

The acute and chronic toxic effects of vitamin A¹⁻⁴

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ABSTRACT

The acute and chronic effects of vitamin A toxicity are well documented in the literature. Emerging evidence suggests that subtoxicity without clinical signs of toxicity may be a growing concern, because intake from preformed sources of vitamin A often exceeds the recommended dietary allowances (RDA) for adults, especially in developed countries. Osteoporosis and hip fracture are associated with preformed vitamin A intakes that are only twice the current RDA. Assessing vitamin A status in persons with subtoxicity or toxicity is complicated because serum retinol concentrations are nonsensitive indicators in this range of liver vitamin A reserves. The metabolism in well-nourished persons of preformed vitamin A, provided by either liver or supplements, has been studied by several research groups. To control vitamin A deficiency, large therapeutic doses are administered in developing countries to women and children, who often are undernourished. Nevertheless, little attention has been given to the short-term kinetics (ie, after absorption but before storage) of a large dose of vitamin A or to the short- and long-term effects of such a dose given to lactating women on serum and breast-milk concentrations of retinol and its metabolites. Moreover, appropriate dosing regimens have not been systematically evaluated to ascertain the quantitative improvement in vitamin A status of the women and children who receive these supplements. The known acute and chronic effects of vitamin A toxicity have been reported previously. However, further research is needed to ascertain the areas of the world in which subclinical toxicity exists and to evaluate its effects on overall health and well-being. *Am J Clin Nutr* 2006;83:191–201.

KEY WORDS Vitamin A toxicity, public health, supplements, bone

INTRODUCTION

Dietary vitamin A is obtained from preformed vitamin A (ie, retinyl esters from animal foods, fortified foods, and pharmaceutical supplements) as well as from provitamin A carotenoids from plant sources. Preformed vitamin A is efficiently absorbed and utilized by humans at absorption rates of 70–90%. Up to 75% of dietary vitamin A in Europe, the United States, and other industrialized nations is preformed vitamin A (1, 2), which is largely

derived from multivitamins, fish liver oil, and the fortification of foods such as milk, butter, margarine, breakfast cereals, and some snack foods. In developing nations, however, 70–90% of vitamin A is obtained from provitamin A carotenoids in plant foods. These are absorbed much less efficiently, at rates of 20–50%, depending on each person's vitamin A status and other dietary and nondietary factors (3, 4). The cleavage of provitamin A carotenoids to retinal is a highly regulated step, and vitamin A toxicity from provitamin A sources is largely impossible. In contrast, absorption and hepatic storage of preformed vitamin A occur very efficiently until a pathologic condition develops.

Nearly all retinyl esters are hydrolyzed to retinol in the intestinal lumen. Retinol is absorbed by intestinal epithelial cells, where it is reesterified to long-chain fatty acids and incorporated into chylomicra, which circulate in the intestinal lymph before moving into the general circulation. As the chylomicra lose their triacylglycerol and other constituents, most of the retinyl esters remain within, and the particles become chylomicron remnants. Chylomicron remnants are cleared mainly by the liver, but extrahepatic uptake of the remnants may be important in the delivery of vitamin A to mammary tissue, bone marrow, adipose tissue, and spleen (5–7). Retinyl esters in serum are normally below 0.2 $\mu\text{mol/L}$ in the fasting state (8), but they increase significantly after a large influx of vitamin A, such as occurs after a vitamin A-rich meal. Retinyl esters can be distinguished from

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retinol in serum and other tissues and quantified with the use of methods such as HPLC (9). Once in the liver, retinol binds to retinol-binding protein (RBP) and is transported from the liver to tissues as the *holo*-RBP complex. Serum retinol concentrations are not associated with hepatic vitamin A over a wide range of liver values because *holo*-RBP is under homeostatic control (10).

Research on vitamin A toxicity has been carried out primarily in animals, and most studies have been short-term and have focused on acute effects (11–13). Many studies used intramuscular or venous injections of various forms of vitamin A (14, 15), which cannot be extrapolated to physiologic conditions because injections bypass gastrointestinal effects. In developed nations, the increasing availability of and interest in fortified foods and supplements resulted in a large percentage of the population with preformed vitamin A intakes higher than recommended (16). Indeed, observational studies suggest that more than 75% of people may be routinely ingesting more than the recommended dietary allowance (RDA) for vitamin A, much of it as preformed vitamin A (16). Until recently, little research investigated hypervitaminosis A in these situations.

Fossilized skeletal remains of early humans suggest that bone abnormalities may have been caused by hypervitaminosis A (17, 18). From these and other reports, vitamin A toxicity is known to be an ancient phenomenon. Several comprehensive reviews and case studies of vitamin A toxicity, which discuss both acute and chronic excess, have been published (19–21). Whereas vitamin A deficiency is decidedly the greater problem, especially in developing nations, and has received much attention in terms of public health initiatives, excessive vitamin A intake may be a growing but underappreciated problem.

VITAMIN A METABOLISM AND TOXICITY

Plasma vitamin A metabolites after supplementation

The range of serum retinol concentrations under normal conditions is 1–3 $\mu\text{mol/L}$ (2), and, because of homeostatic regulation, that range varies little with widely disparate vitamin A intakes (10). Case reports of vitamin A toxicity have shown serum retinol concentrations within normal limits (22–24), which suggests that serum retinol is not a good measure of vitamin A status during toxicity. The serum retinol concentration is regulated by a number of mechanisms, including the formation of derivative retinoids from retinol and retinoic acid and the postprandial or postsupplemental increase in circulating retinyl ester concentrations.

