 Corasil or silica Corasil II chromatographic materials (Waters Assoc., Milford, Mass.).

Vitamin Determinations

Vitamin A, as all-trans retinol, was determined by injecting the unsaponifiable fraction onto the C-18 column and eluting with methanol/water (95:5) at a flow rate of 1.0 ml min^{-1}. The UV absorbance detector was set at 325 nm. The same system was used for determination of d-α-tocopherol except the flow rate was 1.2 ml min^{-1} and UV absorbance was 295 nm. For vitamin D_3, a C-18 column cleanup was used prior to silica column determination. The unsaponifiables were injected onto a C-18 column and eluted with methanol (100%) at 1.0 ml min^{-1}. The area of the chromatogram corresponding to vitamin D_3 elution was collected in a 10 ml screw cap tube. The cholecalciferol from two 200 μl injections of the unsaponifiables was thus collected, dried with a stream of nitrogen, and redissolved in 500 μl hexane/isopropanol (97.5:2.5). This solution was injected onto a silica column eluted with hexane/isopropanal (97.5:2.5) at a rate of 1.5 ml min^{-1} and the detector set at 265 nm. Recoveries for all three vitamins were calculated using authentic standards and the standard additions technique.

Statistical Analysis

Analysis of variance (completely random design), correlation coefficients and multiple liner regression (using the step-down technique) was done using the Statistical Analysis System package (SAS Institute Inc., Cary, N.C., U.S.A.) on a NAS-AS9160 mainframe computer.

Results and Discussion

Mean vitamin recoveries and standard errors are illustrated in Table I. The HPLC methods proved satisfactory for all three vitamins. Several clean-up methods other than saponification were examined but proved unsatisfactory (O'Keefe, 1984). The quantitative transfer of unsaponifiables during the saponification procedure required practice to achieve precise results.

The vitamin contents are expressed as retinol equivalents (R.E.), μg cholecalciferol, and μg d-α-tocopherol for vitamins A, D_3 and E respectively, as suggested by Bieri and McKenna (1981). The minimum vitamin potencies required to reach US Pharmacopoeia standards are 850 and 85 IU per gram of oil for vitamins A and D. This translates to 850 R.E. and 2.13 μg vitamin D. The average vitamin content of the livers would not be expected to equal the value of the pooled oil from the livers (i.e. vitamin concentration and amount of oil from individual livers are both important).

Table I Recoveries of added Vitamins A, D_3, and E from Cod Liver Oil.

Vitamin	Mean Recovery (%)*	Std. Dev.
A	93.1	1.3
D_3	80.1	3.0
E	81.3	2.0

* three replications

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The biological data for the cod are summarized in Tables II, III, and IV. Cod liver oil was found to have from 210 to 4180 R.E. g^{-1} liver oil. Bailey et al. (1952) reported a range of 165-3000 R.E. g^{-1} liver oil. The average values for Yarmouth (Y), Lower Prospect (LP), and Canso (C) were 1100, 420, and 850 R.E. g^{-1} oil.

The average vitamin D_3 values obtained were 1.56, 0.88, and 0.88 $\mu g\ g^{-1}$ oil for Y, LP and C. The vitamin potencies ranged from 0.11 - 6.21 $\mu g\ g^{-1}$ oil. Although some of the literature values for vitamin D in cod liver oil are for commercial oils, which may have been blended or spiked to achieve a certain vitamin concentration, most of the values reported are wtihin the range cited by Cruickshank (1962), which is 0.5-0.7 $\mu g\ g^{-1}$ oil. The range of the present study also agrees with the older values.

Vitamin E in fish liver oils has been overlooked by most researchers. There are no standards for vitamin E content of cod liver oil. The range of potencies was 51 to 1669 $\mu g\ g^{-1}$ oil. Literature values for cod liver oil range from 115 to 430 $\mu g\ g^{-1}$ oil (Ackman and Cormier, 1967; Cruickshank, 1962; Stancher and Zonta, 1983) and burbot (the freshwater cod *Lota lota*) liver oil has been reported to contain 340 $\mu g\ g^{-1}$ oil (Syvaoja et al., 1985).

There were differences in the vitamin contents of the oils from the three Nova Scotian locations. The difference for vitamin D but not A or E was significant at the 5% level (F=5.00, 1.28 and 0.28 respectively). Because of the variation in sampling date and location, a cautious interpretation of the data is necessary. There may be a dissimilarity between cod caught inshore and offshore. The offshore cod from Browns Bank would likewise not be expected to accurately represent cod captured in other offshore fishing grounds.

Due to the small number of samples from Canso and Lower Prospect, only Yarmouth data were used for correlation and multiple linear regression. The results for the correlation analysis of Yarmouth data are represented in Table V. It should be noted that, in the cases for all three vitamins, the oil content of the liver by itself explained much of the observed variation (r^2 of 0.58, 0.62, and 0.56 for vitamins A, D_3, and E respectively). The backward multiple regression models contained oil, liver weight and fish length for vitamin D_3, while liver condition was also significant for the other two vitamins and fish weight replaced fish length for vitamin A (Table VI). The importance of oil content is obvious because of the dilution effect the triglycerides have on vitamin concentrations. The importance of fish length, fish weight and liver weight probably reflect the fact that these parameters are elevated in older cod, which have incorporated greater amounts of vitamins in their livers. Very reasonable R^2 values of 0.778, 0.732, and 0.715 for the three vitamins (O'Keefe, 1984) indicate that the variables incorporated in the multiple regression equations explain most of the vitamin variations. This is remarkable in the light of the variations in the physical conditions of the fish studied, but suggests that the three oil-soluble vitamins are not functional in cod. The rapidity with which cod can accumulate fat in the liver has recently been demonstrated (Lie et al., 1986), but despite many years of industrial records (Bailey et al., 1952; Aure, 1967) this is the first comparison interrelating three oil-soluble vitamins.

In this study we have shown that vitamin contents of Nova Scotian cod liver oils are sufficiently high to be important commercially. The low vitamin D content may be of concern but it is unlikely that the data in this study would accurately estimate vitamin potencies in a commercially processed and refined oil. The vitamin contents in the commercial oils would have to be determined before resorting to spiking with concentrates.

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Table II Summary of Data for Cod from Yarmouth.

Variable	Mean	Std. Dev.	Minimum	Maximum
Fish: Length cm	63.5	17.4	39.0	120.0
Weight kg	3.3	2.9	0.7	14.5
Liver: Weight g	157.5	159.7	6.9	634.4
Oil content %	52.9	16.1	1.9	77.0
Condition	4.3	2.1	1.0	9.3
Appearance	1.6	1.0	1.0	4.0
General Condition	1.1	0.1	0.8	1.5
Vitamin: A in R.E. g^{-1} oil	1100	650	340	4180
D_3 in $\mu g\ g^{-1}$ oil	1.56	0.95	0.57	6.21
E in $\mu g\ g^{-1}$ oil	259	260	51	1669

Table III Summary of Data for Cod from Lower Prospect.

