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 CHRIS TEMOVSKY

I, Chris Temovsky, declare and state as follows under penalty of perjury:

1. I am the co-president of Ddrops Company ("Ddrops'"). I am one of the partners of Ddrops.

2. This declaration is based upon personal knowledge and my review of relevant company documents unless otherwise indicated.

3. I earned an Honors Bachelors of Science in Biology degree from the University of Toronto, Canada in 1988. I then went on to earn a Master of Business Administration degree (MBA) in Marketing and Finance from Queens University Ontario, Canada in 1999.

4. I have worked in the pharmacy, health, wellness, and vitamin supplement industry since 1988, for over 35 years. I have often been tasked with understanding the purchasing and consumption behaviors of consumers regarding products and supplements. I use this information to make quality, marketing, and advertising decisions, as well as considerations for future business development and distribution.

5. Between 1988 and 2006, I worked in the pharmaceutical industry at Syntex, Inc. (acquired by Roche under its Pharmaceuticals Division), which was an international pharmaceutical company that manufactured and sold human and animal-grade pharmaceutical products and diagnostic assay systems. At Syntex, Inc., I had a variety of duties and undertook many opportunities within the

company including working in the roles of pharmaceutical sales representative, market research associate, pharmaceutical product manager, and sales manager. After the acquisition by Roche, I was a Marketing Director and International Business Leader for pharmaceutical-grade products.

6. In or about 2004, I was hired as the Vice President of Sales and Marketing of Solvay Pharmaceuticals, where I remained until late 2006 when business negotiations and the partnership for Ddrops began.

7. Since July 2008, I have been co-president of Ddrops. My key areas of responsibility include overseeing sales, marketing, monitoring competitor activities, and distribution of our products globally. Those areas have remained essentially the same since I became co-president of Ddrops. As co-president, I have access to the property, books, financials, and records of Ddrops.

8. Another responsibility I have at Ddrops is to monitor sales and market share in the United States and Canada for Ddrops' various products and its competitors' products. Drops also keeps its own internal financial records.

9. In Canada, Ddrops uses the IQVIA Consumer Health lnsights Canada ("CHIC") service from IQVIA SOLUTIONS CANADA INC. ("IQVIA") for Canadian sales and market share information for vitamins and select probiotics One Shot – National and regional markets that provide online access to IQVIA's OTC Point-of-Sale category data and vital statistics required to track and compare

performance in Markets: National Grocery+Mass+Drug (GDM), National Grocery+Mass, National Drug, National GM+WC, Total West GDM, Ontario GDM, Quebec GDM, Atlantic GDM, SDM National, LCL National, and National Discount markets.

10. Since about 2018, Ddrops has subscribeds and paid for MarketTrack OneShot service from AC Nielsen Company of Canada ("Nielsen") for access to sales and market share information for United States sales of vitamins in the grocery, mass market, and drug channels.

11. As part of my responsibilities at Ddrops I also attend several tradeshow health conferences each year in the U.S., Canada, and internationally, including the American Academy of Pediatrics annual event, and the NAPNAP Nurse Practitioner event, to name a few, on behalf of Ddrops.

12. Ddrops is a partnership registered under the laws of Ontario, Canada, with its principal offices located in Woodbridge, Ontario Canada. Ddrops is a world leader in the manufacturing, sales, and research of vitamin D nutritional supplements and pharmaceutical-grade supplements. Ddrops creates nutritional high-quality supplements that support the healthy growth and development of infants, children, and adults.

13. Elaine and Reinhold Vieth (the "Vieths") are individuals who are citizens of Canada and reside in Toronto, Canada. I know them personally and professionally.

14. The Vieths are inventors and owners of certain inventions, and patents. One of the inventions created by the Vieths resulted in Canadian Patent No. 2,578,881 (the "Canadian Patent"), entitled "Vitamin D Compositions and Method of Administration to a Human Being," which was issued on November 4, 2008.

15. Since January 1, 2007, the Vieths have granted an exclusive, worldwide license under all patents or patent applications relating to this particular invention of "Vitamin D Compositions and Method of Administration to a Human Being," to The Ddrops Company Inc., a Canadian corporation, including the right to enforce the patent against infringers and to collect damages (the "License").

16. As of July 24, 2008, The Ddrops Company, Inc., with the consent of the Vieths, transferred and assigned its rights and obligations under the License to Ddrops. Since the time of the assignment, The Ddrops Company, Inc. has not engaged in business operations.

17. On June 30, 2015, the United States Patent and Trademark Office (U.S.PTO) issued U.S. Patent No. 9,066,958 ("the '958 Patent"), entitled "Vitamin

D Compositions and Method of Administration to a Human Being," to the Vieths. The Vieths are the owners of the '958 Patent.

18. Ddrops sells vitamin D supplements, including vitamin D supplements for infants. Ddrops sells its Baby Ddrops® product and method ("Patented Product") under the registered U.S. trademark "Baby Ddrops®". Ddrops has sold the Patented Product in the United States since at least June 2011. Ddrops also sells Kids Ddrops®, Ddrops® Booster, Ddrops® for Adults, and Vegan Ddrops®, all of which are vitamin D supplements.

19. As co-president of Ddrops, I am aware of the composition, ingredients, properties, instructions for use, manufacturing, bottling, and distribution of the Patented Product. The Patented Product has a composition according to the ingredients listed on the box and has always been sold with instructions. The ingredients are Vitamin D3 (Cholecalciferol) 400 IU Per Drop and Fractionated coconut oil. 400 IU of vitamin D3 equals 10 mcg of vitamin D using a .025 conversion factor as specified by the United States National Institutes of Health. Attached as Exhibit A is a true and correct copy of a screenshot from the United States National Institutes of Health's website (accessed February 3, 2024).

20. From its founding, Ddrops' innovative vision and mission has been to develop vitamins and supplements that would meet and pass even the most rigorous testing and quality protocols.

21. In 2007, Ddrops invested Redacted to build and develop its own NSF-certified Canadian manufacturing facilities, and independently sought to subject its business and products to rigorous health and quality certifications for its products. Ddrops holds two levels of quality NSF International Certification – NSF GMP Facility Certification and NSF Product Certification, and manufactures its products within its Canadian facilities that are also NSF-certified facilities.

22. Ddrops is globally one of the few vitamin and supplement companies that follows and even goes beyond the highest good manufacturing practices and standards (GMPs) of the supplement and therapeutic goods industries, ensuring its products are of the highest quality, requiring NSF certification.

23. By Ddrops independently obtaining NSF certification, its manufacturing facilities and products are subject to, and do undergo annual and random health and safety inspections by Health Canada. Ddrops consistently and routinely passes these health and quality inspections.

24. Although such high-quality standards may be unusual for the less regulated vitamin and supplement industries globally, Ddrops felt it was essential for the health and safety of its end customers. Especially, where Ddrops' largest clientele and potential product consumers are infants, it is crucial that every Ddrops batch is rigorously tested and quality controlled.

25. Aside from its stringent manufacturing quality processes, Ddrops products are specially formulated with science-based, premium nutritional ingredients and supplements, specifically designed to the unique efficacy needs of each infant, mom, and parent. Indeed, from its inception, Ddrops' mission has been to create effective and healthy quality products and supplements that make life a little simpler for moms and parents, so that they can give the best to their families, every single day.

26. Like Ddrops' other products, the contents, concentration, and amounts of every batch of the Baby Ddrops® product are tested by an independent third-party quality testing laboratory before distribution and shipment to retailers and end customers.

27. Not only does this testing ensure quality and safety, but the batch results also confirm that the selected batch tests fall within the concentrations and specifications for specific products. Batch test results of all Ddrops products must conform to the targeted dose and specifications, with all non-conforming goods destroyed. I personally review every batch result of products for Ddrops.

28. Every single formulation of Baby Ddrops' Patented Product is held in quarantine until Ddrops receives a batch test report confirming that the batch conforms with the specifications and concentrations for the Patented Product, which contains a composition matching one or more claims of the '958 Patent.

This conforming concentration then, would be administered according to one or more claims of the '958 Patent. Attached as Exhibit B is a true and correct copy of a sample of batch test report showing the batch conforms to the product specifications for the Patented Product.

29. Since sales of the Patented Product began in the United States, Ddrops has marked the product packaging of the Patented Product with the words "patent pending." Since the issuance of the '958 Patent in 2015, Ddrops has marked the product packaging with the notice, "U.S. Patent 9066958."

30. In or about October 2015, Ddrops began shipping the Patented Product with the '958 Patent number into the United States. Attached as Exhibit C are true and correct copies of the packaging for Ddrops' Patented Product as sold in the United States.

31. The packaging and bottle label for the Patented Product states that its ingredients are cholecalciferol, which is Vitamin D3, and fractionated coconut oil, which is a medium chain triglyceride (MCT). See Exhibit C.

32. The packaging and bottle label for the Baby Ddrops product instructs the end-user on the method for administering vitamin D:

> Directions: Infants less than 1 year old: Place 1 drop daily onto mother's nipple or on a clean surface, such as a washed finger or a pacifier, and allow baby to suck for at least 30 seconds. Or mix one drop daily with milk, juice or other food.

See Exhibit C.

33. The packaging and bottle label for the Patented Product states: "Serving Size: 1 drop (0.028mL or 0.001 fl. oz.)." See Exhibit C.

34. The Patented Product provides 400 IU of vitamin D3 per drop. See Exhibit C.

35. Ddrops' Patented Product was truly revolutionary in the infant and baby vitamin and supplement industry. Before Ddrops introduced its Patented Product, no other company had ever offered in the United States or Canada a product that has a composition that includes only vitamin D3 in MCT oil (fractionated coconut oil) and instructs that one drop is to be placed on the exterior surface of an object that it adheres to, and giving the object to allow the drop to be sucked or licked.

36. Before 2006, and even after Ddrops entered the market, the existing vitamin D supplements for infants generally consisted of one-half to one mL of a detergent-solubilized aqueous liquid emulsion and required additional tools for administration.

37. These kinds of predecessor products were typically given directly into the mouth by use of a tool or mixed into milk or other food for consumption. Instructions for administering these types of predecessor vitamin D products typically involved inserting a plastic or glass dropper into the container with the vitamin concentration, drawing out the desired dose of concentration, and then

taking that syringe and squirting that dose directly into an infant's mouth, or into the infant's food by placing force on the syringe to release the concentration for delivery.

38. When Ddrops began selling its Patented Product, many of the other predecessor vitamin D products available at the time were also not tasteless and had an odor. It was a known problem that some of the predecessor products used oils comprised of unsaturated fatty-acid triglycerides that became rancid over time. It was also known that these products carried an unpleasant or displeasing flavor and had a greasy feel to touch, which can coat the inside of the mouth.

39. In the vitamin and supplement industries it is well known that certain oils like sunflower oil, corn oil, fish oil, or olive (commonly used as carrier mixing oils), and other oils have their own independent flavor profiles and smells, separate from anything these oils might be mixed with to formulate specific supplements and concentrates.

40. For example, cod oil is highly detectable in smell, and gives off a "fishy after-taste" flavor and taste profile. Another example is sunflower oil, which has a naturally bitter and sharp flavor, and can also leaving a slightly yellow residue, with possible staining. Similarly, olive oil carries an earthy detectable scent and flavor profile, and also can leave a yellow residue that may cause staining.

41. Because certain oils like corn oil, sunflower oil, olive oil, and fish oil have naturally stronger flavor profiles independently, those profiles are often exacerbated when stored over long periods of time, which results in pungent and even rancid tasting and smelling products. Therefore, a common problem with the predecessor products was due to the detectable and unsavory flavor and odor profiles of these products, making them even less tolerable to the taste or smell.