Early studies proposed that fasting retinyl ester concentrations > 10% of total circulating vitamin A (retinol + esters) could be a biomarker for toxicity (8, 25, 26). Once vitamin A is ingested as a supplement or in a meal, serum esters increase as retinol is first esterified in the intestinal mucosa and then circulated in chylomicra. This process is particularly effective when the meal or supplement dose is provided with fat, which aids the esterification and packaging of retinol into chylomicra. The esterification process may exist to prevent large increases in retinol and retinoic acid, both of which are known to be potentially toxic forms of vitamin A. Myhre et al (27) performed a meta-analysis, from which they concluded that the ingestion of large amounts of vitamin A as liver or oil-based supplements caused an increase in retinol, retinoic acid, and related retinoids, but not as great an increase as that resulting from the ingestion of comparable doses

in water-miscible and emulsified forms. A postprandial increase in serum retinol concentration may be blunted when vitamin A is ingested with either food or ample dietary fat, whereas a significant amount of free (unesterified) retinol may circulate when vitamin A is consumed without dietary fatty acids, which leads to excessive production of retinoic acid (28). Thus, the immediate increase in retinyl esters that normally follows a high dose of vitamin A is not necessarily a concern. Nevertheless, of particular interest is the age-related increase in postprandial circulating retinyl ester concentrations, which suggests that the plasma clearance of chylomicra is delayed in older people (29). Whether this is a feature of normal aging or of disordered vitamin A metabolism requires further investigation.

An acute elevation of retinoids other than retinyl esters—eg, retinoic acid—occurs after the ingestion of a large amount of vitamin A, possibly because the intestinal absorptive capacity is overwhelmed, which leads to the oxidation of retinol to retinoic acid by the intestinal enterocytes (30) and to the rapid formation of retinoic acid from retinol in certain cells (5). Whereas retinoic acid can be produced from excentric cleavage of β -carotene in humans (31), it is generally considered a minor contributor to circulating concentrations, at least in normal, healthy persons.

The metabolism of vitamin A supplementation has been well studied in swine, which, because of intestinal tract similarities, are a good model for humans (30, 32). After ingestion of vitamin A from a single supplement or from liver, several retinoids and metabolites were detected in the plasma. These included retinol; retinyl esters; anhydroretinol; 14-hydroxy-4, 14-*retro*-retinol; various isomers of retinoic acid; 4-oxoretinoic acid; retinoyl β -glucuronide; and retinyl β -glucuronide (30). The structural schemata for these biosynthetic transformations are outlined in **Figure 1**. The polar metabolites of vitamin A—retinoic acid and its derivatives—are transported via the portal vein. Accordingly, the pig portal vein concentrations were higher than the central vein concentrations of both *all-trans*-retinoic acid and retinyl β -glucuronide, which suggests that a threshold of vitamin A intake may exist, wherein the intestine itself contributes systemically to toxic retinoids and metabolites.

Eckhoff et al (33) observed an increase in several plasma retinoids and proposed metabolic pathways for the formation of retinoid metabolites after the provision of a single oral dose of retinol or retinyl ester to *Cynomolgus* monkeys. Blood was sampled up to 32 h afterward. Increases in serum concentrations were observed for retinyl esters, retinol and its metabolite retinyl β -glucuronide, and retinoic acid and its metabolites, including *all-trans*-4-oxoretinoic acid, 13-*cis*-4-oxoretinoic acid, and retinoyl β -glucuronide. These results concur with those of recent work elucidating the metabolism of retinol and retinoic acid (34).

Few human studies have looked at the acute effects of a large dose of vitamin A on circulating vitamin A concentrations. Eckhoff and Nau (35) examined plasma concentrations of vitamin A metabolites in men ($n = 6$) after the ingestion of 833 IU $\cdot$ kg body $\text{wt}^{-1} \cdot \text{d}^{-1}$ as retinyl ester for 20 d—eg, 50 000 IU [15 000 retinol equivalents (RE)]/d for a 60-kg man. The current RDA for men is set at 900 μg retinol activity equivalents per day (36), which is a small fraction of the dose Eckhoff and Nau used. Blood was collected multiple times within 6 h of the dose and for 20 d thereafter. Transient increases in retinoic acid (both the 13-*cis* and *all-trans* isomers) and in 13-*cis*-4-oxoretinoic acid were observed. In contrast to the earlier study in monkeys (32), significant interindividual variability was noted in the current study.