Variable	Mean	St. Dev.	Minimum	Maximum
Fish: Length cm	69.5	8.9	63	90.0
Weight kg	3.4	1.3	2.27	5.90
Liver: Weight g	115.3	84.4	36.3	295.4
Oil content %	46.8	9.4	32.1	59.4
Condition	3.6	1.6	1.9	6.4
Appearance	1.5	0.8	1.0	3.0
General Condition	0.9	0.1	0.8	1.1
Vitamin: A in R.E. g^{-1} oil	420	150	210	670
D_3 in $\mu g\ g^{-1}$ oil	0.88	0.57	0.11	2.36
E in $\mu g\ g^{-1}$ oil	286	64	198	389

Table IV Summary of Data for Cod from Canso.

Variable	Mean	Std. Dev.	Minimum	Maximum
Fish: Length cm	N.D.*			
Weight kg	N.D.			
Liver: Weight g	71.0	24.1	23.7	101.4
Oil content %	56.0	9.5	42.7	67.6
Condition	N.D.			
Appearance	N.D.			
General Condition	N.D.			
Vitamin: A in R.E. g^{-1} oil	850	780	360	1210
D_3 in $\mu g\ g^{-1}$ oil	0.88	0.27	0.54	1.34
E in $\mu g\ g^{-1}$ oil	320	104	117	415

* not determined

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Table V Correlation Coefficients.

	Fish Length	Fish Weight	Liver Weight	Oil %	Appearance	A	D_3	E
Fish Length	1.00	0.96	0.79	-0.02	0.10	0.37	0.30	0.32
Fish Wt.	0.96	1.00	0.77	-0.10	0.15	0.38	0.37	0.39
Liver Wt.	0.79	0.77	1.00	0.32	-0.18	-0.01	-0.08	-0.05
Oil %	-0.02	-0.10	0.32	1.00	-0.87	-0.76	-0.79	-0.75
App	0.10	0.15	-0.18	-0.87	1.00	0.69	0.71	0.64
A	0.37	0.38	-0.01	-0.76	0.69	1.00	0.77	0.75
D_3	0.30	0.27	-0.08	-0.79	0.71	0.77	1.00	0.74
E	0.32	0.39	-0.05	-0.75	0.64	0.75	0.74	1.00

Table VI Multiple Regression Analysis.

Vitamin	R^2	Significant parameters	b
A	0.778		
		intercept	1.13
		oil %	-0.035
		liver weight	-0.002
		fish length	0.024
		liver condition	0.154
D_3	0.732	intercept	3.29
		oil %	-0.038
		liver weight	-0.002
		fish weight	0.167
E	0.715	intercept	613.5
		oil %	-11.625
		liver weight	-1.084
		fish weight	64.294
		liver condition	50.429

Acknowledgements

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Exhibit K

Biomechanics of milk extraction during breast-feeding

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How do infants extract milk during breast-feeding? We have resolved a century-long scientific controversy, whether it is sucking of the milk by subatmospheric pressure or mouthing of the nipple–areola complex to induce a peristaltic-like extraction mechanism. Breast-feeding is a dynamic process, which requires coupling between periodic motions of the infant's jaws, undulation of the tongue, and the breast milk ejection reflex. The physical mechanisms executed by the infant have been intriguing topics. We used an objective and dynamic analysis of ultrasound (US) movie clips acquired during breast-feeding to explore the tongue dynamic characteristics. Then, we developed a new 3D biophysical model of the breast and lactiferous tubes that enables the mimicking of dynamic characteristics observed in US imaging during breast-feeding, and thereby, exploration of the biomechanical aspects of breast-feeding. We have shown, for the first time to our knowledge, that latch-on to draw the nipple–areola complex into the infant mouth, as well as milk extraction during breast-feeding, require development of time-varying subatmospheric pressures within the infant's oral cavity. Analysis of the US movies clearly demonstrated that tongue motility during breast-feeding was fairly periodic. The anterior tongue, which is wedged between the nipple–areola complex and the lower lips, moves as a rigid body with the cycling motion of the mandible, while the posterior section of the tongue undulates in a pattern similar to a propagating peristaltic wave, which is essential for swallowing.

submental ultrasound | sucking pressure | computational model | fluid-structure interaction

Breast-feeding is strongly publicized and encouraged by many societies and communities. It is well accepted that breast milk provides the infant both nutrients and immunities required for growth and development during the first months after birth. It is less known that breast-fed infants exercise and prepare their orofacial muscles for future tasks of speaking and chewing (1), and also have higher oxygen saturation than bottle-fed infants (2). Breast-feeding is the outcome of a dynamic synchronization between oscillation of the infant's mandible, rhythmic motility of the tongue, and the breast milk ejection reflex that drives maternal milk toward the nipple outlet. First, the infant latches onto the breast and nipple so that the nipple, areola, and underlying mammary tissue and lactiferous ducts are drawn into the infant's mouth with the nipple tip extended as far as the hard–soft palate junction (HSPJ). Then, the infant moves its mandible up and down, compressing the areola and the underlying lactiferous ducts with its gums in a suckling process that extracts the milk into its mouth (3, 4). Simultaneous with compression, spontaneous undulating motions of the infant tongue channel the milk posteriorly and trigger the swallowing reflex (5). During breast-feeding, suckling, swallowing, and breathing are coordinated by the central nervous system in a way that allows for the infant's continuous feeding without breathing interruptions (2, 6, 7).

The physical mechanisms that enable the infant to extract milk from the breast have intrigued scientists for more than a century (8). The two proposed mechanisms that have been a subject of scientific controversy to this day are (*i*) sucking—emptying of the nipple–breast contents by development of subatmospheric pressures within the infant oral cavity (9–12) and (*ii*) mouthing—squeezing out of the nipple–areola contents by compression between the jaws or other mouth parts (3). With the appearance of cine–X-ray and ultrasound (US) imaging modalities, a significant role was also attributed to tongue undulation which was naturally referred to as "tongue peristalsis" while chewing the nipple (13, 14). However, advanced computational modeling has not yet been used along with imaging data to perform hypothesis testing on the underlying explanations of the suckling behavior during breast-feeding.

We have explored the physical aspects of infant feeding via noninvasive visualizations of the moving components in the oral cavity and a biophysical model. An objective dynamic analysis of submental US imaging of the midsagittal cross-section of the oral cavity during infant feeding was used to study the dynamic characteristics of tongue motion with respect to the rigid upper palate. A 3D fluid–structure interaction (FSI) biophysical model was developed to simulate milk extraction during breast-feeding.

Results

Tongue Motion During Breast-Feeding. Midsagittal submental US movie clips recorded during infant breast-feeding were dynamically analyzed. The tracked contours representing the palate (in red in Fig. 1*A*) and the upper surface of the tongue (in green in Fig. 1*A*) were tracked on each frame (Figs. S1–S3) and are demonstrated for several frames (Fig. 1*A*). The tip of the tongue is not visible on submental US images of breast-feeding. Assembling the results from all frames (about 150) of the movie clip

Significance

We have resolved a century-long scientific controversy and demonstrated with a 3D biophysical model that infants suck breast milk by subatmospheric pressures and not by mouthing the nipple–areola complex to induce a peristaltic-like extraction mechanism. Analysis of ultrasound (US) movies demonstrated that the anterior tongue, which is wedged between the nipple–areola complex and the lower lips, moves as a rigid body with the cycling motion of the mandible, while the posterior section of the tongue undulates like a peristaltic wave, which is essential for swallowing. The computational simulations of breast-feeding successfully mimicked the dynamic characteristics observed in US imaging and also predicted the subatmospheric pressure required to draw the nipple–areola complex into the infant mouth during latch-on.