42. These issues also created potential problems with administration. The odor and taste issues with the predecessor vitamin D products created frustrating problems with administering a precise and accurate therapeutic dose to the infant. Further, these problems in the predecessor products also presented a risk that during the administration process, an infant could potentially gag on or inhale some of the liquid upon administration due to the taste, smell, or texture. This could result in toxic overdose, harm, or even death to the infant.

43. When Ddrops introduced its Patented Product in the food/mass/drug channels, vitamin D supplements for infants were dominated by 1 mL products. Enfamil® D-Vi-Sol, produced by Mead Johnson, was the infant vitamin D market leader.

44. Enfamil® D-Vi-Sol vitamin D drops was and is still marketed as a naturally fruit flavored liquid supplement with a manual dropper for administration to infants and babies containing vitamin D and glycerin, water, polysorbate 80

(emulsifier), citric acid (antioxidant for vitamin D), natural flavor, sodium citrate. Attached as Exhibit D are true and correct screenshots of the product photos, specifications, and instructions for Enfamil® D-Vi-Sol vitamin D drops.



45. Enfamil® D-Vi-Sol vitamin D drops is made of 50 mL of vitamin D mcg and claims to provide 400IU, meeting the daily amount of vitamin D recommended by the American Academy of Pediatrics for infants daily. Enfamil D-Vi-Sol vitamin D drops are administered through a syringe directly to the infant or into food:

> Shake the bottle well and fill the dropper to 1 mL line (daily serving) unless doctor recommends another dosage; Dispense gently into mouth towards inner cheek; a small amount will remain in the tip; To increase acceptance, mix with breast milk, formula, juice, cereal or other foods.

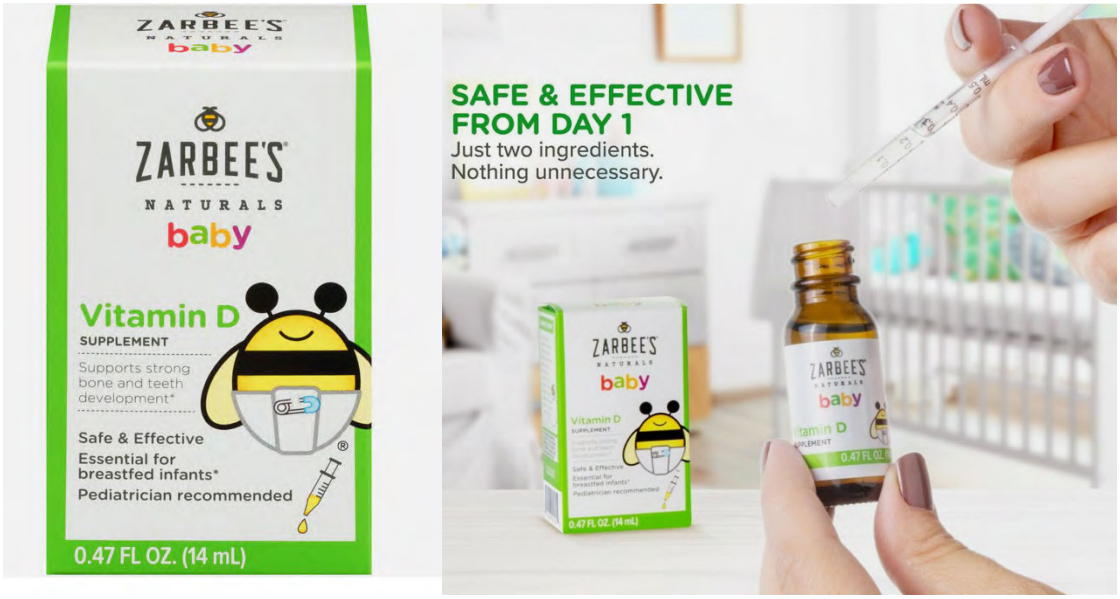
See Exhibit D.

46. Enfamil® D-Vi-Sol vitamin D drops formula has a distinct odor that is present from opening the product, and also has a greasy texture when applied to the skin. This greasy texture does not dissolve easily without wiping or cleaning the skin with a damp cloth. Customers have complained about these problems on Enfamil®'s own website regarding its D-Vi-Sol vitamin D product:

> The flavor is just awful. My daughter could not keep it down ever. So I tasted it myself and it went right in the trash. There are alternatives that cost way less, have no flavor and you only need one drop (bottle, pacifier, etc.)
> …
> Tastes horrible. I'd search around and get something else.
> …
> I definitely agree with the other reviews. My daughter always seems to get an upset tummy on the days we give this to her. She hates the taste even though I gave it a try and it didn't taste bad nor did it taste good. I was hoping that this would work out because I like the fact that it has a dropper but I won't be repurchasing this sadly.
> …

Attached as Exhibit E is a true and correct collection of screenshots of customer reviews online regarding odor and taste issues with the Enfamil® D-Vi-Sol vitamin D drops.

47. Another competitor at the time Ddrops entered the market was Zarbees Baby Vitamin D supplement, which is still sold today. Attached as Exhibit F are true and correct screenshots of the product photos, specifications, and instructions for the Zarbees Baby Vitamin D product.



48. Zarbees Baby Vitamin D supplement contains Vitamin D3 in medium chain triglycerides (MCT), and claims to meet the daily recommended dose of 400IU of vitamin D for infants by the American Academy of Pediatrics.

49. However, Zarbees is administered directly into the mouth or mixed with food via syringe: "Administer 0.25 mL daily, using the enclosed syringe. Can be administered directly into the mouth or mixed with food or drink."



See Exhibit F.

50. When Ddrops entered the market, none of the competitors in the baby vitamin D market sold a product and method of use that was the same as the claims in the ‘958 Patent.

51. Ddrops product was an innovative solution to giving babies vitamin D. For the first time, parents and caregivers could now easily administer just one drop of liquid vitamin D, precisely at feeding time – providing the intended therapeutic dose.

52. Ddrops’ Patented Product solved many of the notable issues in predecessor products related to pungent or undesirable flavors and odors. Ddrops’ Patented Product also solved the administration problems with previous products, by not requiring any additional tools or syringes that rendered previous administration methodologies as clumsy, unpredictable, and frustrating at best.

53. Ddrops’ Patented Product contains no artificial preservatives, allergens, or artificial flavors. From inception, the Patented Product was designed to have no detectable taste or odor, so as to not interfere with natural feeding.

54. When Ddrops first entered the market and attended trade shows, it was clear to me how spectacularly innovative the one-drop administration of vitamin D was.

55. Throughout the years, I have had the honor to speak with customers, consumers, parents, and physicians, who provide in-person testimonials about the Patented Product. Individuals often approach me with success stories regarding the efficacy and ease of use of the Ddrops patented method and product. These comments mirror reviews Ddrops has received regarding the Patented Product. Attached and Exhibit G is a true and correct copy of customer reviews of the Patented Product.

56. Ddrops' Patented Product isn't just highly regarded and praised by customers, it has also become a research staple to the clinical and medical industries. Since 2008, Ddrops has provided vitamin D products to universities, medical professionals, and researchers globally for clinical studies. This has earned Ddrops a reputation for being the "go-to" vitamin D tester product for vitamin D in controlled clinical research settings.

57. Ddrops products have been selected and requested for testing in more than a dozen clinical trials around the world, demonstrating efficacy and safety in all ages, from infants to adults. These studies were completed in partnership with some of the best educational and research institutions across North America and Europe.

58. Attached as Exhibit H is a true and correct copy of the study by Lontchi-Yimagou, Eric, et al. "Insulin-Sensitizing Effects of Vitamin D Repletion

Mediated by Adipocyte Vitamin D Receptor: Studies in Humans and Mice." Molecular Metabolism, vol. 42, Dec. 2020.

59. Attached as Exhibit I is a true and correct copy of the study by Kimball, Samantha M., et al. "Effect of a Vitamin and Mineral Supplementation on Glycemic Status: Results from a Community-Based Program." Journal of Clinical & Translational Endocrinology, vol. 10, Dec. 2017.

60. Attached as Exhibit J is a true and correct copy of the study by Rueter, Kristina, et al. "In "High-Risk" Infants with Sufficient Vitamin D Status at Birth, Infant Vitamin D Supplementation Had No Effect on Allergy Outcomes: A Randomized Controlled Trial." Nutrients, vol. 12, no. 6, 11 June 2020.

61. Attached as Exhibit K is a true and correct copy of the study by Burt, Lauren A, et al. "Adverse Effects of High-Dose Vitamin D Supplementation on Volumetric Bone Density Are Greater in Females than Males." Journal of Bone and Mineral Research, vol. 35, no. 12, 16 Sept. 2020.

62. The Patented Product has also received prestigious awards, including the Mom's Choice Gold Award; a Parent tested – Parent approved™, awarded on September 27, 2012. The Mom's Choice Awards ("MCA") is based in the United States, and evaluates products and services created for children, families and educators. MCA is globally recognized for establishing the benchmark of excellence in family-friendly media, products and services, and has reviewed

thousands of items from more than 60 countries. The MCA evaluation process uses a methodology in which items are scored on a number of elements including production quality, design, educational value, entertainment value, originality, appeal and cost, where each item is judged on its own merit. Attached as Exhibit L is a true and accurate copy of Ddrops' 2012 Mom's Choice Gold Award; a Parent tested – Parent approved™ award.

63. In 2015, Ddrops also won the Cribsie Awards New Arrival "Academy Star" Award. The Cribsie Awards are the definitive awards for the parenting industry that offer parents a secure forum for parents to provide real reviews and recommend their favorite brands and services from a curated list of best-of-the-best finalists across seven different categories. The Cribsie Awards states that its mission is to "recognize the best brands, products, services, and websites for babies, tots, and the parents who adore them." Attached as Exhibit M is a true and accurate copy of Ddrops' 2015 Cribsie Awards New Arrival "Academy Star" Award.

64. Ddrops also won the Bounty Mums Love "Commended" Award on January 28, 2015. Bounty is an established and trusted parenting Australian brand that has been providing information and support to expecting parents for over 35 years, from the first trimester of pregnancy, through to parents with toddlers and children. Bounty awards are judged by Bounty's "expert panel" as well as real

parents. The Bounty Baby Awards provides consumers with a list of the top baby products from pregnancy through to parenthood. The Awards are promoted across each Bounty platform, reaching over 500,000 pregnant women and new mothers. Attached as Exhibit N is a true and accurate copy of Ddrops' 2015 Bounty Mums Love "Commended" Award.

65. In 2015, Ddrops was the Well.ca Green Choice Award Winner. Well.ca is a Canadian e-commerce health and wellness company that specializes in health, wellness, beauty, baby, home, and green and natural products. Every year Well.ca awards carefully curated, peer-reviewed and trusted products to its customers, providers, parents, caregivers, and children. Attached as Exhibit O is a true and accurate copy of Ddrops' 2015 Well.ca Green Choice Award.

66. In 2016 Ddrops was the National Parenting Product Awards ("NAPPA") Winner. NAPPA awards and showcases products loved and used by families, and products are evaluated by real parents and caregivers. In order to be a NAPPA Award winner, a product must meet specifically the "parent test" – do they feel comfortable using this product for their own child, would they buy it for themselves, do they feel that this is a quality product with safe materials and good design? family approved and rated products. NAPPA Awards has been celebrating the best in the industry for 34 years, and prides itself on showcasing the best toys,

games, books, baby and family products. Attached as Exhibit P is a true and accurate copy of Ddrops' National Parenting Product Awards 2016.

67. Based on my 35 years of experience in the vitamin and supplement industry, each of the above referenced awards occurred because of Ddrops' innovative features—specifically the use of the 1 drop method of administration of Vitamin D to deliver a therapeutic dose. Ddrops' innovative features are also praised by parents and physicians, as will be further identified below.

68. Ddrops' Patented Product is so popular that it was even unexpectedly featured on "The Price Is Right" television program in 2015 during the "It's In The Bag" pricing game. Ddrops did not know its product would be used on the program. Attached as Exhibit Q are true and correct copies of screenshots showing the Ddrops' featured on "The Price Is Right" television program from 2015.