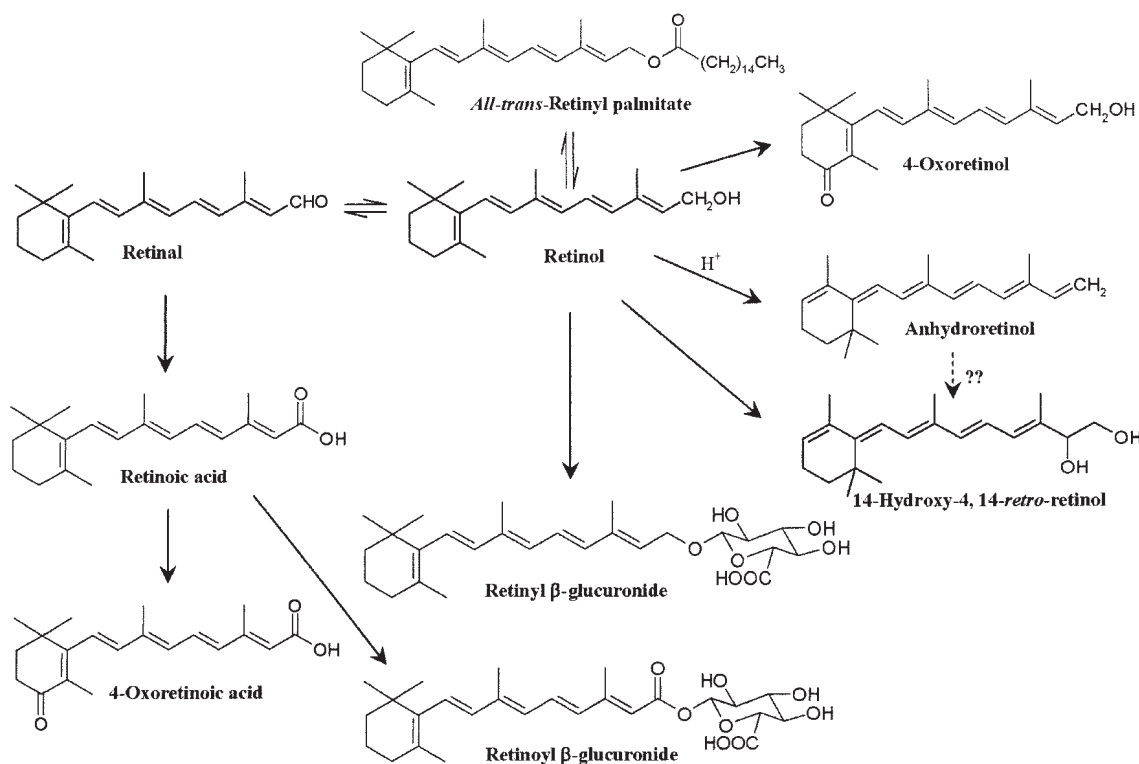


FIGURE 1. The biosynthetic transformations of preformed vitamin A that occur in the human body. Dietary preformed vitamin A is typically as retinyl esters. The retinyl esters undergo hydrolysis to retinol. Retinol can be oxidized to retinal or 4-oxoretinol. Retinal can be further oxidized to retinoic acid, which can be oxidized to 4-oxoretinoic acid. The β -glucuronides can be synthesized from both retinol and retinoic acid. Other metabolic forms identified in humans include anhydretinol and 14-hydroxy-4, 14-*retro*-retinol. Not pictured are the 4-hydroxy derivatives of both retinol and retinoic acid. Provitamin A carotenoids are cleaved to retinal in the intestinal mucosa. All structures are shown in the *all-trans* configuration. ??, the biosynthetic pathway has not yet been completely elucidated.

Measurements of plasma retinoic acid concentrations and those of related compounds may serve as biomarkers for subchronic vitamin A intoxication in an evaluation of the systemic generation of retinoic acid metabolites (32–34), which may help to establish safe dosages of vitamin A.

Buss et al (37) studied the effects of 5 different vitamin A doses on plasma vitamin A and its metabolites in women ($n = 10$). The single supplements were provided as either retinyl palmitate (15 000 and 45 000 RE) or an equivalent dose in fried calf liver. Blood was collected at intervals within 12 h of dosing and thereafter for 6 d. The results showed substantial increases in plasma retinyl palmitate, 13-*cis*- and *all-trans*-retinoic acid, and 13-*cis*- and *all-trans*-4-oxoretinoic acid. Women who received the supplement had significantly higher concentrations of retinoids than did those who received the liver, possibly because the food matrix may have ameliorated the absorption rate or altered the circulating forms of vitamin A. However, plasma retinol changed only slightly, which supports the concept that this method is not an appropriate means by which to evaluate a vitamin A supplementation trial.

Van Vliet et al (38) compared the metabolism of vitamin A from liver paste with that of a retinyl palmitate in oil supplement and measured the formation of retinoic acid and its metabolites in women ($n = 35$). Vitamin A was provided as 3000, 7500, or 15 000 RE. Blood was collected 2–24 h after supplementation. As in previous studies, serum retinol concentrations were unaffected by treatment. Increases in 2 isomers of retinoic acid and of

4-oxoretinoic acid were observed, and large individual differences were noted. However, unlike the study of Buss et al, which used higher vitamin A doses, the study of van Vliet et al found no significant difference between the supplement and liver paste.

To clarify the effects of liver intake on circulating vitamin A, Arnhold et al (39) studied the formation of retinoids in men ($n = 10$) after consumption of 1000 RE/kg body wt from turkey liver (2 g liver/kg body wt). This resulted in greatly increased plasma concentrations of retinyl palmitate, 5 isomers of retinoic acid, *all-trans*-4-oxoretinoic acid, and 14-hydroxy-4, 14-*retro*-retinol, the last of which (Figure 1) was identified for the first time in humans. The results of this study, which showed significant elevation over endogenous concentrations of several toxic as well as putatively nontoxic retinoids and metabolites, suggest the possibility that high vitamin A from supplements or vitamin A-rich foods leads to a toxic response in vivo.