Author contributions: D.E., S.D., and L.B.S. designed research; E.B., S.D., M.Z., and L.B.S. recruited subjects; D.E., P.K., O.B., M.Z., and L.B.S. performed research; D.E., A.F.L., and M.J.P. contributed new reagents/analytic tools; D.E., A.F.L., M.J.P., E.B., S.D., and L.B.S. analyzed data; and D.E., A.F.L., and S.D. wrote the paper.

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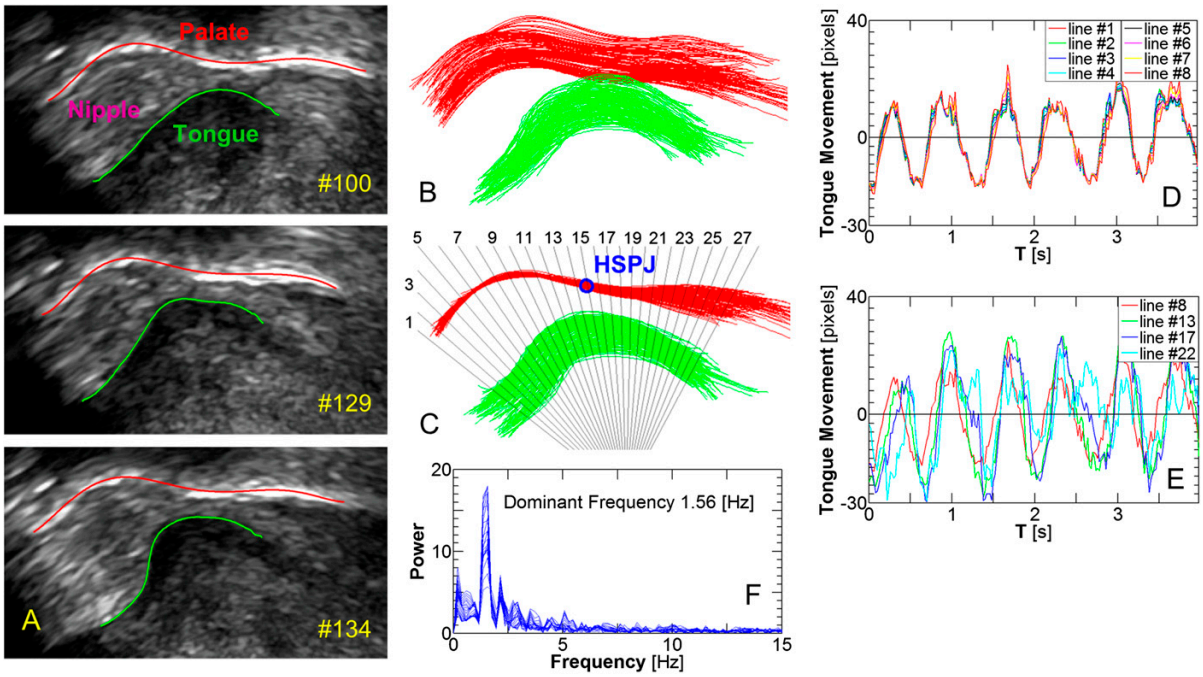


Fig. 1. Tongue motility of a healthy infant during breast-feeding. (*A*) Submental US images with traced contours of the palate and the tongue's upper surface in different frames of the movie clip (subject 24). (*B*) Contours of all of the palate (red) and the tongue (green) from 150 frames of subject 24. (*C*) The contours of *B* after rigid registration around the rigid palate and the imposed polar coordinates. (*D*) Motility of the anterior tongue around anterior coordinates 1–8 after scaling (data from subject 41). The pattern fits the motility of a rigid body. (*E*) Motility of the posterior tongue around polar coordinates 8, 13, 17, and 22 after scaling (data from subject 41). The pattern fits the motility of a peristaltic wave. (*F*) Frequency distribution of the tongue contours around all of the polar coordinates. All regions of the tongue have the same dominant frequency of 1.56 Hz (i.e., 0.64 s per suckling cycle) (data from subject 41). The original and processed movie is provided in Movie S1. HSPJ, hard–soft palate junction.

on a single plane revealed that both contours moved with time within the image plan (Fig. 1*B*). These movements may be due to time-dependent deformations of the palate and tongue, as well as movements of the mother, infant, and technician during data acquisition. Because the hard palate is rigid, its contour does not change during US imaging and can be used for rigid registration of all of the images (15) to eliminate the noise induced by movements not related to breast-feeding. After rigid registration, the tongue and palate contours are depicted in Fig. 1*C*. A movie clip of the original US recording and the final identified tongue and palate outlines can be found in Movie S1. More examples can be found at www.eng.tau.ac.il/~elad/Lab/movies.html.

Dynamic analysis of tongue motility during breast-feeding was performed with respect to a system of polar coordinates that was imposed on the registered data in the spatial coordinate system (Fig. 1*C*). The origin of this system was set at the bottom of the image in the vicinity of the chin surface (16, 17). Tracing the intersections between the palate and tongue contours and the polar coordinates in consecutive images yielded the local time-dependent motility of each structure. Tracing the movements within the hard palate region revealed significant and periodic motility for the tongue (i.e., about 30 pixels), while the palate was randomly oscillating within a band of 4–5 pixels, which represented the noise resulting from the system. The location of the HSPJ on the polar coordinates was identified at a location where the band became wider than 5 pixels as shown by the blue circle on Fig. 1*C*.

The time-dependent motility of the anterior tongue around polar lines 1–8 collapsed on each other, which denoted a rigid body motion in a direction perpendicular to the hard palate (Fig. 1*D*). On the other hand, drawing the motility of a more posterior section of the tongue around polar lines 8, 13, 17, and 22 (Fig. 1*E*) clearly demonstrated an ordered posteriorly spreading of the curves with a phase shift, similar to a pattern of propagating peristaltic waves toward the posterior end of the tongue.

An important outcome of this dynamic analysis is that the anterior part of the tongue oscillated like a rigid body against the rigid palate. This was further supported by analyzing the time delay between lines 1–8, which revealed zero phase shifts. According to the classic theory of breast-feeding, the infant draws the nipple into its mouth, with the areola and the underlying enlarged and branching lactiferous ducts held between the infant's upper gums. Then, a roller-like peristaltic wave of contraction throughout the surface of the tongue squeezes milk from the ampullae into the esophagus (3, 13). Simultaneous recordings of orokinetogram and pressure near the nipple during breast-feeding similarly led to conclusions that oral activity was associated with nipple squeezing (18, 19). The results shown in Fig. 1*D* provide objective support that movement of the anterior part of the tongue, which lies under the nipple, is controlled by the periodic movement of the mandible and definitely does not represent a peristaltic wave of contraction. That is to say, the whole nipple is periodically compressed against the hard palate.