69. Independent third-party studies also show the efficacy of the Patented Product. BMC Public Health, a peer-reviewed journal that considers articles on the epidemiology of disease and the understanding of all aspects of public health, published an article that concluded: "Interestingly 80% of care-givers reported giving their infants D-Drops® versus only 16% who supplemented with D-Vi-Sol. The reason for the popularity of D-Drops® may be their ease of administration requiring only a single drop that can be placed on the mother's breast prior to nursing, versus the need to use a dropper to administer D-Vi-Sol®." Crocker, B.,

Green, T.J., Barr, S.I. et al., "Very high vitamin D supplementation rates among infants aged 2 months in Vancouver and Richmond, British Columbia, Canada," BMC Public Health 11, 905 (2011). A true and correct copy of this article is attached as Exhibit R.

70. Ddrops' Patented Product has also been a commercial success in the marketplace. Every year, millions of mothers, parents, and caregivers across Canada, the U.S. and the world choose to rely on Ddrops' baby vitamin D drops product. Indeed, Ddrops products are sold in thousands of pharmacies, health food stores, and groceries throughout Canada, the United States, Europe, and Asia.

71. Since 2008, Ddrops has grown to become Canada's #1 infant vitamin brand.

72. From 2009 to present, Ddrops has tracked its Canadian sales information and change in growth internally with raw factory sales numbers. Attached as Exhibit S is a true and correct chart showing Ddrops sales and market numbers in Canada from 2009-2016 which was created using Ddrops' internal confidential financial records.

73. Ddrops' Canadian sales numbers show that when Ddrops entered the market with its Baby Ddrops® product covered by the Canadian Patent in 2008, it made up zero percent of the market share. Ddrops ended 2008 with [redacted]% of the Canadian market for infant vitamin D. Then, in its first full year of sales in 2009,

Baby Ddrops® jumped to make up [redacted]% of the Canadian market for infant vitamin D. See Exhibit S.

74. From there, sales quickly grew due to the innovative features of the product and the Patented Product became the market leader in Canada. In 2010, Baby Ddrops® made up [redacted]% of the Canadian market for infant vitamin D. By 2011, Baby Ddrops® was the market leader in Canada with a [redacted]% market share for the infant vitamin D market. In 2012, Ddrops captured [redacted]% of the Canadian market. By 2013, Ddrops estimates it captured over [redacted]%) of the Canadian Infant vitamin D market. Since 2013, Baby Ddrops® has continued to be the top infant vitamin D market leader in Canada. See Exhibit S.

75. Ddrops began selling the Patented Product in the United States in 2015. Similar to the success in Canada, United States sales of the Patented Product similarly began as a great success.

76. Between 2015-2018, Ddrops tracked its U.S. sales information and change in growth internally with raw factory sales numbers. See Exhibit S, which contains a true and correct chart showing Ddrops sales and market change in the U.S. from 2015-2018, which was created using Ddrops' internal confidential financial records.

77. Since 2018, Ddrops has subscribed to various U.S. sales and market shares data services from AC Nielsen Company ("Nielsen"), including the Answers

on Demand service, the Byzzer data service, and MarketTrack OneShot services, from which provides Ddrops access to U.S. sales data and market share information for the sales of vitamins in the grocery, mass market, and drug channels. Ddrops' routinely relies on information and data included in Nielsen reports for its business practices and market analyses. .

78. By 2018, Ddrops had captured Redacted% of the U.S. infant vitamin D market. Ddrops' U.S. market share reached Redacted% for the infant vitamin D market in 2019. See Exhibit S.

79. However, the U.S. growth changed. In 2021, Ddrops' previous market gains dropped by Redacted of its previous gains, with Ddrops making up just Redacted% for the infant vitamin D market. See Exhibit S.

80. I attribute the decrease in Ddrops' market share in the U.S. in 2021 and thereafter to Mommy's Bliss changing its baby vitamin D product composition to be the same as our Patented Product. I also do not believe that Ddrops' drop in U.S. market share was a result of the global COVID-19 Pandemic, because Ddrops sales in Canada did not suffer a similar decline. As I describe further below, the change in Mommy's Bliss product in 2021 made it identical to Ddrops' Patented Product, with the same composition, same one-drop feature, and same instructions to place one drop on a mother's nipple or pacifier. Ddrops had not faced another product in the market identical in all respects to its product since it had

successfully sued two distributors who copied our product in 2015 and 2016, as I describe further below.

81. I attribute the financial successes to the innovation of formula and administration instructions contained in the Ddrops' Patented Product. Ddrops' Patented Product includes only vitamin D3 in MCT oil (fractionated coconut oil) and a hassle-free method of administration of the composition to an infant by applying one drop to an exterior surface of an object, such as a mother's nipple or a clean surface, to allow the drop to be sucked or licked from the object.

82. Ddrops has always believed as a core business principle that the products speak for themselves, including Ddrops' Patented Product. Ddrops, therefore, has not expended significant funds to promote the Patented Product. Therefore, it is undeniable that the success of Ddrops is due to the innovative composition and administration method that delivers an effective and therapeutic dose of the Patented Product.

83. In fact, since 2016, Ddrops Redacted [redacted]. Instead, Ddrops relies on customer testimonials, reviews, and authentic social media interactions, and word of mouth testimonials through customers and parents, such as the following:

Easiest Vitamin D! I am glad I found these vitamins. After researching the better brands for infant vitamin D, I saw this one recommended over and over. I love that it is just one drop vs the vitamin D we were prescribed (where you have to administer an entire syringe). I find the vitamin comes out at a good rate and I haven't had any issues with it. It's free from any of the gross and unnecessary stuff (dyes, etc). Easy for breastfeeding or just put a drop on a pacifier [*sic*]. I'll continue to use this brand.

…

Better than prescription drops!

Our doctor sent in a prescription to our pharmacy for some infant oral vitamin d that was total garbage (one dose was a huge amount for her at 1ml, full of preservatives, vitamin d was 8th on the list of ingredients, and it contained butterscotch flavoring... yuck). I felt terrible at the idea of giving something with artificial flavoring in it to my exclusively breastfed 3w old infant, and shocked those would be prescribed by our dr. After hearing other parents' recommendations, and what their pediatricians recommended, I decided to order these. These drops pretty much taste and smell like nothing, only require one tiny drop, and have just 2 straightforward ingredients! Only downside is that the dropper can be kind of tricky for my partner and he sometimes accidentally does more than one drop and has to start a new bottle (I find it easy to get one drop because the dropper is similar to essential oil bottles I've used). I'll save the other drops with flavoring in them for when my baby is over 6mos, and I'll definitely be reordering these.

…

Baby Loves It.

My baby's pediatrician recommended this brand and a few others to try. This was the first I ordered and it was a success. I think my baby actually enjoys the taste.

…

The best d drops

We have used these with all 3 of our kids and they are great. Always a quality product, easy to either drop a drop in babies mouth directly or place on the mom breast before breastfeeding. The bottle is easy to use and none of our babies ever had a negative reaction Would definitely recommend.

…

> Easy to use!
> These are easy to use quickly drop some vitamin D on your nip before baby latches on. Bottle lasts forever too! Buy these!!

Attached as Exhibit T is a true and correct copy of a sample of screenshots showing online reviews by Ddrops' customers; also see Exhibit G.

84. Customer reviews, orders, and return customers provide reasonable evidence to me that the genius and efficacy of the Patented Product is the profound reason for its success.

85. Not only is Baby Ddrops Patented Product easy to administer, but it is also effective. Customer, parent, and physician word of mouth regarding the efficacy of Ddrops Patented Product has been the biggest driving influencer of sales of the Patented Product – not some clever expensive marketing and advertising ploy.

86. Ddrops' Patented Product has been so successful that infringers and copiers have taken note. Copying became an issue for Ddrops in Canada and later in the United States, almost immediately when Ddrops launched in each market.

87. Since November 4, 2008, Ddrops and the Vieths have successfully enforced the Canadian Patent against various infringers in Canada that have resulted in judgements and settlements.

88. On March 18, 2013, Ddrops Company, and the Vieths entered into a settlement agreement with Swiss Herbal Remedies Limited.

89. On May 28, 2015, Ddrops Company and the Vieths entered into a confidential settlement with Atrium Innovations Inc.

90. Ddrops Company, and the Vieths entered into a confidential settlement agreement with Jamp Pharma Corporation and Wampole Inc. dated November 30, 2015.

91. On February 17, 2016 Ddrops Company and the Vieths entered into a confidential settlement agreement with Nutralab Canada Corp-Wal-Mart.

92. On September 9, 2020, Ddrops Company and Naka Sales Limited, and Naka Herbs & Vitamins Ltd (d/b/a/ Bioabsorb Neutraceuticals) entered into a confidential settlement agreement.

93. Each of the defendants in these actions have Redacted [redacted] (Under Canadian law, Ddrops is precluded from filing the Canadian Settlements listed above, unless court ordered by the PTAB.)

94. Since 2015, Ddrops and the Vieths also have successfully enforced the '958 Patent against several infringers in the United States.

95. From approximately September 2015 until about May 4, 2017, Ddrops' distributors Walgreen Co. and Drugstore.com sold or offered for sale a

product that infringed one or more claims of the ‘958 Patent and Ddrops Patented Product.

96. Walgreen Co. and Drugstore.com had been distributors of Ddrops’ Patented Product. They had access to our product before they copied it, in my opinion because they were selling a lot of our product and believed they could make more money by copying and selling our product.

97. October 30, 2015, Ddrops filed an action for infringement against Walgreen Co. and Drugstore.com in the United States District Court for the District of Minnesota, Case No. 15-cv-3981. We filed a motion for a preliminary injunction in that action.

98. Before the preliminary injunction motion was decided, Redacted [redacted]. Attached as Exhibit U is a true and correct copy of the confidential Settlement Agreement between Ddrops and Walgreens Co. and Drugstore.com, dated April 8, 2016.

99. From approximately August 2016 until about June 17, 2017, another of Ddrops’ distributors, iHerb Inc. and Madre Labs, Inc., d/b/a California Gold Nutrition, sold or offered for sale a product that infringed one or more claims of the ‘958 Patent.

100. iHerb Inc. and Madre Labs, Inc., d/b/a California Gold Nutrition had been distributors of Ddrops' Patented Product. They therefore had access to the Patented Product before they copied it, again likely because of the large volume of sales they were making of Ddrops' Patented Product.

101. On January 19, 2016, Ddrops filed an action for infringement against iHerb Inc. and Madre Labs, Inc., d/b/a California Gold Nutrition, in the United States District Court for the District of Minnesota, Case No. 16-cv-04278. We again filed a motion for a preliminary injunction. Before that motion was decided, Redacted Attached as Exhibit V is a true and correct copy of the confidential Settlement Agreement between Ddrops and iHerb, Inc. and Madre Labs, Inc., d/b/a California Gold Nutrition, dated June 15, 2017.

102. Because I monitor competitor products, I learned about Mommy's Bliss baby vitamin D product sometime after it started selling its product in the U.S. in about 2016 or 2017.

103. The original product ingredients for Mommy's Bliss baby vitamin D product were sunflower oil and vitamin E. Attached as Exhibit W are true and accurate screenshots of Mommy's Bliss baby vitamin D product containing sunflower oil and vitamin E product.

104. Because the Mommy's Bliss baby vitamin D product contained sunflower oil and vitamin E, it was not the same as Ddrops' Patented Product. We did not consider the Mommy's Bliss product a direct competitor of ours in the marketplace. Its entry into the market did not appreciably affect our sales or market share.

105. In fall 2021, while I was conducting routine monitoring of competitors, online products, and retailer stores inventory, I discovered that Mommy's Bliss had changed its formula for its Mommy's Bliss baby vitamin D product.