The formation of derivative retinoids, such as *all-trans*-4-oxoretinoic acid and retinoyl β -glucuronide from retinoic acid and *all-trans*-4-oxoretinol and retinyl β -glucuronide from retinol, has been studied (32, 34, 40, 41). Although few in number, these studies identified the metabolites and derivatives that help to regulate vitamin A and provide clues about potential detoxifying mechanisms. Research in this area may lead both to a better understanding of how the detoxification of retinol and retinoic acid occurs in vivo and to safe and appropriate recommendations for the intake of preformed vitamin A from food and supplements. Collectively, the studies cited above suggest that systemic

concentrations of several retinoids and metabolites are elevated after ingestion of a single high dose of vitamin A from both synthetic and natural sources and that a toxic response is elicited if the dose is high enough. The data from these studies also suggest that biomarkers other than plasma retinol—eg, retinoic acid and its metabolites—may be useful in evaluating the risk for vitamin A toxicity.

Effect of chronic high vitamin A intakes on circulating vitamin A and tissue storage

Acute toxicity, which occurs when adults and children ingest $> 100\times$ and $> 20\times$ the RDA, respectively, for vitamin A over a period of hours or a few days (2), is less of a problem than is chronic toxicity from preformed vitamin A. Chronic toxicity results from the ingestion of high amounts of preformed vitamin A for months or years. Daily intakes of $> 25\,000$ IU for > 6 y and $> 100,000$ IU for > 6 mo are considered toxic, but there is wide interindividual variability for the lowest intake required to elicit toxicity (19, 20, 42). Children are particularly sensitive to vitamin A, with daily intakes of 1500 IU/kg body wt reportedly leading to toxicity (19, 20, 43). Similarly, the elderly may be at significantly greater risk of toxicity than are younger adults with a chronic high intake of preformed vitamin A, but the mechanisms for this greater risk are not known. Possibilities include reduced chylomicron clearance of vitamin A, increased intestinal absorption of vitamin A (44, 45), and other nutritional factors, such as zinc deficiency, a condition that is common among the elderly. Individual tolerances to different amounts of vitamin A ingested on a chronic basis have not been adequately studied. The role that genetics may play in this regard is unknown (2, 20, 42). If hypervitaminosis A is indeed a growing problem, more efforts are needed to characterize the circulation and storage of vitamin A at different stages of the life cycle.

Retinyl ester metabolism differs between species. Studies by Schweigert (46) suggested that strict carnivores and birds have a high percentage of vitamin A circulating as retinyl esters, and this appears to be a major way of transporting vitamin A in some species. In contrast, herbivores and omnivores normally circulate vitamin A as retinol bound to RBP. Elevated amounts of retinyl ester (ie, $> 10\%$ of total circulating vitamin A) in the fasting state have been used as markers for chronic hypervitaminosis A in humans (2, 8, 24, 47, 48) and monkeys (49). The mechanisms for this phenomenon are inconclusive; candidate mechanisms include decreased hepatic uptake of vitamin A (50) and the leaking of esters into the bloodstream from saturated hepatic stellate cells (8, 43, 51). Evidence from dogs suggests that retinyl esters may be released from the liver in LDL particles (52), but vitamin A metabolism in dogs may not represent that in humans and other primates.

The liver is the major storage site for vitamin A, containing $\approx 80\%$ of total body reserves during normal vitamin A status (51, 53). The hepatic uptake and storage of vitamin A under normal conditions has been well described in animals and humans (54–56). Hepatic storage of vitamin A will continue until a pathologic liver condition develops (20, 57). The vitamin A storage capacity of other tissues, however, has not been fully investigated. The existence of stellate cells in other organs, such as the kidneys and the lung, suggests that they may be fully capable of and adapted to storing vitamin A as retinyl esters (5, 58–60). The possibility that extrahepatic tissue stores vitamin A as free retinol or other forms is not completely elucidated (61). Because vitamin A is

involved in many biological processes, it is conceivable that extrahepatic stores contribute to the regulation of vitamin A homeostasis (5). In fact, adipose tissue is an important storage site for retinol (62). In normal chow-fed rats, the amount averaged 20 nmol vitamin A/g adipose tissue as both retinol and retinyl esters (63). Because adipose tissue and liver represent 15% and 4%, respectively, of the total body mass of normal-weight rats, the total amount of vitamin A in the adipose tissue is $\approx 14\%$ of that in the liver, which contributes significantly to total body stores in normal-weight animals.