Analysis of posterior tongue motility further revealed a peristaltic-type motion toward the esophagus with time delays of about 0.14 s between lines 8 and 22 (Fig. 1*E*). The time delay was computed with Matlab (MathWorks) "finddelay" function that performed a cross-correlation between two signals of a similar pattern. This peristalsis motility was similar for all subjects, but varied in size and pattern between subjects and was essential for swallowing of the milk extracted from the breast. In this work we

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tracked the anterior section of the soft palate (i.e., velum) which plays an important role in swallowing (Fig. 1*C*), but did not analyze its motility. Observation of movie clips of breast-feeding infants revealed mandible movements without motions of the buccinator muscles, which may alter mouth volume and pressure. Hence, the peristalsis of the posterior tongue, as well as changes in mouth volume due to mandible oscillations, are most likely the generators of the pressure fluctuations measured near the nipple tip (12, 19, 20), which was located a few millimeters anterior to the HSPJ (21).

The frequency domain of tongue motility was analyzed by using a fast Fourier transform algorithm on the local time-dependent movements around each polar line (Fig. 1*F*). The distribution of frequencies was almost the same for all polar lines and the dominant frequency was 1.56 Hz, corresponding to an average time length of 0.64 s per suckling cycle. This means that the whole tongue was undulating with the same frequency; however, the displacements differ along the tongue to yield different types of motions in the anterior and posterior sections. The dynamic characteristics computed from US movie clips recorded from nine healthy infants during nutritive breast-feeding were similar to those depicted in Fig. 1 (Table S1). The dominant frequencies for all of the infants (i.e., aged 14–120 d) revealed suckling rates in the 1.17–1.95 Hz range. It should be noted that the age differences between infants does not change the physical principles used by the infant to extract milk from the breast. It certainly has an effect on the efficiency and process characteristics due to development of the relevant muscles. Measurements with a Doppler US flow transducer installed in the tip of a latex nipple shield in infants aged 5–9 d showed frequencies under 1 Hz for continuous (i.e., nutritive) suckling and about 2 Hz for intermittent (i.e., nonnutritive) suckling (22). It is obvious that using a latex shield between the nipple and the infant mouth does not represent natural breast-feeding and it has been known to cause disturbances (23). Acquisition of orokinetogram signals in 1- to 2-mo-old infants revealed oral movements at frequencies under 1 Hz during continuous suckling and a band of 4–8 Hz for intermittent suckling (19). The differences may be the result of the different experimental techniques.

Nipple Movement During Breast-Feeding. Observation of cine–X-ray film (13) and US movies recorded during breast-feeding clearly revealed an anterior–posterior movement of the nipple with respect to infants' mandible oscillations. The nipple is moving posteriorly (i.e., toward the inside of the mouth) while the tongue and mandible are lowered, and anteriorly (i.e., toward the outside of the mouth) when the tongue and mandible are moving upward and compressing the nipple against the hard palate (3, 24). The US movie clips recorded from subjects 4, 6, and 41 (Table S1) clearly revealed the nipple motion, and accordingly we applied the same tracking algorithm and extracted the outline of the nipple tip in each frame, in addition to the tongue and palate contours. The results for subject 41, after registration of the images with respect to the hard palate, are shown in Fig. 2. Aiming to measure the time-dependent displacement of the nipple, we first represented the band of the registered hard palate by a single averaged curve (i.e., black curve in Fig. 2*A*) and marked the HSPJ obtained earlier. We then identified the nipple tip (i.e., the deepest point the nipple reached in the infant's mouth) and calculated its distance from the HSPJ as shown in Fig. 2*B* for eight frames of a complete suckling cycle. A clip of nipple movement during breast-feeding can be found in Movie S2. More examples can be found at www.eng.tau.ac.il/~elad/Lab/movies.html.

Once the time-dependent motion of the nipple tip with respect to the HSPJ is known, one can evaluate its dynamics with respect to the anterior part of the tongue, which was found to undulate as a rigid structure. The anterior tongue motility curves were averaged with respect to the hard palate (i.e., lines 1–8 in Fig. 1*D*), as shown by the green curve in Fig. 2*C*, and in comparison with the purple curve of the nipple tip motility (i.e., the distance between the nipple tip and HSPJ in Fig. 2*B*). It should be noted that identification of the nipple tip in US images is sometimes difficult because of increased echogenicity as a result of milk expressed from the breast (24), which may explain the noisy sections on the nipple movement curve. Nevertheless, a very similar pattern between the tongue and nipple movements was also obtained for two other subjects (subjects 4 and 6). The important facts emerging from the results depicted in Fig. 2 were (*i*) the range of nipple tip motion was about 3.7 mm (25 pixels) with respect to the HSPJ, whereas the range of rigid movement of the anterior tongue was 4.4 mm (30 pixels) with respect to the rigid palate; and (*ii*) the nipple tip lags about 0.08 s after the anterior tongue motion, which might be due to the viscoelastic properties of the nipple and tongue tissues.

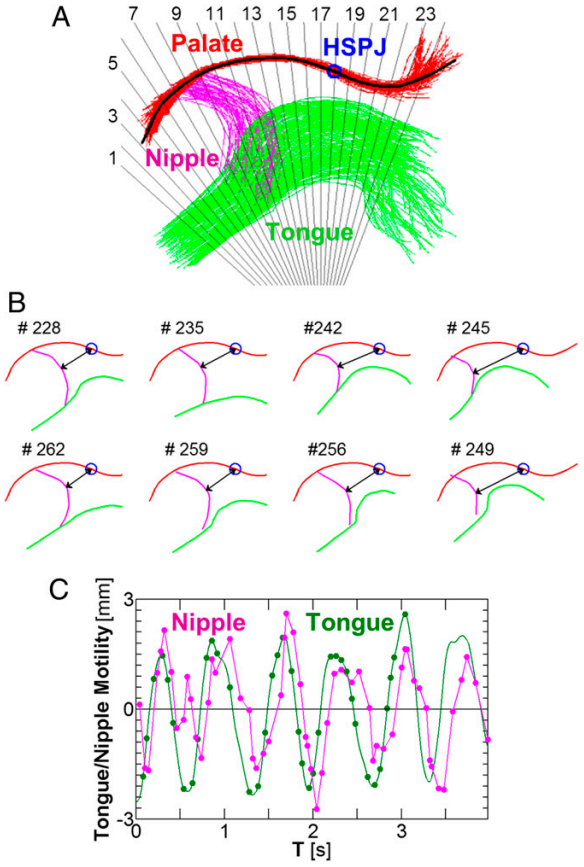


Fig. 2. Nipple movements in the anterior–posterior direction during breast-feeding (data from subject 41). (*A*) Contours of the tongue (green) and nipple distal surface (purple) after rigid registration around the rigid palate. The black curve represents the average of all contours of the rigid palate. (*B*) Contours of the palate (red), tongue (green), and nipple (purple) of several frames during a single suckling cycle. The black arrow represents the distance between the nipple tip and the HSPJ (blue circle). (*C*) The scaled motility of the nipple tip (purple, length of black arrows in *B*) in comparison with the rigid motility of the anterior tongue (green, average of the motility over lines 1–8 as in Fig. 1*D*). Nipple movement is provided in Movie S2. HSPJ, hard–soft palate junction.