106. I noticed that instead of containing sunflower oil and vitamin E, the formula for the first time contained Vitamin D3 (cholecalciferol) 10 mcg (400 IU) per serving sourced from "Organic Medium Chain Triglyceride (MCT) oil." Attached as Exhibit X are true and correct images of the Mommy's Bliss baby vitamin D product made with MCT oil from 2021.

107. According to the 2021 packaging of the Mommy's Bliss baby vitamin D product, the only ingredients are Vitamin D3 (cholecalciferol) 10 mcg (400 IU) per serving and, "Organic Medium Chain Triglyceride (MCT) oil." See Exhibit X.

108. The Mommy's Bliss baby vitamin D product instructions state, "Place one drop daily onto mother's nipple or a pacifier, or mix with formula, breast milk, juice, or other foods." See Exhibit X.

109. The ingredients for the Mommy's Bliss baby vitamin D product were then the same as the Patented Product. The Mommy's Bliss baby vitamin D product also directs its users to administer the product in the same way as Ddrops' Patented Product.

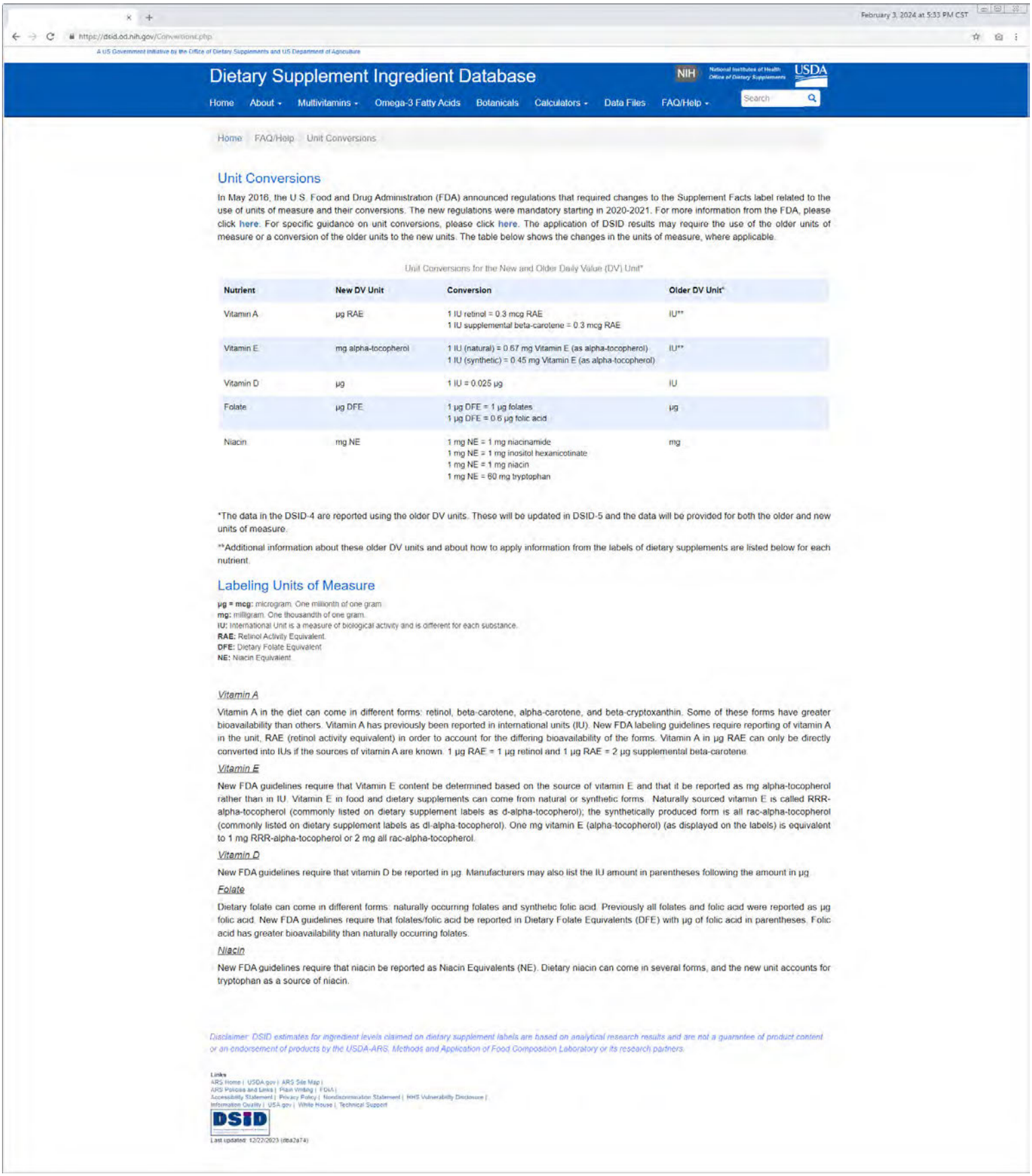
I declare that all statements made herein on my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment,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

Dated: February 8th, 2024.



Chris Temovsky

Exhibit A



February 3, 2024 at 5:33 PM CST

https://dsid.od.nih.gov/Conversions.php

A US Government initiative by the Office of Dietary Supplements and US Department of Agriculture

Dietary Supplement Ingredient Database

NIH National Institutes of Health Office of Dietary Supplements USDA

Home About Multivitamins Omega-3 Fatty Acids Botanicals Calculators Data Files FAQ/Help Search

Home FAQ/Help Unit Conversions

Unit Conversions

In May 2016, the U.S. Food and Drug Administration (FDA) announced regulations that required changes to the Supplement Facts label related to the use of units of measure and their conversions. The new regulations were mandatory starting in 2020-2021. For more information from the FDA, please click here. For specific guidance on unit conversions, please click here. The application of DSID results may require the use of the older units of measure or a conversion of the older units to the new units. The table below shows the changes in the units of measure, where applicable.

Unit Conversions for the New and Older Daily Value (DV) Unit*

Nutrient	New DV Unit	Conversion	Older DV Unit*
Vitamin A	µg RAE	1 IU retinol = 0.3 mcg RAE 1 IU supplemental beta-carotene = 0.3 mcg RAE	IU**
Vitamin E	mg alpha-tocopherol	1 IU (natural) = 0.67 mg Vitamin E (as alpha-tocopherol) 1 IU (synthetic) = 0.45 mg Vitamin E (as alpha-tocopherol)	IU**
Vitamin D	µg	1 IU = 0.025 µg	IU
Folate	µg DFE	1 µg DFE = 1 µg folates 1 µg DFE = 0.6 µg folic acid	µg
Niacin	mg NE	1 mg NE = 1 mg niacinamide 1 mg NE = 1 mg inositol hexanicotinate 1 mg NE = 1 mg niacin 1 mg NE = 60 mg tryptophan	mg

*The data in the DSID-4 are reported using the older DV units. These will be updated in DSID-5 and the data will be provided for both the older and new units of measure.

**Additional information about these older DV units and about how to apply information from the labels of dietary supplements are listed below for each nutrient.

Labeling Units of Measure

µg = mcg: microgram. One millionth of one gram.
mg: milligram. One thousandth of one gram.
IU: International Unit is a measure of biological activity and is different for each substance.
RAE: Retinol Activity Equivalent.
DFE: Dietary Folate Equivalent
NE: Niacin Equivalent

Vitamin A

Vitamin A in the diet can come in different forms: retinol, beta-carotene, alpha-carotene, and beta-cryptoxanthin. Some of these forms have greater bioavailability than others. Vitamin A has previously been reported in international units (IU). New FDA labeling guidelines require reporting of vitamin A in the unit, RAE (retinol activity equivalent) in order to account for the differing bioavailability of the forms. Vitamin A in µg RAE can only be directly converted into IUs if the sources of vitamin A are known. 1 µg RAE = 1 µg retinol and 1 µg RAE = 2 µg supplemental beta-carotene.

Vitamin E

New FDA guidelines require that Vitamin E content be determined based on the source of vitamin E and that it be reported as mg alpha-tocopherol rather than in IU. Vitamin E in food and dietary supplements can come from natural or synthetic forms. Naturally sourced vitamin E is called RRR-alpha-tocopherol (commonly listed on dietary supplement labels as d-alpha-tocopherol); the synthetically produced form is all rac-alpha-tocopherol (commonly listed on dietary supplement labels as dl-alpha-tocopherol). One mg vitamin E (alpha-tocopherol) (as displayed on the labels) is equivalent to 1 mg RRR-alpha-tocopherol or 2 mg all rac-alpha-tocopherol.

Vitamin D

New FDA guidelines require that vitamin D be reported in µg. Manufacturers may also list the IU amount in parentheses following the amount in µg.

Folate

Dietary folate can come in different forms: naturally occurring folates and synthetic folic acid. Previously all folates and folic acid were reported as µg folic acid. New FDA guidelines require that folates/folic acid be reported in Dietary Folate Equivalents (DFE) with µg of folic acid in parentheses. Folic acid has greater bioavailability than naturally occurring folates.

Niacin

New FDA guidelines require that niacin be reported as Niacin Equivalents (NE). Dietary niacin can come in several forms, and the new unit accounts for tryptophan as a source of niacin.

Disclaimer: DSID estimates for ingredient levels claimed on dietary supplement labels are based on analytical research results and are not a guarantee of product content or an endorsement of products by the USDA-ARS, Methods and Application of Food Composition Laboratory or its research partners.

Links
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Information Quality | USA.gov | White House | Technical Support

DSID

Last updated: 12/22/2023 (dba2a74)

Exhibit B

AIPRIME NUTRITIONS PRIVATE LIMITED
114, 1st Floor, Taramandal Complex, 5-9-13,
Saifabad, Hyderabad - 500 004, Telangana State, INDIA. Mobile : +91 - 99481 51116
CIN No. : U74999TG2018PTC123723

CERTIFICATE OF ANALYSIS

DISTRIBUTED BY KRAFT CHEMICAL EST. 1935 kraftchemical.com

Product Name: Organic MCT Oil
Product Code: MCT-COCO
Production Date: MAY 2023.
Expiry Date: MAY 2025.
Batch Number: AIPN/OMCT/23/079
Plant Base: Coconut.

Parameter	Specifications	Test Method	RESULT

Redacted

Microbiological Parameters:

Parameter	Specifications	Test Method	RESULT

Redacted

AIPRIME NUTRITIONS PRIVATE LIMITED
114, 1st Floor, Taramandal Complex, 5-9-13,
Saifabad, Hyderabad - 500 004, Telangana State, INDIA. Mobile : +91 - 99481 51116
CIN No. : U74999TG2018PTC123723

Heavy Metals Parameters:

Parameter	Specifications	Test Method	RESULT
Redacted			

BLQ REFERS TO Below the Limit of Quantitation

AIPRIME NUTRITIONS PRIVATE LIMITED HYD.