Extrahepatic tissue vitamin A is characterized in humans (56), small mammals (58–61, 63–66), fish (55), and birds (67, 68). Most studies have focused on concentrations in the kidneys and the lungs, and a few have assayed other tissue. Results of these studies suggest wide interspecies variations in vitamin A concentrations in extrahepatic tissues. Dogs, marmoset monkeys, and foxes, for example, appear to store appreciable amounts of vitamin A in their kidneys (61, 69, 70), whereas humans and many other primate species do not. The human studies collectively suggest that extrahepatic vitamin A stores vary greatly between persons, most likely as a result of vitamin A status and possible underlying disease processes that may alter vitamin A status or metabolism. Schmitz et al (56) documented vitamin A in the kidneys and lungs at autopsy of 20 humans whose hepatic vitamin A concentration was 0.30 ± 0.28 $\mu\text{mol/g}$. Vitamin A concentrations were 0.052 ± 0.09 and 0.051 ± 0.11 $\mu\text{mol/g}$ in the kidneys and the lungs, respectively, and no correlation with hepatic stores was apparent. The vitamin A concentrations in all tissues ranged widely between the subjects in the study by Schmitz et al, and not all persons were healthy at the time of death. Most of the samples were from persons who had heart disease, cancer, or infections, and these conditions have unknown effects on the distribution of vitamin A among tissues. Therefore, true normal values are unknown.

Correlations between extrahepatic vitamin A stores and status have been shown in a few reports limited to rats (71) and monkeys (61). It is not clear whether vitamin A is stored in extrahepatic tissue when liver stores are high. Some studies suggest that extrahepatic vitamin A stores, such as those in the kidneys, rise during vitamin A deficiency (72), possibly as a reserve for the production of retinoic acid that is required for growth and cellular differentiation. Rhesus and marmoset monkeys at the Wisconsin National Primate Research Center have extremely high vitamin A stores (57, 61), both by human standards (6, 56, 73–75) and in comparison to previously published data from monkeys (76). The hepatic vitamin A stores of various animal species are compared in **Table 1**. Characterization of the extrahepatic vitamin A in these rhesus monkeys, whose hepatic vitamin A concentrations exceed published values ≈ 18 -fold (57, 61), provides information on whether a critical storage limit is reached and on the relative vitamin A concentrations and capacity of these other organs. Considering the extreme liver concentration in these rhesus monkeys (ie, 18.8 ± 6.4 $\mu\text{mol/g}$), the kidney and lung vitamin A concentrations were not remarkable—ie, 0.0100 ± 0.0032 and 0.0061 ± 0.0025 $\mu\text{mol/g}$, respectively (61). Because the rhesus monkey is genetically related to humans, further characterization studies with different vitamin A statuses might serve as a model for evaluating the risks and effects of a high vitamin A intake.

TABLE 1Comparison of published hepatic vitamin A concentrations in fish, primates, and other mammals¹

Animal	Hepatic vitamin A	Reference
	$\mu\text{mol/g}$	
Halibut	36.1	Bendich and Langseth (19)
Polar bear	22.0 ± 7.81^2	Schweigert (46)
	$13.6\text{--}18.9^3$	McDowell (77)
	$7.74\text{--}36.4$	Robbins (78)
	13.6	Robbins (78)
Bearded seal	12.6	Robbins (78)
Arctic fox	0.92	Bendich and Langseth (19)
Antarctic husky	5.04	Robbins (78)
Whale	1.08 and 1.07	O'Toole et al (76)
Rhesus monkey	17.0 ± 6.3	Penniston and Tanumihardjo (57)
	18.8 ± 6.4	Mills et al (61)
	1.25 ± 0.58	Penniston and Tanumihardjo (57)
	1.40 ± 0.44	Mills et al (61)
Human	0.441 ± 0.50	Underwood et al (73)
	0.326 ± 0.15	Furr et al (74)
	0.295 ± 0.28	Schmitz et al ⁴ (56)
	$0.147 \pm 0.14; 0.931 \pm 1.1^5$	Tanumihardjo et al (75)
	0–2.0	Olson et al (79)
	0.026–11.2	Suthutvoravoot and Olson (80)

¹ Conversion factors: 1 IU = 0.3 retinol equivalents (RE); 1 RE = 1 μg retinol = 0.0035 μmol retinol.² $\bar{x} \pm \text{SD}$ (all such values).³ Range (all such values).⁴ Not all subjects enrolled in this study were healthy.⁵ Data from 7 diseased children and 5 healthy adults, respectively. One adult had hepatic vitamin A stores in the toxic range (ie, 2.86 $\mu\text{mol/g}$ liver).

GLOBAL CONCERNS ABOUT CHRONIC AND ACUTE VITAMIN A TOXICITY

Effects of a chronic high intake of preformed vitamin A on bone

Osteoporosis, a disease of low bone mass, has multiple causes and, thus, many potentially modifiable factors. Technological advances in the measurement of bone mineral density have improved the detection and classification of osteoporosis and its forerunner, osteopenia. The World Health Organization's criteria for classifying these diseases are shown in **Table 2**. Recently,

several human studies have suggested an association between chronic high intakes of preformed vitamin A and bone loss that potentially leads to osteoporosis (82–86). The data are convincing, but the studies were observational and can be said to lead to more questions than answers. Data showing trends in the incidence of osteoporosis suggest a prevalence of up to 40% in postmenopausal women and 25% in men (87, 88), which is much less than the percentage of people estimated to have high preformed vitamin A intakes. Thus, interpretation of the aforementioned epidemiologic studies should proceed with caution until their results are confirmed by future research.