Tongue Motion with Respect to the Mandible. Tongue undulation during breast-feeding has been the topic of many studies (4–14). Submental US imaging revealed the complex motion of the

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tongue, as was also demonstrated here. Observation of movie clips of breast-feeding infants revealed steady mandible oscillations without motions of the buccinator muscles. Because both the tongue and the mandible are oscillating during breast-feeding, we were interested in exploring their relative motions. We recently received a movie clip of a cinefilm of radiographic exposure during breast-feeding from the study of Ardran et al. (13), which clearly demonstrated the movement of the nipple, tongue, and mandible. With permission from the Nuffield Department of Obstetrics and Gynecology (University of Oxford, Oxford) we used dynamic analysis to explore the tongue motion with respect to the mandible during breast-feeding. Specifically, we performed rigid registration with respect to the mandible and the tracked outlines of the tongue can be found in Movie S3. The results demonstrated that the rigid movement of the anterior tongue (as demonstrated in Fig. 1*D*) was dictated by mandible oscillations, while the posterior tongue was undulating as shown in Fig. 1*E* to facilitate swallowing and coordination with breathing. The anterior tongue, which is wedged between the nipple–areola complex and the lower lips, is continuously moving with the mandible and also slightly moves anteriorly (i.e., outside the mouth) as the mandible moves down (i.e., the mouth opens) and vice versa. This anterior motility complies with the babies' tongue thrust reflex and may be of major concern in infants with tongue tie.

Physical Model of Milk Extraction During Breast-Feeding. The physical process by which the infant extracts milk from the breast is still unclear and two theories have been proposed in the absence of noninvasive techniques for experimental validation. The results obtained in this study clearly demonstrated that the anterior tongue moves as a rigid body under the nipple, ruling out the hypothesis of a peristaltic squeezing of the nipple. Other unexplained physical issues are related to the deformation of the nipple and the proximal areola. During latch-on, the infant closes its mouth on the nipple and part of the areola to form a teat in the

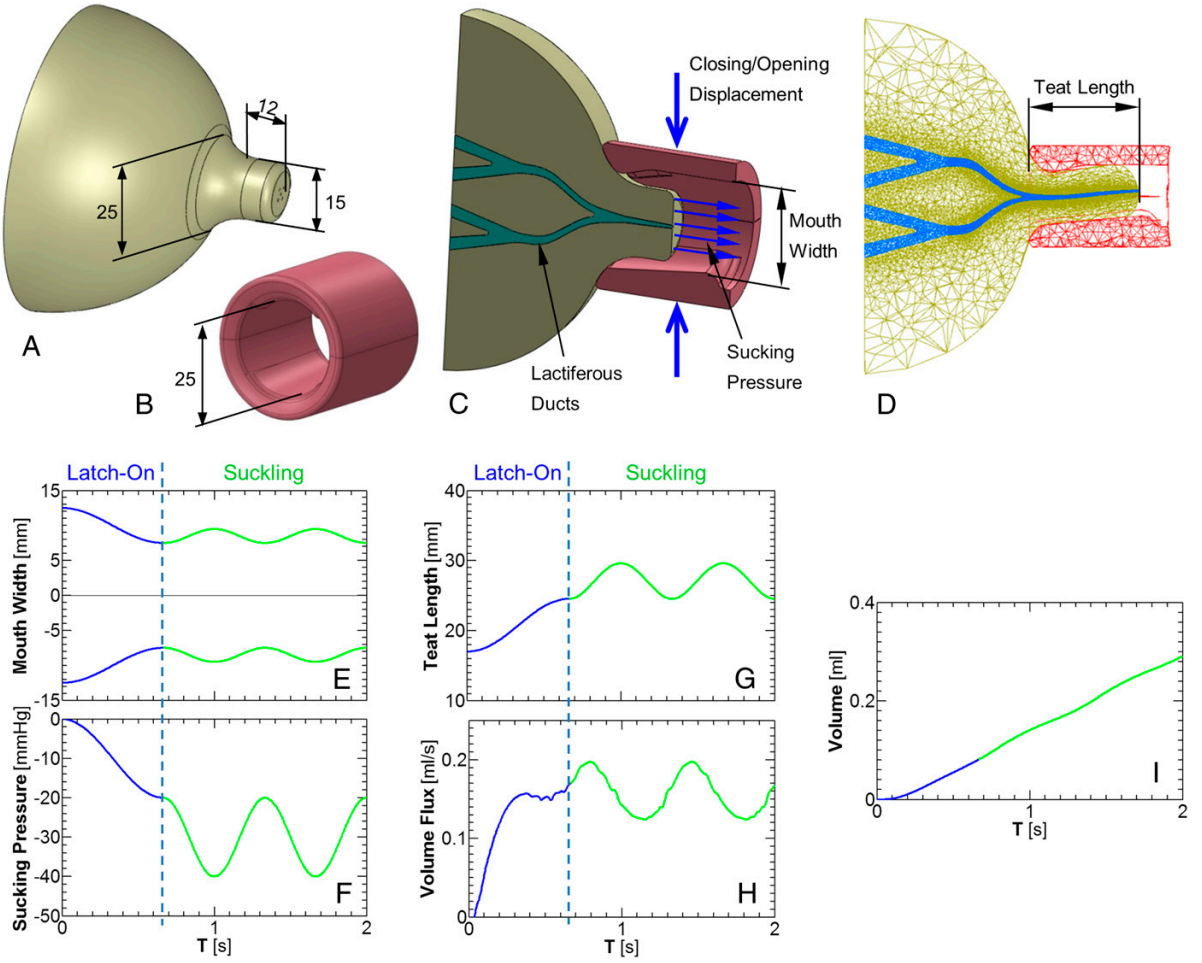


Fig. 3. Physical model of milk extraction during breast-feeding. (*A*) The breast model. (*B*) The infant mouth model at maximal opening. (*C*) Sagittal cross-section of the infant mouth attached to the breast just at the beginning of latch-on with arrows to represent the closing–opening displacements and the subatmospheric sucking pressure. (*D*) Sagittal cross-section of the infant mouth and the breast just at the end of latch-on. (*E*) Vertical maximal width of the infant mouth during latch-on and suckling. (*F*) Sucking subatmospheric pressure during latch-on and suckling. (*G*) The teat length (as shown in *D*) during latch-on and suckling. (*H*) Volume flux of milk extracted by the infant during latch-on and suckling. (*I*) Accumulated milk during the simulation shown here (i.e., latch-on and two suckling cycles), obtained by integration of the data in *H*.

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mouth, which is believed to be two to three times longer than the free lactating nipple (3). Also unexplained is the retraction of the nipple when the mandible and tongue move up and compress the nipple against the palate, and vice versa when the mandible moves down and the volume of the infant's mouth increases. In the absence of negative mouth pressure, one would expect a reversed pattern of nipple deformation during mandible oscillations.