(Q.C)

nutrasource
Pharmaceutical and Nutraceutical Services

PRODUCT TESTING REPORT

400 IU, Lot# 5104369-O

Date received: 14-Sep-2023
NDI#: 25012023

Analysis for:

Ddrops Company

126 Trowers Road
Woodbridge, ON L4L 5Z4
(905) 851-8898 x243
(905) 851-8890

Date: 25-Sep-2023

Kevin Yan, M.Sc.
Vice President,
Certification & Analytics
Dated: 25-Sep-2023

Hyun Ah Kim
Program Manager,
Certification & Analytics
Dated: 25-Sep-2023

CONFIDENTIAL

nutrasource
Pharmaceutical and Nutraceutical Services

Heavy Metals

Component	Analytical Method	Specification	Result	Units	Meets Specification
	ICP-MS				

Redacted

nutrasource
Pharmaceutical and Nutraceutical Services

Physical Properties

Component	Analytical Method	Specification	Result	Units	Meets Specification
Visual Description	Visual	Clear odorless liquid	Conforms	Not Applicable	Yes

nutrasource
Pharmaceutical and Nutraceutical Services

Vitamins

Component	Analytical Method	Specification	Result	Units	Meets Specification
Vitamin D3 (Cholecalciferol)			Redacted		

nutrasource
Pharmaceutical and Nutraceutical Services

Identification

Component	Analytical Method	Specification	Result	Units	Meets Specification

Redacted

nutrasource
Pharmaceutical and Nutraceutical Services

PRODUCT TESTING REPORT

Organic MCT Oil, Lot# AIPNOMCT23079-230710

Date received: 10-Jul-2023
NDI#: 17772023
Interim report--Some tests still require lab sign off

Analysis for:

Ddrops Company
126 Trowers Road
Woodbridge, ON L4L 5Z4
(905) 851-8898 x243
(905) 851-8890

Date: 21-Jul-2023

Kevin Yan, M.Sc.
Vice President,
Certification & Analytics
Dated: 21-Jul-2023

Hyun Ah Kim
Program Manager,
Certification & Analytics
Dated: 21-Jul-2023

nutrasource
Pharmaceutical and Nutraceutical Services

Oxidation Analysis

Component	Analytical Method	Specification	Result	Units	Meets Specification
Redacted					

nutrasource
Pharmaceutical and Nutraceutical Services

Medium Chain Triglycerides

Component	Analytical Method	Specification	Result	Units	Meets Specification

Redacted

nutrasource
Pharmaceutical and Nutraceutical Services

Fatty Acid Identification

Component	Analytical Method	Specification	Result	Units	Meets Specification
Redacted					

nutrasource
Pharmaceutical and Nutraceutical Services

Heavy Metals

Component	Analytical Method	Specification	Result	Units	Meets Specification
Redacted					

nutrasource
Pharmaceutical and Nutraceutical Services

Physical Properties

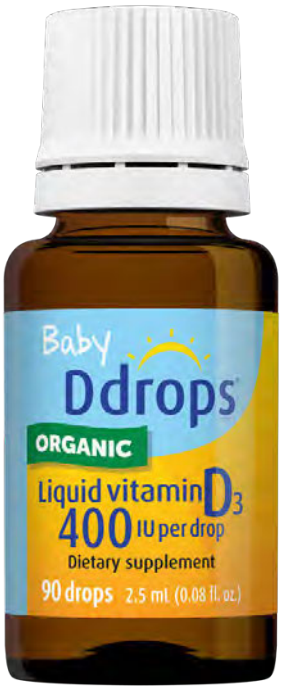
Component	Analytical Method	Specification	Result	Units	Meets Specification

Redacted

nutrasource
Pharmaceutical and Nutraceutical Services

Redacted

Exhibit C


Baby
Ddrops
ORGANIC
Liquid vitamin D3
400 IU per drop
Dietary supplement
90 drops 2.5 mL (0.08 fl. oz.)


ORGANIC
Baby
Ddrops
The sunshine vitamin in just one drop
Liquid vitamin D3
Dietary supplement
90 drops for 90 days
MOMS CHOICE AWARDS
HONORING EXCELLENCE
Babies need vitamin D for healthy growth and development.
Contains no preservatives, no artificial flavors, no coloring.
400 IU per drop
90 drops 2.5 mL (0.08 fl. oz.)
Baby
Ddrops
ORGANIC
Liquid vitamin D3
400 IU per drop
Dietary supplement
90 drops 2.5 mL (0.08 fl. oz.)

Supplement Facts
Serving Size 1 Drop (0.028 mL or 0.001 fl. oz.)
Servings Per Container 90

Amount Per Serving		% Daily Value
Vitamin D3 (Cholecalciferol)	10 mcg (400 IU)	100%

Other ingredient:
Fractionated coconut oil.

Does not contain: Corn, casein, dairy, egg, fish, gluten, lactose, peanuts, shellfish, soy, starch, sugar, sulphites, wheat, yeast, preservatives, coloring or artificial flavors.

Directions: Infants less than 1 year old - Place 1 drop daily onto mother's nipple or on a clean surface, such as a washed finger, and allow baby to suck for at least 30 seconds. Or mix one drop daily with milk, juice or other food.



SPECIFICALLY DESIGNED FOR BREASTFED BABIES.

See insert for more information.

STORE BOTTLE UPRIGHT AND BETWEEN 40°F AND 85°F. KEEP OUT OF REACH OF CHILDREN. DO NOT USE IF SECURITY SEAL ON BOTTLE IS BROKEN.

Vitamin D is important for healthy growth and development in infancy and childhood especially for normal bone growth and muscle strength.*

The American Academy of Pediatrics recommends that infants who are breastfed or partially breastfed should be supplemented with 400 IU of vitamin D daily.*

Ddrops Company
Woodbridge, ON L4L 5Z4 Canada
www.vitaminDdrops.com

Registered TM of Ddrops Company

U.S. Patent 9066958

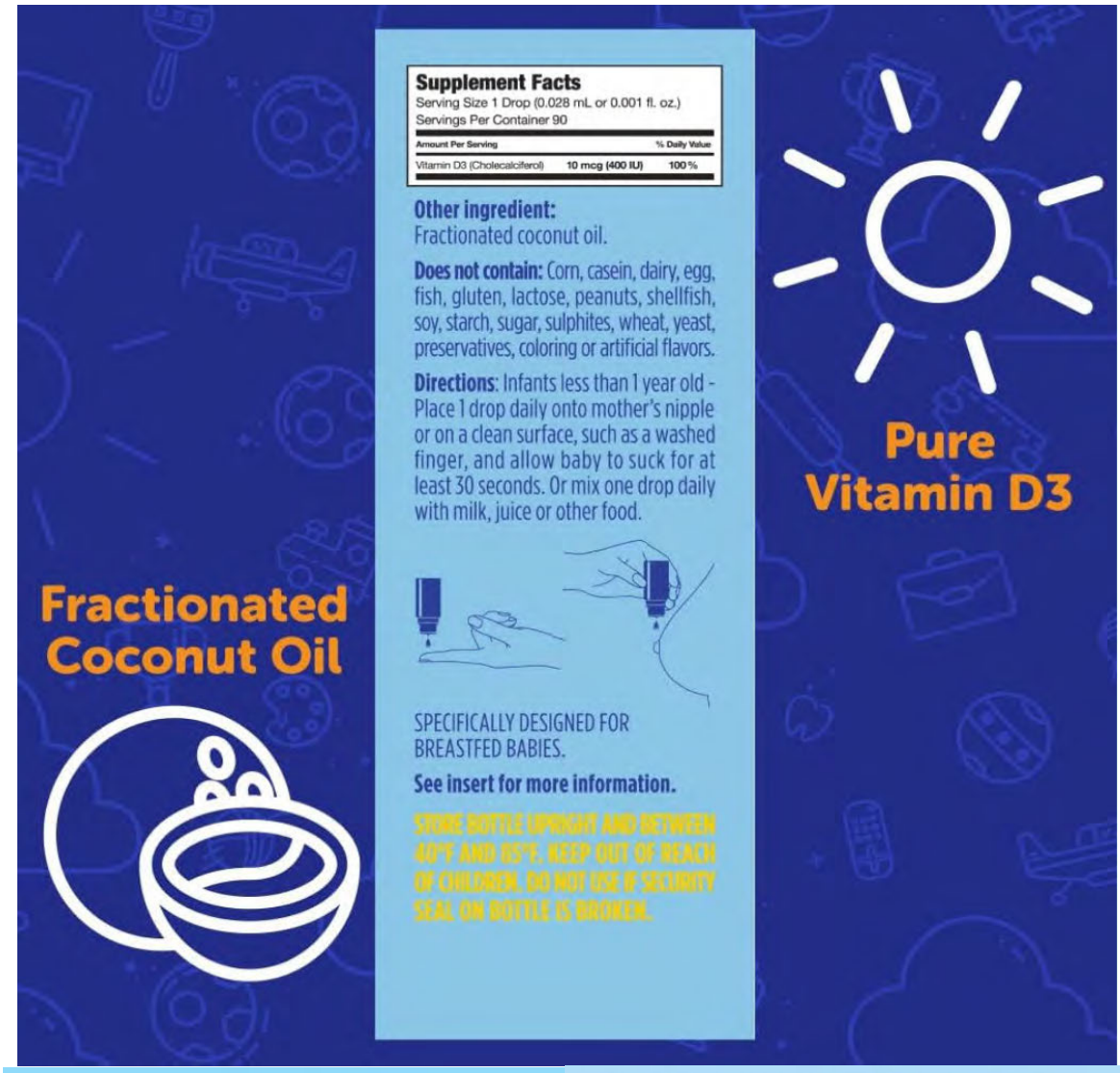
The Mom's Choice Awards Honoring Excellence logo is a trademark of the Mom's Choice Awards.

EURO DDROPPER™ TECHNOLOGY

CONTENTS CERTIFIED NSF

igen NON-GMO TESTED

* These statements have not been evaluated by the FDA. This product is not intended to diagnose, treat, cure or prevent any disease.