Historically, vitamin A toxicity has been associated with bone alterations of various types in many species (89–93), although variable tolerance exists between species (20). Human skeletal remains and reports of toxicity in Arctic people, whose intake of preformed vitamin A has traditionally been high, confirmed bone involvement (17, 18, 94). Clinical observations, such as hypercalcemia and elevated alkaline phosphatase, in persons with vitamin A toxicity clearly suggested that vitamin A affects bone. Synthetic retinoids in humans are reported to alter bone metabolism and increase turnover (95, 96). Case reports in children with hypervitaminosis A revealed altered skeletal development (22, 43, 97–100). Binkley and Krueger (92) noted the consistent occurrence of spontaneous bone fractures associated with hypervitaminosis A and also noted that no compound other than vitamin A is known to be associated with such fractures in animals. Cell-culture studies showed increased bone resorption and decreased bone formation (93, 101), which potentially led to bone loss, as is consistent with observations in humans and animals. Biochemical studies suggest an antagonism between vitamins A and D at the receptor level (102, 103) and an interaction with calcium-regulating hormones, such as parathyroid hormone

TABLE 2World Health Organization criteria for diagnosing osteopenia and osteoporosis¹

Category	Criteria	t Score
Normal	BMD $\leq$ 1 SD of that of average peak in young adults	0 to –1
Osteopenia	BMD between 1 and 2.5 SD below that of average peak in young adults	–1 to –2.5
Osteoporosis	BMD $\geq$ 2.5 SD below that of average peak in young adults	$\geq$ –2.5
Severe osteoporosis	Fragility fractures plus BMD $\geq$ 2.5 SD below that of average peak in young adults	$\geq$ –2.5

¹ BMD, bone mineral density. Information compiled from the *Physician's Guide to Prevention and Treatment of Osteoporosis* (81).

TABLE 3Comparison of 4 prospective observational studies in humans that tested for an association between vitamin A intake and measures of bone loss¹

Study	Subjects		Variables	Outcome measures	Results ²
	Country	Characteristics			
Lim et al (83)	USA (<i>n</i> = 34 703)	Postmenopausal women (median age 61 y)	Vitamin A intake from food and supplements	Hip fractures and all fractures; follow-up of 9.5 y	Slightly greater risk for hip fracture (RR = 1.18) with supplement use; no dose-response relation observed
Michaelsson et al (84)	Sweden (<i>n</i> = 2322)	Men aged 49–51 y	Serum retinol from all men; dietary vitamin A intake from one-half of the men 20 y after serum was obtained	All fractures; follow-up of 30 y	Serum retinol: highest versus lowest quintile, RR = 1.64 for any fracture and 2.47 for hip fracture; diet: retinol $\geq$ 1500 μ g/d was associated with doubled risk of any fracture
Promislow et al (85)	USA (<i>n</i> = 958)	Women aged 55–92 y; men aged 55–92 y	Vitamin A intake from food and supplements	BMD; follow-up of 4 y	Lower BMD was associated with retinol intake up to peak intake of 2000–2800 IU; the effect was more pronounced in women
Feskanich et al (86)	USA (<i>n</i> = 72 337)	Postmenopausal women aged 34–77 y	Vitamin A intake from food and supplements	Hip fractures; follow-up of 18 y	Retinol intake $\geq$ 2000 versus $<$ 500 μ g/d nearly doubled the rate of hip fracture

¹ BMD, bone mineral density; RR, relative risk.² “Retinol intake” refers to preformed vitamin A.

(104). Receptors for retinoic acid are located on both osteoblasts and osteoclasts, which indicates that they are direct vitamin A targets (90).

Whereas excess preformed vitamin A clearly affects bone adversely, few studies have specifically evaluated the association between vitamin A intake and bone status. Only 3 studies used biomarkers of vitamin A status to investigate an association with bone status (82, 105, 106). Two of these studies measured the serum retinol concentration, which is not a good status indicator, and the third measured fasting serum retinyl esters. A correlation between serum retinol and bone loss was found in only one study (82). Studies are needed to assess true vitamin A status, bone turnover markers, bone mineral density, and bone fracture incidence by using measures specific to vitamin A status (107).

Four large, prospective, observational studies (83–86), conducted in Scandinavia and the United States, where the incidence of osteoporosis is high (108), found associations between preformed vitamin A intake and hip fracture or osteoporosis. The studies differed in many design factors (eg, subject sex and age

and years of follow-up), which makes direct comparisons difficult (Table 3). Nonetheless, the findings generated much interest, because calcium intake is generally high in both these areas. It is interesting that the amount of dietary vitamin A associated with this effect was relatively low at 1500 RE, which is much lower than the amount traditionally associated with risk of toxicity and lower than the tolerable upper intake level (ie, 3000 RE), which is the highest amount thought to pose no risk of adverse health effects in the general population (36). These studies suggest that intakes much lower than 10 times the RDA, the amount conventionally thought to lead to toxicity (2, 20), are needed to increase risk for osteoporosis—ie, $\approx 2 \times$ RDA. The findings in these 4 studies contradicted other studies (106, 109, 110) reviewed by Myhre et al (27), which did not link vitamin A intake to a risk of osteoporosis. The Expert Group on Vitamins and Minerals (111) nonetheless concluded that the risk of hip fracture is a continuous graded response that includes exposure levels within dietary intake levels. Moreover, they were not able to establish a safe upper limit for vitamin A because of overlap with reasonable dietary intakes (111). The effect of vitamin A on bone

at the population level should be investigated further to ascertain whether the vitamin per se or other synergistic nutritional or nonnutritional influences enhance bone fragility.