A 3D FSI breast-feeding model was developed to obtain better insights into the mechanical aspects occurring during breast-feeding. For this purpose we developed an elastic breast model (Fig. 3*A*) that represented a lactating breast with an areola and a 12-mm long nipple with a diameter of 15 mm (25). A complete lobe of branching lactiferous tubes running from the proximal alveoli toward a single outlet in the nipple tip was simulated by a branching network of tubes that ended at an outlet 0.7 mm diameter (Fig. 3*C* and Fig. S4) (26–29). The material elasticity of the breast, including nipple and areola, was assumed to behave as a Mooney–Rivlin hyperelastic material with coefficients that fit in vitro force-deformation tests of breast tissue (30). The infant mouth, including the tongue and gum, at maximal opening before starting to latch-on the breast was modeled as an elastic cylinder made of two halves with 25-mm inner diameters and 30 mm long (Fig. 3*B*). The gum was assumed to bulge 1.9 mm from the hard palate. Following the analysis of the cine–X-ray movie clip, the tongue was assumed to be connected to the lower jaw, whereas the proximal part of the tongue was 3 mm higher than its distal part. The closure of the mouth was obtained by applying vertical forces via the compression of the cylinder by two opposing flat plates (Fig. 3*C*). Mouth elasticity was much stiffer than that of the nipple–areola complex but allowed partial closure when subjected to the opposing vertical forces. We assumed that milk is available in the breast or supplied as needed in the simulation of breast-feeding as a result of let-down reflux.

Simulation of the infant feeding on the mother's breast was performed in two stages: first, latch-on to the nipple–areola region to extend the nipple–areola complex into the infant's oral cavity, followed by periodic suckling maneuvers by the infant's mandible while holding the skull and palate stationary and the mouth sealed to the breast. Explorations of the physical forces that the infant exerts on the breast to extract milk were performed for a reference breast model with a single lobe of branching lactiferous tubes and at a suckling rate of 1.5 Hz. For simulation of latch-on, we assumed mouth closure from a vertical opening of 25 mm to 15 mm with simultaneous generation of a subatmospheric sucking pressure of −20 mmHg in the infant mouth (blue curves in Fig. 3 *E* and *F*). Then, for nutritive suckling, we assumed periodic cycles of mouth opening/closing within a range of 5 mm (i.e., between 15 and 20 mm) and simultaneously synchronized with the subatmospheric pressure cycling between −20 and −40 mmHg (green curves in Fig. 3 *E* and *F*). Throughout the simulations we assumed full contact between the mouth model "lips" and the breast areola to ensure the mouth sealing observed during in vivo breast-feeding.

Simulations of the latch-on phase during mouth closure on the nipple and part of the areola revealed the generation of a 24.5-mm-long teat within the infant's mouth, which is twice the length of the free nipple (i.e., 12 mm), for a subatmospheric pressure of −20 mmHg (Fig. 3 *D* and *G*). Reducing the subatmospheric pressure to −10 mmHg reduced the teat length to 21.5 mm and a larger subatmospheric pressure of −30 mmHg increased the teat length to 27.4 mm. For a more flexible nipple–areola tissue, the length of the teat will be larger and vice versa for stiffer tissue.

The simulation of breast-feeding for the reference case, while the infant performed cyclic mouthing (i.e., mandible oscillations) and periodic sucking (i.e., oscillatory subatmospheric pressure) depicted by the green curves in Fig. 3 *E* and *F*, revealed anterior–posterior cyclic displacements of the nipple tip in the 4.9 mm range and a breast milk flow rate of 0.105 mL per cycle of suckling (Fig. 3 *G* and *H* and Movie S4). The simulated motion of the nipple tip with respect to mouth opening/closing was similar to the observations in US imaging (Fig. 2*C* and Movie S1). The accumulation of milk volume with time is demonstrated in Fig. 3*I*, which is similar to Woolridge's measurements (22).

Several simulations with single changes with respect to the reference case were conducted to explore the relative contribution of mouthing and sucking. Simulation of mouthing only (as in Fig. 3*E*) while a latch-on subatmospheric pressure of −20 mmHg was held constant revealed a flow of 0.116 mL per cycle. Leaving the mouth closed at 15 mm (as at the end of latch-on) while a latch-on subatmospheric pressure of −20 mmHg was held constant yielded a flow of 0.114 mL per cycle. On the other hand, simulation of mouthing only (i.e., between 25 and 15 mm) without any subatmospheric mouth pressure but with a let-down oscillating pressure of 1 mmHg revealed the significantly lower flow rate of only 0.023 mL per cycle. This analysis clearly demonstrated that the infant can extract milk from the breast simply by applying subatmospheric pressure in its mouth. However, this does not take into account the natural reflexes of swallowing and breathing which require mandible oscillation and tongue undulation. This analysis also mimicked the periodic anterior–posterior displacement of the nipple tip observed in US imaging.

The contribution of breast elasticity and lactiferous tube size were also analyzed. Simulations with a 20% softer breast increased the teat length to 32 mm (i.e., 2.65 times the free nipple length) and reduced the milk flow rate to 0.086 mL per cycle (i.e., 18% reduction) due to reduction of the lactiferous ducts cross-sectional areas. Assuming a 20% stiffer breast elasticity yielded a milk flow of 0.116 mL per cycle, which is 10% greater than the reference case. This pattern was expected due to the collapse of the lactiferous tubes when the mouth closed and because of subatmospheric pressures. Simulating a model with a branching network of tubes that ended at an outlet of 0.5 mm diameter (instead of 0.7 mm) yielded a milk volume of 0.068 mL per cycle during a single nutritive suckling. It should be noted that the milk flow rate for a rigid model of the lobe of lactiferous tubes subjected to the same subatmospheric pressure oscillations was almost three times greater, which clearly indicated that the major resistance to breast milk flow is in the deformed region of the areola–nipple complex.

Repeating the simulation for a breast model with two branching networks that simulated two lobes of lactiferous tubes yielded a milk flow of 0.208 mL per cycle, which was almost twice the flow rate obtained with a single lobe. A more complex simulation of a more realistic breast with five lobes of branching lactiferous tubes with five outlets in the nipple tip (Fig. S5 and Movie S5) yielded the same anterior–posterior motion of the nipple and milk outflow rate of 0.513 mL per cycle, which is almost five times the flow obtained with a single lobe. The existing data on the flow rate of milk intake by infants during breast-feeding were measured either with a special Doppler US device (22) or by weighing the infants before and after breast-feeding (11, 31) and varied in the range of 0.03 to 0.5 mL per cycle of sucking. Obviously, a wide variability of the amount of the extracted milk is expected due to the functional variability of the breast (e.g., elasticity, number and structure of lobes, and number and size of outlets at the nipple tip), as well as the mode and level of driving forces exerted by the infant during breast-feeding. We clearly demonstrated that changing the characteristics of the lactiferous network, breast elasticity, and the infant's suckling forces leads to the range of variability that was measured in vivo.

Discussion

Breast-feeding requires synchronized manipulation of the jaws, tongue, and lips. The employment of an objective and dynamic analysis of US movie clips acquired during breast-feeding, as well as a physical simulation with a complex biomechanical model, revealed the dynamic characteristics of mouth structures and the

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exerted forces required for milk extraction during breast-feeding. It has been known for decades that breast-feeding required an infant's latch-on to the nipple–areola complex, which is then transformed into a long teat in the infant's mouth, and then suckling, during which the teat moves posteriorly into the mouth during the mouth's opening (i.e., mandible moving down) and vice versa during the mouth's closure (i.e., mandible moving up). We demonstrated that transforming the nipple–areola complex into a teat twice as long as the free lactating nipple requires closure of the infant's lips on the breast and the development of a subatmospheric pressure of about −20 mmHg. This pressure must be maintained throughout breast-feeding to continuously keep the teat in the infant's mouth. Once the infant starts suckling on the nipple, its anterior tongue moves like a rigid body due to the cycling motion of the mandible. We have shown via the complex biophysical simulation that milk extraction from the breast was the result of cycling subatmospheric pressures within the infant's mouth in the range of −20 to −40 mmHg and not due to the chewing of the breast nipple.