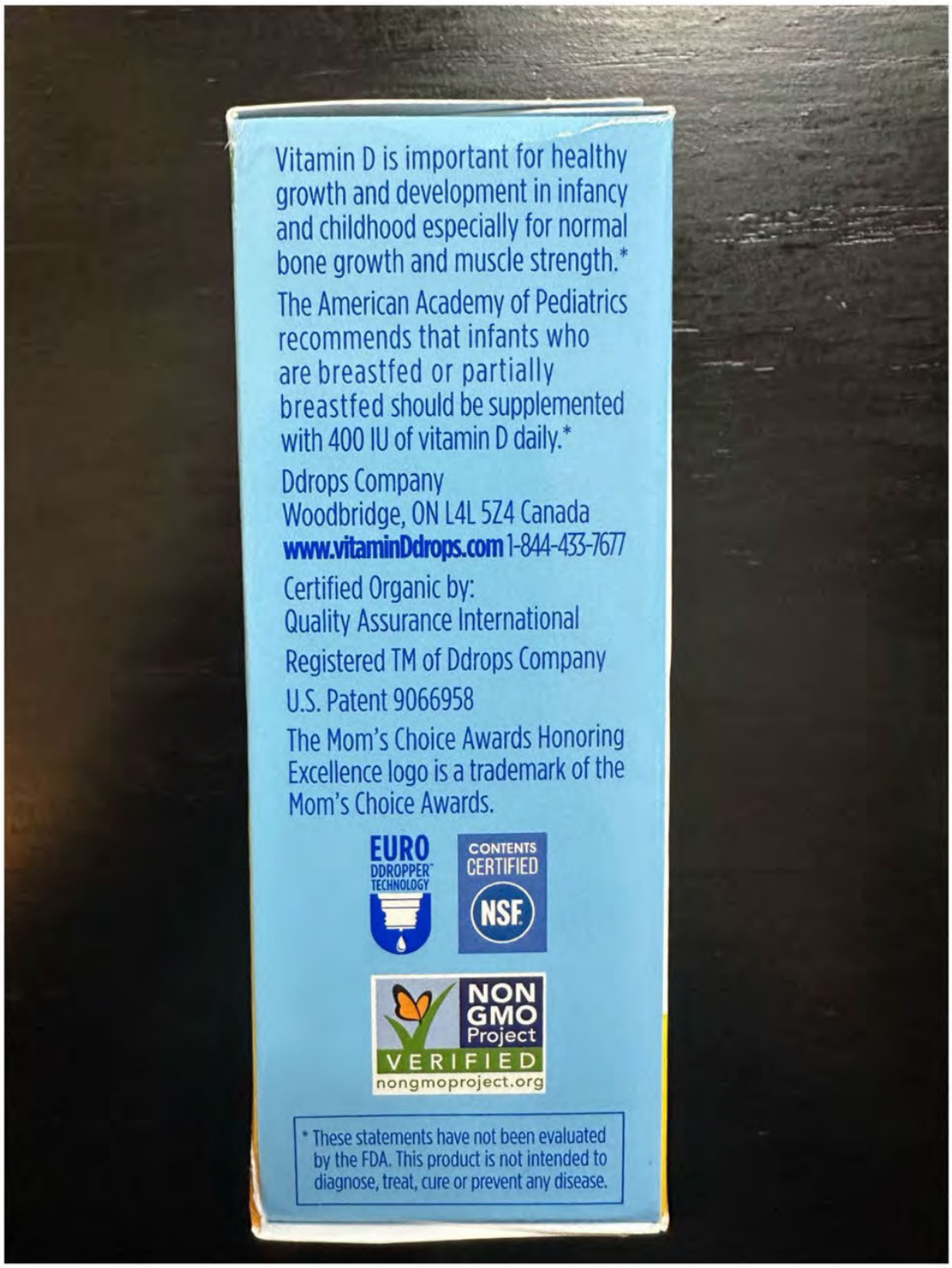

Supplement Facts
Serving Size 1 Drop (0.028 mL or 0.001 fl. oz.)
Servings Per Container 90
Amount Per Serving
% Daily Value
Vitamin D3 (Cholecalciferol)
10 mcg (400 IU)
100 %
Other ingredient:
Fractionated coconut oil.
Does not contain: Corn, casein, dairy, egg, fish, gluten, lactose, peanuts, shellfish, soy, starch, sugar, sulphites, wheat, yeast, preservatives, coloring or artificial flavors.
Directions: Infants less than 1 year old - Place 1 drop daily onto mother's nipple or on a clean surface, such as a washed finger, and allow baby to suck for at least 30 seconds. Or mix one drop daily with milk, juice or other food.
SPECIFICALLY DESIGNED FOR BREASTFED BABIES.
See insert for more information.
STORE BOTTLE UPRIGHT AND BETWEEN 40°F AND 85°F. KEEP OUT OF REACH OF CHILDREN. DO NOT USE IF SECURITY SEAL ON BOTTLE IS BROKEN.
Pure Vitamin D3
Fractionated Coconut Oil


Ddrops
By design, the bottle is filled to an exact number of doses and is not filled to the top.
The bottle is chosen for its:
• ease of use
• safety
• label readability


Simply Place One Drop:
Directly onto nipple
Into a drink
Onto a clean surface


ORGANIC
Baby
Ddrops®
The sunshine vitamin
in just one drop®
Liquid
vitamin D3
Dietary supplement
90 drops for 90 days
MOM'S CHOICE AWARDS
HONORING EXCELLENCE
Babies need
vitamin D for healthy
growth and development.
Contains no preservatives,
no artificial flavors,
no coloring.
USDA
ORGANIC
400 IU per drop
90 drops 2.5 mL (0.08 fl. oz.)


Supplement Facts
Serving Size 1 Drop (0.028 mL or 0.001 fl. oz.)
Servings Per Container 90
Amount Per Serving
% Daily Value
Infants 0-12 months
Vitamin D3 (as Cholecalciferol) 10 mcg (400 IU) 100%
Other ingredient:
Organic fractionated coconut oil.
Does not contain: Corn, casein, dairy, egg, fish, gluten, lactose, peanuts, shellfish, soy, starch, sugar, sulphites, wheat, yeast, preservatives, coloring or artificial flavors.
Directions: Infants less than 1 year old - Place 1 drop daily onto mother's nipple or on a clean surface, such as a washed finger, and allow baby to suck for at least 30 seconds. Or mix one drop daily with milk, juice or other food.
SPECIFICALLY DESIGNED FOR BREASTFED BABIES.
STORE BOTTLE UPRIGHT AND BETWEEN 40°F AND 85°F. KEEP OUT OF REACH OF CHILDREN. DO NOT USE IF SECURITY SEAL ON BOTTLE IS BROKEN.
SCAN TO LEARN MORE



DDROPS-0022427


ORGANIC
Baby
Ddrops®
The sunshine vitamin
in just one drop®
Liquid
vitamin D3
Dietary supplement
90 drops for 90 days
MOM'S CHOICE AWARDS
HONORING EXCELLENCE
Babies need
vitamin D for healthy
growth and development.
Contains no preservatives,
no artificial flavors,
no coloring.
USDA
ORGANIC
400 IU per drop
90 drops 2.5 mL (0.08 fl. oz.)

Exhibit D


#1 Brand Recommended by Pediatricians
Enfamil
D·Vi·Sol
Essential for All Breastfed Infants
Vitamin D
Supplement Drops
1 2/3 FL OZ (50 mL)


EASY TO USE SYRINGE
NEW LOOK!
Enfamil
D
·VI·SOL
Liquid Vitamin D
Supplement
400 IU
per mL
50 mL
For
Breastfed
Infants


NEW Look
Same Great Formula
NEW!
Enfamil
VITAMIN
Breastfed Infant
D·VI·SOL
VITAMIN D FOR SUPPORTING
STRONG BONES & TEETH*
DIETARY SUPPLEMENT
0.25 FL OZ
(7.5 mL)
Enfamil
D·VI·SOL
Vitamin D Drops
STRONG
BONES
AAP recommended 400 IU
for breastfed infants
0-12 MONTHS • DIETARY SUPPLEMENT
1 BOTTLE with EASY-TO-USE DROPPER
1 2/3 FL OZ (50 mL)

DIRECTIONS: Shake bottle well and use 1 mL a day. See other side panel for additional information.

Supplement Facts

Serving Size 1 mL Daily
Servings Per Container 50

Amount Per 1 mL	% Daily Value Infants through 12 Months of Age	% Daily Value Children 1 through 3 Years of Age
Vitamin D 10 mcg (400 IU) (from cholecalciferol)	100%	67%

OTHER INGREDIENTS: GLYCERIN, WATER, POLYSORBATE 80 (EMULSIFIER), CITRIC ACID (ANTIOXIDANT FOR VITAMIN D), NATURAL FLAVOR, SODIUM CITRATE.

Distributed by:
MEAD JOHNSON & COMPANY, LLC
EVANSVILLE, IN 47721 U.S.A.
Made in Canada
©2022 Mead Johnson & Company, LLC

FROM THE MAKER OF ENFAMIL
#1
PEDIATRICIAN RECOMMENDED
INFANT FORMULA BRAND

#1 INFANT
#1
VITAMIN BRAND

HOW TO USE

1. Shake bottle well, fill dropper to 1 mL line (daily serving)
2. Dispense gently into mouth
3. Can mix with breast milk, formula, or food



SEE PACKAGING FOR FULL INSTRUCTIONS



Mix with Food, Breast Milk, or Infant Formula


Naturally Fruit
Flavored Drops

Mead Johnson Nutrition
HEALTHCARE PROFESSIONAL
RESOURCE CENTER



Enfamil ® D-Vi-Sol ® (Liquid)

Vitamin D Supplement

Liquid vitamin D supplement for all full-term breastfed infants. ©D NPN 00762881

Indication

Liquid vitamin D supplement for all full-term breastfed infants. Enfamil ® D-Vi-Sol ® provides breastfed infants with vitamin D which helps in the normal development and maintenance of bones and teeth. Helps to prevent vitamin D deficiency. A joint statement of Health Canada, Canadian Paediatric Society, Dietitians of Canada, and Breastfeeding Committee for Canada states that a daily vitamin D supplement of 10 mcg (400 IU) is recommended for exclusively and partially breastfed infants, from birth to one year of age.

Rationale and Special Characteristics

Enfamil® D-Vi-Sol® liquid provides 400 IU per mL of vitamin D to meet the recommended daily intake of vitamin D. It is sugar-free, gluten-free, and lactose-free.

DOSAGE AND ADMINISTRATION

Daily Dosage for all full term breastfed infants: 1 mL (fill provided syringe to 1.0 mL line). Dispense slowly into child's mouth, toward inner cheek. If preferred the liquid may be slowly mixed with formula, juice, cereal, or other food and fed within one hour

Medicinal Ingredients (per 1 mL)		
Vitamin D3	(cholecalciferol)	400 IU (10 mcg)

NON-MEDICINAL INGREDIENTS

Glycerin, polysorbate 80, caramel, citric acid anhydrous, sodium citrate dihydrate, sodium hydroxide, artificial flavour and purified water. Store at room temperature 15-30°C (59-86°F).

PRECAUTIONS

Keep this and all medications out of the reach of children. Safety sealed bottle. Do not use if seal is broken.

REFERENCES

1. Health Canada, Canadian Pediatric Society, Dietitians of Canada and Breastfeeding Committee for Canada. Nutrition for Healthy Term Infants: Recommendations from Birth to Six Months, 2012.

Product Forms

Ingredients

REDACTED Vieth Ex 2071 IPR2023-00726 62



Hospital customers: **(1) 800 263-1143**
To Place an Order: Please contact your on-site Hospital Purchasing Department
Clinic and Pharmacy Inquiries: **(1) 877 711-8901**

Enfamil.ca

Privacy Policy

Terms

Site map

Intended for Healthcare Professionals Only
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REDACTED Vieth Ex 2071 IPR2023-00726 63

Exhibit E

Home > Products > Enfamil® D Vi Sol® Vitamin D Drops

Vitamin D for supporting strong bones & teeth

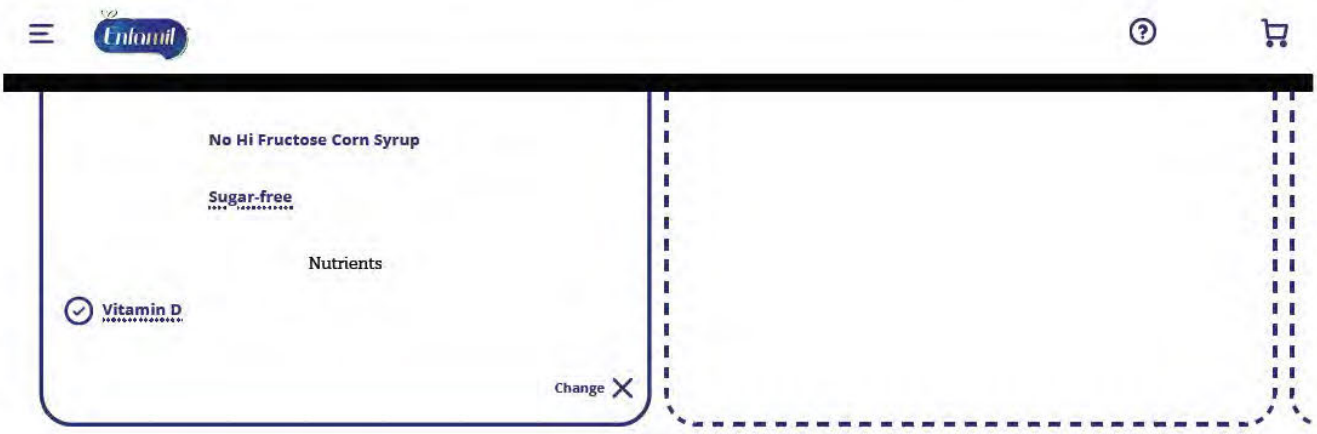


Format

Chat with Emma

$11.99 *In Stock*

Add to cart



Ratings & Reviews

See what other parents are saying about
Enfamil® D Vi Sol® Vitamin D Drops

Reviews

Authentic Reviews

Rating Snapshot

Select a row below to filter reviews.