Nutritional influences on the accrual and maintenance of bone mass were reviewed elsewhere (112, 113). Reported dietary data are an indirect measure of nutrient status. Calcium and vitamin D are necessary for achieving and maintaining optimal bone mass through the life cycle. However, given that the incidence of osteoporosis appears to be highest in countries with adequate calcium and vitamin D intakes and where the intake of preformed vitamin A is the highest, it is probable that other nutrition factors play a role. Clearly, more research is warranted, and studies should be designed to assess both vitamin A intake and status in persons with bone loss to clarify the remaining questions about the role of preformed vitamin A on bone in humans.

Vitamin A supplementation and fortification programs in developing countries

The control of vitamin A deficiency has relied largely on 1 or 2 megadoses of preformed vitamin A twice a year, which is an amount great enough to be considered toxic. The benefits of supplementation with high doses of preformed vitamin A in combating vitamin A deficiency in women and children are documented. Indeed, a strong body of literature describes decreased morbidity and mortality with vitamin A supplementation, especially in young children (114–118). Few studies, however, have characterized and confirmed the safety of these doses in humans, and the practice has relied instead on clinical measures of improvement specific to vitamin A [eg, curing night blindness and reversing other ocular pathologic conditions, improving maternal health, and enhancing serum retinol concentrations (114, 115, 119, 120)].

The World Health Organization currently recommends that lactating women in developing nations be given large doses of preformed vitamin A to reduce the incidence of deficiency both in themselves and in their nursing infants. Currently, the International Vitamin A Consultative Group recommends 2 doses of 200 000 IU vitamin A administered at least 24 h apart as soon after delivery as possible. Recent trials of one dose each of 300 000 (121) and 400 000 (122) IU were conducted in Africa. In an animal model (123), single large doses of preformed vitamin A increased maternal liver stores dose dependently, but the theoretical contribution to the nursing infant (123) and the actual increase in piglet liver vitamin A was not enhanced with increasing dosage (124). These dosages meet the criterion for acute toxicity in humans— $100 \times$ RDA (2, 19, 20). Nevertheless, these supplementation dosages and regimens in humans have not been adequately studied with respect to the short- or long-term safety for the mother who receives the dose or to the amount of additional vitamin A available to the nursing infant. Whereas a small incidence of transient toxic effects may be expected and tolerated through vitamin A administration in these programs, the mortality in India after supplementation of children with apparently excessive vitamin A (125) underscores the need to understand the dose levels and the frequency of administration that are safe and effective in humans.

Vitamin A is delivered to breast milk by chylomicra in the form of retinyl esters (126, 127). The process is not highly regulated, and, thus, milk concentrations of vitamin A may reflect the mother's vitamin A status (128) and recent dietary intake (129). Women in developing nations with poor nutrition have

relatively low breast-milk vitamin A concentrations (ie, $\approx 1 \mu\text{mol/L}$; 118, 130–132), whereas women in more prosperous nations with high intakes of vitamin A from foods or supplements (or both) have higher vitamin A concentrations (ie, $\approx 2 \mu\text{mol/L}$; 118, 131, 133). Lactating women in developing nations are provided supplemental vitamin A to enhance milk concentrations for nursing infants. Few data are available to show whether these high doses of vitamin A enhance breast-milk concentrations for an appreciable length of time. The current body of literature on this topic is focused largely on the use of breast milk as an indicator of response to postpartum vitamin A supplementation and not necessarily on the duration of the effect or the amount of vitamin A available to a nursing infant.

A few studies have examined both the total vitamin A concentrations in human milk after supplementation with a single large dose of preformed vitamin A and the duration of the effect. Rice et al (134) provided Bangladeshi women with 200 000 IU vitamin A 1–3 wk after delivery and found the highest milk vitamin A concentration (retinol plus retinyl esters) at 0.5 mo, a precipitous drop at 3 mo, and a decline to the same concentrations as in the placebo group at 6 and 9 mo after delivery. In a separate study, 300 000 IU vitamin A as retinyl palmitate was provided to Indonesian women ($n = 153$) 1–3 wk after delivery. The vitamin A treatment group had significantly lower vitamin A milk concentrations at 0.5 mo but significantly higher concentrations at 1–8 mo than did the placebo group (135). However, the vitamin A status of the treatment group was significantly lower than that of the placebo group at baseline, despite randomization, and this may have resulted in larger treatment effects.

Roy et al (117) examined breast-milk vitamin A concentrations in Bangladeshi women ($n = 50$) after providing a single oral dose of 200 000 IU vitamin A or placebo 24 h after delivery. Elevated breast-milk vitamin A concentrations were most pronounced at 24 h (11 and $3 \mu\text{mol/L}$ in supplemented and control groups, respectively). The elevation of vitamin A in the breast milk of the supplemented group at 1 mo ($1.92 \mu\text{mol/L}$) was markedly lower than it had been at 24 h, but it still was significantly higher than that in the control group. Treatment effect was still observed at 3 and 6 mo and amounted to a difference of 0.22 and $0.33 \mu\text{mol/L}$ from the control values, respectively. It is interesting that breast-milk vitamin A at 9 mo was significantly lower in the supplemented than in the control group. Similarly, in Indian women ($n = 300$) who were randomly assigned to receive either 200 000 IU vitamin A or placebo, breast-milk vitamin A was elevated at 24 h and then decreased gradually over 4 mo, at which time breast-milk vitamin A concentrations were the same as those in the control subjects (116).