In view of the results of the present study it is worth noting the valuable observations and critical speculations reported in earlier studies (9, 13). Hytten (9) showed that sucking was associated with a relatively small swing of subatmospheric mouth pressures of about 10 mmHg, with maximal sucking pressure lower than 50 mmHg. Hytten (9) further demonstrated that using an undamped manometer and a pin-hole teat yielded violent pressure oscillations when the infant's tongue sealed off the teat (or tube) and that a valve-like action caused negative pressures that greatly exceeded the true intraoral pressure. Ardran et al. (13) studied many cineradiographic films via observation and provided accurate descriptions of tongue and nipple motions. The present study further supports his observations and his proposed mechanism with an accurate physical model.

Our study demonstrated a new method that allows exploration of the tongue kinematics from recorded US images and then modeling milk extraction from the breast during breast-feeding. The important outcomes of this work are the subatmospheric pressure oscillations to suck milk and the motility pattern of the anterior tongue. Visual observation of infants during breast-feeding revealed very relaxed oscillations of the mandible without buccal motions and almost no effort. Thus, the subatmospheric pressure oscillations required to extract milk from the breast are most likely generated by changes in mouth volumes due to the mandible oscillations and the posterior tongue peristalsis. The anterior tongue is moving as a rigid body that follows posterior undulations.

Methods

US Imaging. Midsagittal submental US imaging of infants during breast-feeding was performed with a portable GE Vivid *i* US system using a high multifrequency (5–8 MHz) transducer with a single focal zone and a small convex curved face. The study was approved by the Ethical Review Board of the Tel Aviv Sourasky Medical Center (0498–10-TLV). The parents were informed of all aspects of the procedure, and signed a written informed consent form. The best observable sequence of four to six suckling cycles of nutritive breast-feeding was selected for the analysis. The palate and tongue contours in each frame were tracked using an active contour model (32, 33). Dynamic analysis was performed after rigid registration of all of the images (15) and by superimposing a polar grid system (16, 17).

Biophysical Model. Simulations of infant breast-feeding were performed with computational tools for FSI that consider the physical deformation of the nipple–breast complex simultaneously with milk flow in the lactiferous tubes. The physics of the problem is controlled by the simultaneous solution of the dynamic governing equations of the motion and deformation of both the fluid and the structure while coupled with boundary conditions. The 3D models were discretized into thousands of elements and simultaneously solved on a high-speed computer using Automatic Dynamic Incremental Nonlinear Analysis (ADINA) commercial software. Details of the model are provided in *SI Methods*.

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Exhibit L

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The British 'Limeys' Were Right: A Short History of Scurvy



Scurvy, a disease caused by lack of vitamin C, sickened sailors who had no access to fresh food supplies, and killed more than 2 million sailors between the 16th and 18th centuries alone.

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Scurvy was the scourge of sailors for thousands of years. An estimated 2 million sailors died of the disease [https://www.usni.org/magazines/naval-history-magazine/2021/february/finding-cure-scurvy] between the 16th and 18th centuries alone, often decimating entire ship crews.

Scurvy is a dietary deficiency caused by a lack of vitamin C. The body does not naturally make vitamin C, so it has to come from foods rich in the vitamin such as citrus or from dietary supplements. Before modern refrigeration,

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sailors on long voyages relied mostly on salted or nonperishable food and had no regular access to fresh green vegetables or fruits that contain the vitamin.

The symptoms of scurvy are dramatic and terrible. In his 1820 textbook on nautical medicine [https://collections.nlm.nih.gov/catalog/nlm:nlmuid-2566035R-bk], Navy surgeon Usher Parsons unflinchingly described the signs:

- "The gums become soft, livid and swollen, are apt to bleed from the slightest cause, and separate from the teeth, leaving them loose.
- About the same time the legs swell, are glossy, and soon exhibit foul ulcers.
- The same appearances follow, on other depending parts of the body.
- At first the ulcers resemble black blisters which spread and discharge a dark colored matter. These ulcers increase.
- Emaciation ensues.
- Bleeding occurs at the nose and mouth.
- All the evacuations from the body become intolerably fetid.
- Death closes the scene."

British 'Limeys' and the Cure for Scurvy

The U.S. Navy continued to struggle with scurvy into the 19th century even though the Royal Navy cracked the mystery of the disease in the 18th century thanks to surgeon James Lind. The British began storing citrus fruits on board all of its ships. The British Navy gave its sailors limes or lemon juice rations to ward off scurvy – earning them the nickname of "Limeys" among the American sailors who didn't know about or believe in the preventative treatment.

In his article on the history of scurvy in the U.S. Navy, naval medical historian [https://www.dvidshub.net/news/385894/navys-fight-against-scurvy] André Sobocinski, wrote: "In 1809, Dr. William Paul Crillon Barton, a young Philadelphia-born Navy surgeon, took on the fight against scurvy while aboard USS United States, then under the command of Commodore Stephen Decatur. Turning to the medical literature out of Great Britain, Barton administered a citrus concoction to the most severely affected crewmembers and curing them of their symptoms."

Over the ensuing years Barton was the leading figure in the fight against scurvy. He lobbied the Secretary of the Navy and ship captains with what he described as a "clarified lemon-lime ration."

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Despite Barton's success, "the decision to adopt vitamin C-rich rations would remain in the hands of individual fleet commanders, ship captains, and their consulting surgeons for well into the nineteenth century. And even if a ship did take necessary preventive measures against scurvy, long deployments could exhaust shipboard provisions leading to a host of nutritional diseases like scurvy," Sobocinski wrote.

The Navy Today

Scurvy, of course, is not a problem for today's Navy.

Jennifer Person, program manager and dietitian for the Navy Food Service, said there are many items on Navy menus to prevent the disease. For example, she said:

- Food service personnel are encouraged to provide 2.5 cups of vegetables and two cups of fruit daily to service members.
- Foods containing vitamin C are abundant.
- Navy menus include a combination of fresh, frozen, and canned fruits and vegetables.
- Vegetables and fruits offered include oranges, red peppers, kiwi, strawberries, broccoli, cantaloupe, tomatoes, potatoes, cauliflower, Brussel sprouts, kale, lemons, and grapefruit juice.
- Many more vitamin C options are available in a wide range of flavors that make it easy for sailors with different preferences to get their daily requirement.

"Leaders across the DOD recognize the importance of fueling our service members for performance with quality foods," Person said. "Galley menus are written according to DOD Menu Standards for Human Performance Optimization that ensure an array of foods are provided daily."

Scurvy Hasn't Gone Away Altogether

Today, scurvy still occurs in developed countries [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2517958/] despite the widespread availability of multiple enriched and fortified foods.