5 stars	543
4 stars	83
3 stars	21
2 stars	22
1 star	83

Overall Rating

4.3 ★★★★☆ 752 Reviews

519 out of 607 (86%) reviewers recommend this product

Review this Product

☆ ☆ ☆ ☆ ☆

Adding a review will require a valid email for verification

Average Customer Ratings

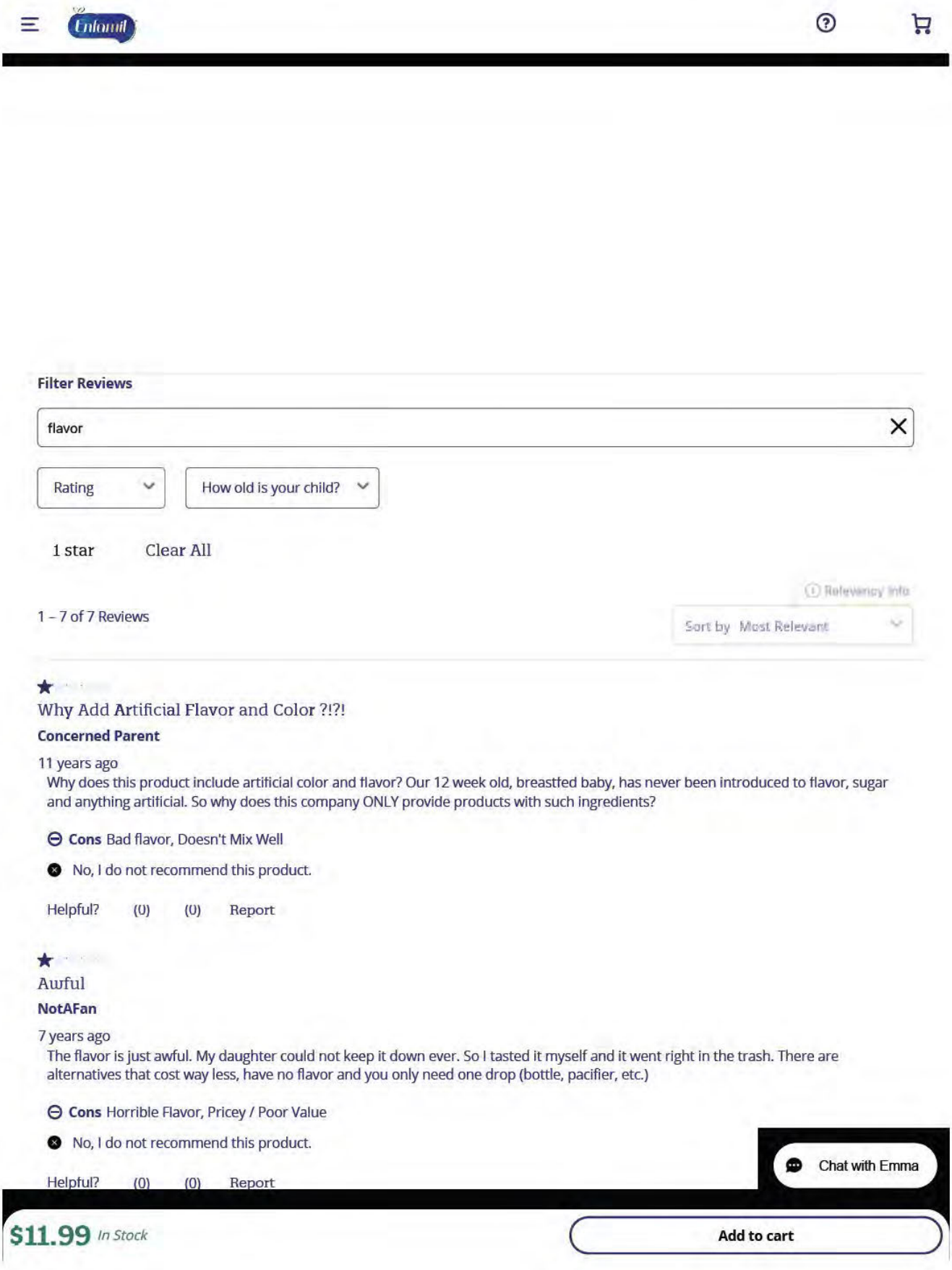
How was your Shopping Experience? 4.8

Online Shopping Experience (if you bought on Enfamil.com) 4.6

How likely are you to purchase from Enfamil Online Shop?

Chat with Emma

$11.99 *In Stock* Add to cart


Filter Reviews
flavor
Rating
How old is your child?
1 star
Clear All
1 – 7 of 7 Reviews
Sort by Most Relevant
Why Add Artificial Flavor and Color ?!?!
Concerned Parent
11 years ago
Why does this product include artificial color and flavor? Our 12 week old, breastfed baby, has never been introduced to flavor, sugar and anything artificial. So why does this company ONLY provide products with such ingredients?
Cons Bad flavor, Doesn't Mix Well
No, I do not recommend this product.
Helpful? (0) (0) Report
Awful
NotAFan
7 years ago
The flavor is just awful. My daughter could not keep it down ever. So I tasted it myself and it went right in the trash. There are alternatives that cost way less, have no flavor and you only need one drop (bottle, pacifier, etc.)
Cons Horrible Flavor, Pricey / Poor Value
No, I do not recommend this product.
Helpful? (0) (0) Report
Chat with Emma
$11.99 In Stock
Add to cart

I am sorry to learn of your daughter's experience with our D-Vi-Sol. Our Product Information Team would be happy to get some additional feedback from you. At your earliest convenience please call 1-800-222-9123 (Option 1) or visit Enfamil.com/ContactUs. Thank you! - Erin

★

DO NOT BUY THIS PRODUCT!

Mama

10 years ago

I was astonished when I read the ingredients label and saw that there are ARTIFICIAL flavors and colors in this product! What a terrible thing to give an infant! I wasted my money and will never purchase this again.

No, I do not recommend this product.

Helpful? (0) (0) Report

★

Disappointed in Ingredients

Mother Goose

9 years ago

Very disappointed to read that there is artificial coloring and flavoring in this product. We used it for two months and after reading about the effects of caramel coloring, I've decided to seek a better source of D3 for my little lady.

Cons Artificial Coloring, Artificial Flavoring

No, I do not recommend this product.

Helpful? (0) (0) Report

Response from :
8 years ago

Enfamilpwr

Hello, and thank you for taking the time to share your comments. Mead Johnson does not add un-needed or an excessive amount of artificial flavors in our vitamin and mineral products. We chose the artificial colors and flavors used in our vitamin and mineral drops based on the superior stability and uniformity these ingredients offer. Each of the ingredients used in our vitamin and mineral drops has been carefully studied and is considered safe by the U.S. Food and Drug Administration (FDA) for inclusion in products of this type. I hope that this helps! -Ashley

★

Not my choice of vitamin d

Mia

10 years ago

I really hate this vitamin d, there are much easier drops to use, to big of a dose for my infant to take and it's flavored, why would I want my infant to start likening sweet things?!

Cons Big Dose, Flavored

No, I do not recommend this product.

Helpful? (0) (0) Report

Chat with Emma

$11.99 *In Stock*

Add to cart

newborn, has to be mixed in milk and it changes the flavor of it. Also caused lots of gas and made my baby throw up. I wish I knew about the one drop d vitamins availabilty, which works perfectly, only one drop is the serving size.

Cons Bad odor, Caused Gas, Caused vomiting

No, I do not recommend this product.

Helpful? (0) (0) Report

★

ARTIFICIAL INGREDIENTS

Concerned Mommy

12 years ago

I used this for my exclusively breastfed baby because it was recommended by her doctor. I was happy to give her these drops because my daughter never seemed to mind the taste. However, I just read the ingredients on the bottle and I am EXTREMELY DISAPPOINTED!!! It contains Artificial Ingredients and Artificial Coloring... and Preservatives. I chose to breastfeed my daughter because I wanted her to have the best nutrition possible. I feel the D-Vi-Sol has interfered with my efforts (I now have to live with the fact that for 5 months straight my daughter was fed Glycerin, Polysorbate 80, Citric Acid, Sodium Citrate, Sodium Hydroxide, Artificial Flavor, Artificial Caramel Color). Never again will I trust the opinion of the Dr. without checking it out for myself. How can this be #1 recommended brand? I will now be purchasing vitamin D drops without any of these unnecessary chemicals. I hope to see this change in the future for Enfamil, but until then I will no longer be a customer

Cons Artificial Coloring, Artificial ingredients, Preservatives

No, I do not recommend this product.

Helpful? (0) (0) Report

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Email*

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Chat with Emma

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Kayyyra19

KY

Review **1**

Vote **1**

Gender **Female**

Age **25 to 34**

★★

Never heard my baby scream like that

3 years ago

I have used these drops twice. In the 48 hours that my son has had them in his system he has been extremely uncomfortable and miserable. He's been spitting up constantly all day today and tonight he screamed in a way I've never heard him scream before with tears running down his face.. he couldn't even catch his breath he was in so much pain. WHAT IS IN YOUR PRODUCT? Surely you can see these comments about all these babies being inconsolable after using the liquid. Something isn't right. I am so disappointed in the quality of this product.

Online Shopping Experience (if you bought on Enfamil.com) 1.0

Value 3.0

Any comments about your online shopping experience? I bought this product at Walmart because it was less expensive than others.

No, I do not recommend this product.

Helpful? (1) (0) Report

Response from Enfamil: 3 years ago

Enfamil Customer Care

Hello! We would love to speak with you directly about your experience with our Poly-Vi-Sol! One of our team members will be reaching out to you via email, but if you would like to speak with us immediately please give us a call at 1-800-222-9123, select option 1 and reference #611570. We look forward to speaking with you and seeing how we can help! -Brooke

David

New York, NY

Review **1**

Votes **0**

★★

new package needs bottle adapter

9 years ago

I have been giving D-VI-Sol to my 2 children for over 4 years. In the original bottles, it came with an oral bulb dispenser that can screw on to bottle. Now, the new bottle comes with an oral syringe. Yes the oral syringe may provide a more accurate dose but it does not come with a bottle adapter. This means you can drop the whole syringe into the bottle. Also, 2 hands are require to hold the oral syringe. I'm afraid one day, I will accidentally tip the bottle and spill everything. With a bottle adapter, the oral syringe tip can be inserted into the adapter and invert the bottle to draw out the amount desired- no worry about spilling. Please, provide a bottle adapter for each bottle. If not go back to the bulb oral syringe.

Yes, I recommend this product.

Helpful? (0) (0) Report

lanp3

Reviews **2**

Votes **0**

★★

Upset baby's belly– Do not buy!

lanp3

a year ago

We purchased these drops after our pediatrician recommended Vitamin D drops for our newborn. Our baby is a relatively calm, easy going little guy who doesn't cry much. After purchasing these drops, though, bedtime became awful. He would wail at the top of his lungs for over an hour, and nothing that we tried would calm him down. Finally we realized that he started this when we started giving him the drops. Read the label - there are so many inactive ingredients in these drops, and at least one of them was upsetting our baby's stomach. We stopped giving him these drops, and he's been back to his usual happy self. We feel terrible that we were the ones giving him these drops that were hurting him, but at least we figured it out.

influenster Originally posted on influenster.com

Ally's mom

Chicago, IL

Review **1**

Votes **0**

★★☆☆☆

Disappointed in ingredients

11 years ago

We have been using this for three months and have had no problems, BUT I am actively searching for another brand because this contains artificial colors and flavoring. SO disappointing. Enfamil: these ingredients seem totally unnecessary--especially the coloring. Who cares what color it is?? Please keep the garbage out of our children's systems. I am otherwise happy with the product, but like other reviewers I will be discontinuing use for this reason. Please consider removing these ingredients to keep your customers (and keep our kids healthy!)

⊖ **Cons** Artificial Coloring, Artificial Flavoring

⊗ No, I do not recommend this product.