More recently, Bahl et al (136) measured the effect of 200 000 IU vitamin A (as retinyl palmitate), given 21–42 d after delivery, on the breast milk of 924 women from Peru, India, and Ghana. At 2 mo after delivery, supplementation increased breast-milk vitamin A, but the effect was not sustained at 6 and 9 mo. There were site-specific effects that suggest a highly variable response. Breast-milk vitamin A concentrations did not differ between the women in Ghana and Peru, which showed that the effect was due solely to the breast milk of the women in India. Moreover, breast-milk vitamin A concentrations were significantly lower in the supplemented women than in the control subjects at all 3 sites 9 mo after delivery.

The lack of a consistent and sustained effect on breast-milk vitamin A concentrations in these studies has prompted some

researchers to suggest that 200 000 IU vitamin A is too low (134), even though few studies of the effect of a higher dose have been conducted. Research by Ayah et al (121) in Kenya suggested that an increase to 400 000 IU vitamin A would not be a solution, because a consistent effect beyond 4 wk was not observed when breast-milk vitamin A was corrected for fat content.

Furthermore, no data on the metabolism of vitamin A in mammary tissue after a large dose of preformed vitamin A have been published. Such data would be important to ensure that high doses of vitamin A do not result in an increase in retinoic acid concentrations in the breast milk. One report has documented the excretion of acitretin, as well as its 13-*cis* metabolite, into the breast milk of a woman who received oral acitretin therapy for a skin disorder (137). It is possible that, as in plasma, milk retinoic acid concentrations rise after a large dose of preformed vitamin A. If so, retinoic acid would be available to the nursing infant, who may have limited capacity to metabolize it to less-toxic metabolites because of the infant's immature renal and hepatic systems.

A lactating sow–nursing piglet model was used to better define the effects of acute large vitamin A doses on both maternal and infant vitamin A status. High doses of preformed vitamin A were given to lactating sows to emulate doses administered to lactating women in developing countries (32, 123, 124). The circulation of serum retinyl esters increased after the dose (32); however, sow milk concentrations did not differ significantly between dose groups (123). On the basis of a theory that the benefit to infants may not differ between 400 000 and 200 000 IU when vitamin A is given as a single bolus (123), a separate study was performed in which piglets were killed at 2 time points after the administration of the same doses to sows. As predicted, piglet liver vitamin A reserves did not differ between the high- and low-dose treatment groups (124). Moreover, less-toxic metabolites of retinol and retinoic acid were elevated in the sows after high-dose supplementation (Figure 1). Postdose increases were observed for total vitamin A and retinyl esters, 4-oxoretinol, retinoyl β -glucuronide, and retinyl β -glucuronide but not retinoic acid (32). From this series of studies, it appears that higher doses given to human mothers may not be more beneficial than are lower doses for their infants when given as a single bolus (124), and the higher doses may result in the formation of a greater amount of detoxifying metabolites in the mothers (32).

Given the above results, it would appear that a single large dose of preformed vitamin A may not be as beneficial as a long-term food-based strategy or low-dose vitamin A supplements given for a longer period of time. Research in rats suggests that sustained chylomicron delivery may be the most important means by which vitamin A is delivered to mammary tissue (126). Moreover, the large doses of vitamin A may be toxic or teratogenic (or both), and, therefore, the timing of the dose is critical. Women in developing nations tend to become pregnant very soon after lactation amenorrhea (132), and, if responsiveness to vitamin A dosage is truly best between 5–9 wk after delivery (132), questions remain about the effectiveness of vitamin A doses provided to women $\leq$ 6 wk after delivery as currently recommended.

Vitamin A supplementation efforts were initiated as an immediate action to control vitamin A deficiency while other more long-term, sustainable interventions could be developed and implemented. Examples of the latter include food fortification, diet

diversification, and biofortification. Sugar-fortification programs are in place in a number of countries, including Zambia, El Salvador, Guatemala, Honduras, and Nicaragua. A recent evaluation of the program in Nicaragua (138) suggested a positive effect, but continued monitoring of the program will be necessary to ensure that sustained greater intakes of preformed vitamin A from sugar pose no risk of adverse health effects (139). Several children in that study had estimated liver reserves above those considered normal—ie, 300 μ g/g liver—as ascertained by using a stable isotope method. Because diet diversification and biofortification typically provide provitamin A carotenoid sources, toxicity will not occur. Moreover, carotenoid sources of vitamin A, which have antioxidant capacity, may confer to the nursing infant other benefits that are not available with preformed vitamin A.

SUMMARY

Vitamin A is an essential nutrient and is added to a variety of foods in the developed world and to specific foods in developing countries. Ideally, vitamin A status is monitored as part of public health programs to prevent the occurrence of both subclinical deficiency and toxicity. The deleterious effects of vitamin A deficiency are known, but further research is needed to ascertain whether subclinical toxicity exists and, if so, what are its effects on overall health and well-being.



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