According to the National Institutes of Health, those most at risk for scurvy are:

- living alone
- institutionalized with diets devoid of fresh fruits and vegetables
- missing teeth or toothless
- showing poor self-care

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- lacking social support
- displaying destructive behaviors such alcoholism, drug abuse, or smoking
- mentally or chronically ill

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Exhibit M

The Aspirin Story

Introduction (https://www.aspirin-foundation.com/history/the-aspirin-story/)
Timeline
Chemistry

The story of Aspirin – a versatile medicine with a long history

Herbal medicine has used salicylic acid, the natural substance related to synthetic aspirin, from myrtle, willow and meadow sweet, since ancient times (at least 2500 BCE). Ancient history has many examples of humans using salicylic acid for medicinal purposes; there are clay tablets from the Assyrians in the Sumerian period (around 4000 years ago) in which willow leaves are recommended for rheumatic disease, the Egyptians describe the use of willow leaves or myrtle for joint pain or inflammation and Hippocrates (460-377 BCE) recommended an extract of willow bark for fever, pain and child birth. Ancient Chinese, Roman and Native American civilizations have all long recognised the benefits of plants containing salicylic acid for their medicinal benefits[1,2,3,4].





Meadow sweet, another source of acetylsalicylic acid, was one of three most sacred herbs to the Druid Celts (the other two are water-mint and vervain) and is one of the 50 ingredients in the drink ‘Save’ mentioned in Chaucer’s 14th century Knight’s Tale (known as Medwort or Meadwort)[5].

It was an English clergyman, Reverend Edward Stone who carried out the first scientific study of the benefits of willow bark when he used it successfully to treat fever ‘ague’ in 50 of his parishioners. He documents this in a letter to the President of the Royal Society in 1763 [6].



In 1828, Johann Andreas Buchner, Professor of Pharmacology at the University of Munich purified salicin from willow bark. A number of scientists worked on refining the process, but it was Professor Hermann Kolbe, at Marburg University who first worked out the chemical structure

of salicylic acid and made it synthetically in 1859. Unfortunately, it had an unpleasant taste and irritated the stomach[1]. The first clinical trial of salicylate was recorded in 1876, by Thomas MacLagan, a doctor from Dundee who used salicin to obtain complete remission of fever and joint inflammation in his patients who were suffering from acute rheumatism[1].



Salicylic acid is a benzene ring with a phenol (HO) group and a carboxylic acid (COOH) group. Whereas other scientists had focused on the carboxylic acid group, Dr Felix Hoffman, a German chemist at Friedrich Bayer and Co, concentrated on the phenol group and managed on August 10th 1897 to acetylate the phenol group and produce pure stable acetylsalicylic acid (ASA) for the first time[1,2]. In this work he was supported and inspired by a number of other scientists including Arthur Eichengrün, Carl Duisberg and Wilhelm Siebel[1,2,4]. Dr Hoffman's discovery was the first time a drug had been made synthetically and was the birth of both aspirin and the pharmaceutical industry [2]. After recognising the importance of Hoffman's discovery Professor Heinrich Dreser, Head of the Pharmacology Institute at Bayer, tested it first on himself, then in a series of animal experiments before successful clinical trials in humans[1].

The new compound was named and registered Aspirin on February 1st1899. The 'A' comes from acetyl and 'spir' from the first part of *Spirea ulmaria (*Meadowsweet) a botanical source of salicylic acid[1].

Aspirin in cardiovascular disease prevention

In the 1950s Laurence Craven, a general practitioner from California, published his work using aspirin to prevent vascular events. Craven recommended aspirin for patients 45-65 years of age who were at risk of a heart attack due to weight or sedentary lifestyle. He found that in those who took aspirin no myocardial infarctions (MIs) occurred. Craven also observed that none of his patients taking aspirin experienced a stroke[6].

Many different scientists contributed to our understanding of how aspirin effects platelet function. Dr Harvey Weiss in the late 1960s reported that aspirin displayed a rapid and irreversible inhibition of platelet aggregation[7]. It was Sir John Vane's research group who were first to discover the role of prostaglandins in haemostasis [4]. In 1971, Vane described aspirin's inhibition of prostaglandin synthesis in a Nature publication[8]. In 1982 he was awarded the Nobel Prize for Medicine in recognition of this work and aspirin started to become established as a drug for treating and preventing cardiovascular. In 1985 Carlo Patrono with his research group showed that low dose aspirin permanently inhibits thromboxane[9].

In 1974 realising the growing potential for aspirin and the importance of uniting the research community to study its benefits Nick Henderson FIPR HonFRCVS, established the

International Aspirin Foundation to increase the knowledge and understanding of the drug. He remained an active member up until his death, age 92 in December 2018.

Professor Richard Peto, an epidemiologist at Oxford University, published in 1980 a meta-analysis of 6 trials and showed a highly significant 23% reduction in vascular disease mortality for those taking aspirin[10]. Interestingly this work also inspired Archie Cochrane to set up the Cochrane collaboration with the aim of organising medical research findings and facilitating evidence-based choices for clinicians, patients and policy makers[2].

In 1988 the Second International Study of Infarct Survival ISIS-2[11] showed the benefits of aspirin after a heart attack. As a result, cardiologists start using aspirin as immediate standard practice for suspected MI. ISIS-2 also showed the importance of large randomised trials with thousands of patients in order to demonstrate these lifesaving benefits.

Also, in 1988 Physicians Health Study provided evidence that long term low dose aspirin resulted in a reduction in MI and a role for aspirin in the primary prevention ischemic heart disease[12].

In 1994 The Antiplatelets Trialist Collaboration confirmed that aspirin could prevent further cardiovascular events and death in people with established vascular disease[13]. Then, in 2002, The Antithrombotic Trialist' Collaboration[14] publish a meta-analysis demonstrating the antiplatelet effect of aspirin and its role in

preventing death, myocardial infarction and stroke in high risk patients. Aspirin also has an important role in the immediate treatment of minor stroke in order to prevent recurrent stroke[15,16]. Reviewing both its preventative effect for the two big killers cardiovascular disease and cancer the US Preventative Task Force[17] in 2009 recommend aspirin for primary disease prevention in certain groups.

A 2020 publication looking at the secondary prevention of cardiovascular disease has shown that aspirin is still one of the best options[18].

Aspirin in cancer prevention and management

1988 Professor Gabriel Kune noticed that people taking aspirin had a 40% lower risk of developing cancer of the large intestine[19]. Other research has further supported this finding and a time dependent reduced incidence in colorectal cancer has been observed[20-22]. In 2011, Sir John Burns[23] showed the role of aspirin in helping to prevent cancer in carriers of hereditary colorectal cancer.

Further studies are ongoing to fully understand aspirins role in cancer prevention including the role of aspirin in reducing the risk of metastatic spread[24,25] in established cancer.

Primary disease prevention

Results from recent primary prevention trials such as ASCEND, ASPREE, ARRIVE, AspECT seAFOod etc. have triggered a lot of debate and discussion[26] and prompted refinement of further

research studies. It is becoming apparent that an individualised approach is important as well as careful consideration of age and all risk factors. Individualised dosing regimens will be needed to fully exploit the potentially lifesaving benefits of this fascinating drug. A one size fits all approach is not appropriate and factors such as increased platelet turnover in certain disease conditions e.g. diabetes leads to 'unblocked' platelets in the circulation when a once daily aspirin regime is used[4], in addition body weight can effects aspirin effectiveness [27].

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