Helpful? (0) (0) Report

Excited to be a mom ★★

Canton, GA

Review **1**

Votes **0**

Please bring back dropper

9 years ago

With the new syringe, I now require my husband's help to give the vitamins to my daughter. Before, with the dropper, I could do it with one hand. Now, because the syringe falls apart easily when you get to 1ml and because it's difficult to hold in the bottle and pull the blue part out, I need two hands. Two steady hands are not always available with an infant in your arms. The other thing that was easier with the dropper was dispensing into the child's mouth. I could control the release a lot better. I find myself squirting too much for her to swallow with the syringe because of the friction and it gets wasted. We've scoured the store for the old dropper to use going forward since we threw our old one out before we knew the design changed.. sadly, we haven't found one.

⊕ **Pros** Vitamins Baby Needs

⊖ **Cons** Falls apart easily, Inconvenient Syringe, Too Much In Baby's Mouth

⊗ No, I do not recommend this product.

Helpful? (0) (0) Report

Response from : 8 years ago

Enfamilpwr

Thank you for sharing your comments with us. We changed from a dropper to a syringe for easier measuring and dispensing of our supplement products. A syringe gives you more control over the amount of product you draw up and dispense. I am sharing your message regarding the new syringe with the appropriate personnel. Thank you again for taking the time to contact us about your experience. -Ashley

S

Omaha, NE

Review **1**

Votes **0**

★★☆☆☆

Miss My Dropper

9 years ago

I use this for my son every morning and he had bee doing great with it. Then we opened a new box this morning to find a syringe instead of the convenient dropper. It now requires an extra hand and extra cleanup to give my son his vitamins. What a disappointment. We will be searching for a new brand with infant-friendly dosing after we finish up this bottle using the dropper from our previous one.

⊕ **Pros** None, Syringe Is Messy

⊖ **Cons** Syringe Is Messy

⊗ No, I do not recommend this product.

Helpful? (0) (0) Report

Response from : 8 years ago

Enfamilpwr

Hello, and thank you for taking the time to share your comments regarding the syringe that came in your new packaging. I am happy to share your comments with the appropriate personnel. -Ashley

ginag90

Review **1**

Votes **0**

★★

My daughter hated this

ginag90

2 years ago

My daughter hated this so much, but she was very picky about medicine or anything that wasn't milk. She honestly threw up everytime I tried to give this to her and it was just so frustrating. It doesn't seem like a large volume but trying to give it to a 2- month old who clearly didn't want it was challenging and I ended up switching so something with much less volume that worked 1000x better

influenster Originally posted on influenster.com

Response from Enfamil: 2 years ago

Enfamil Customer Care

Hello! We are sorry to hear your little girl didn't like our Enfamil D-Vi-Sol, and thank you for providing your feedback. We would like to speak with you directly to learn more about her experience. One of our team members will be reaching out to you via email, but if you would like to speak with us immediately please give us a call at 1-800-222-9123, select option 1 and reference #637979. We look forward to speaking with you and thank you for being a part of our Enfamil family! -Brittany

DropperSqueezer

Los Angeles, CA

Review **1**

Votes **0**

★★☆☆☆

The Dropper Is Lacking

13 years ago

While the supplement seems fine, the dropper included with it does not work effectively. While the tube of the dropper easily accommodates the 1ml dose, the bubble up top is not large enough to easily draw in enough of the supplement. This is quite annoying. I hope the folks at Enfamil look into this and replace the dropper with one that can easily handle the recommended dose.

⊗ No, I do not recommend this product.

Helpful? (0) (0) Report

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Rating How old is your child?

1 star **Clear All**

Relevancy Info

1 – 8 of 81 Reviews Sort by Most Relevant

TarellJrmommy

MA

Review **1**

Votes **0**

★

Not worth babies stress. Newborn son

9 years ago

I used this product for 2 days after my son (preemie 34wks 5pounds at 8oz at time of birth) was discharged from hospital a week after birth. I was told by his nicu nurse to get him Vitamin D drops because I breatfeed. No specific brand though. My husband bought Enfamils brand. I gave my son 1 dose for 2 days. Day 1 his stool started to become greenish. Day 2 He started having hard time passing his bowel which he never had. So I immediately stopped use. Last night he was fussy would not sleep because he could not pass his bowel. I am actually currently looking on ways to help his constipation and came across this.

⊖ **Cons** Changed Normal Stool, Constipation, Fussiness, Green Thick Bowel Movemen, Stomach Ache, Too Busy Pushing No Sleep

✕ No, I do not recommend this product.

Helpful? (0) (2) Report

BMC011313

Nh

Review **1**

Votes **4**

Gender **Male**

Age **35 to 44**

★☆☆☆☆

Awful product

2 years ago

Gave my newborn horrendous diarrhea. Seeing her in pain was absolutely heart wrenching. There is more bad reviews of this product than good reviews and it hasn't been pulled? How is this even possible! The guilt I feel for not researching this product first is overwhelming. . . .

Online Shopping Experience (if you bought on Enfamil.com) 1.0

Value 1.0

✕ No, I do not recommend this product.

Helpful? (4) (0) Report

Response from Enfamil: 2 years ago

Enfamil Customer Care

Hello! We are sorry to hear your little girl did not do well with our D-Vi-Sol drops. We would like to speak to you directly to learn more about her experience. One of our team members will be reaching out to you via email, if you would like to speak with us immediately please give us a call at 1-800-222-9123, select option 1 and reference #630985. We look forward to hearing from you! -Stephanie

Sleepless in Seattle ★☆☆☆☆

Caused horrific gas in 3 month old

Review **1**

Votes **2**

Gender **Female**

Age **25 to 34**

3 years ago

I switched to this brand a week ago, and since then my 3 month old has been sleeping horribly and in so much pain from awful gas. She was sleeping 7-8 hour stretches but now I have to hold her through the night. I can hear her stomach gurgling and she arches her back each time she has gas. The only change was the gas drops. They obviously are very hard on infant stomachs and I'm not the only one to notice. Please Enfamil change your formula and pull this junk off the market.

Online Shopping Experience (if you bought on Enfamil.com) 1.0

Value 1.0

✕ No, I do not recommend this product.

Helpful? (2) (0) Report

Response from Enfamil: 3 years ago

Product Information & Compliance

Hello! We are so sorry to hear your little girl has been experiencing problems while using our Enfamil D-Vi-Sol Liquid Vitamin D Supplement! We would love to speak with you directly about her experience with our product! When you have a moment please give us a call at 1-800-222-9123, select option 1 and reference #599618. We look forward to speaking with you and seeing how we can help! - Emily

Momoftwo

Lancaster, PA

Review **1**

Votes **2**

Gender **Female**

Age **25 to 34**

★

Terrible reaction

3 years ago

Was prescribed to use this product from pediatrician. My daughter took it for 1 week and was extremely constipated and urinating frequently and in so much pain. After figuring out it was either this product, breast milk or Enfamil Neuro Pro formula. I started with this product first to rule out. After reading reviews on this site with people experiencing the same symptoms and after 2 days off the product our 2 week old daughter is back to her normal self.

Online Shopping Experience (if you bought on Enfamil.com) 1.0

Value 1.0

Any comments about your online shopping experience? Fine

✕ No, I do not recommend this product.

Response from Enfamil: 3 years ago

Product Information & Compliance

Hello! We are sorry to hear that that your little girl was having constipation and discomfort while using Enfamil D-Vi-Sol. We would love to speak with you directly about her experience while using our product! When you have a moment please give us a call at 1-800-222-9123, select option 1 and reference #596231. We look forward to speaking with you and seeing how we can help! -Kristi

Chelsea

New york

Review **1**

Votes **0**

★☆☆☆☆

Don't give this to your baby!!

5 years ago

Full of bad ingredients for your baby when looking more closely at the label. Vitamin D is second to last ingredient. Why all the other junk?! There are much better more natural options out there. Gave to my newborn week 2-4 and we didn't need know why she was screaming in pain and up all night inconsolably crying. Had so much gas and was so irritable. We stopped the drops and she is a different and happy baby, not crying anymore at night from gas pain and is now actually sleeping. DO NOT USE THIS PRODUCT UNLESS YOU WANT A BABY THAT IS UP ALL NIGHT SCREAMING. Enfamil, please do everyone a favor and take another look at this product with awful additives you are making to give to our baby's.

⊖ **Cons** Caused Gas, Caused Stomach Aches

⊗ No, I do not recommend this product.

Helpful? (0) (0) Report

Response from : 5 years ago

Enfamilpwr

We are sorry your baby had this experience with our D-Vi-Sol. At your earliest convenience, please contact our team at Enfamil.com/ContactUs or call 1-800-222-9123 (Option 1). Thank you! -Jennifer, Enfamil Expert

Sam

Las Vegas

Review **1**

Votes **0**

★

STAY AWAY

5 years ago

My pediatrician recommended these drops for my 10 day old son. Before taking the drops he was a happy baby. Today is the second day I've given it to him and he's turned into a whole different child. My poor son has been awake for 8 hours! Nothing could console him, he's had gas and an upset stomach. I must have changed his diaper 3 times within two hours. He was breathing heavily, to the point where I thought I might have to take him to the ER. He finally fell asleep, hopefully the Vitamin D is out or almost out of his system. I can't believe my pediatrician recommended this trash product to us.

⊖ **Cons** Caused Gas, Caused Stomach Aches

Helpful? (0) (0) Report

Response from : 5 years ago

Enfamilpwr

I'm sorry about your son's experience with the Enfamil D-Vi-Sol. Our Product Information Team would like to hear more about it. If you could please contact them at 1-800-222-9123 (option 1) or by email at Enfamil.com/ContactUs and reference number 02777081. Thank you. --Amilia, Enfamil Expert 8/24/18

Kylie

Basking Ridge, Nj

Review **1**

Votes **0**

★☆☆☆☆

New design is terrible

8 years ago

The new design is terrible. It must be a cost saver bc otherwise I don't understand the change from the dropper. Now I have to wash the flimsy syringe after every use which just adds an extra step. Not to mention I need to make sure I don't lose it. This may not seem like a big deal if I just had one kid, but with two small kids at my heals, while caring for a newborn it's just annoying. The dropper was so much more convenient. I read it was to ensure accuracy of dosage, but that's ridiculous. It wasn't hard to measure with the dropper. I'll be looking for a new vitamin D supplement.

⊗ No, I do not recommend this product.

Helpful? (0) (1) Report

Response from : 7 years ago

Enfamilpwr

Kylie, I am sorry to learn of your dissatisfaction with the syringe that is included with our D-Vi-Sol. We changed from a dropper to a syringe for easier measuring and dispensing of our supplement products. A syringe gives you more control than a dropper over the amount of product you draw up and dispense.

Thank you for your feedback regarding the syringe. Please know that I have forwarded your comments to our Product Education and Safety Team. - Erin

JessicaU

Detroit Area

Review **1**

Vote **1**

★

Terrible side effects

6 years ago

Gave this to our 2 week old from a sample from the pediatrician. Within an hour or two, he was crying inconsolably and thrashing around for HOURS. Had terrible gas pains and ended up getting constipated. It was awful. He's now three weeks old and that hasn't happened since, it was definitely from this stuff. Our pediatrician acted so surprised he had this reaction, almost arguing with me that it probably wasn't from this. He must get a kick back for giving out these samples So upsetting.

⊖ **Cons** Caused Gas, Caused Stomach Aches, Caused Constipation

⊗ No, I do not recommend this product.

Helpful? (1) (0) Report

Response from : 5 years ago

Enfamilpwr

Hi Jessica. Our Product Information Department would like to get some additional feedback from you. If you would, please call 1-800-222-9123 and choose Option 1 or visit Enfamil.com/ContactUs. ~Nora

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Csw

Lenoxville PA

Review **1**

Votes **0**

★☆☆☆☆

Beware

5 years ago

Awful product it's too oily makes baby cough puke and start choking- thankfully my baby is ok but these drops need to be pulled from market

No, I do not recommend this product.

Helpful? (0) (0) Report

Response from : 5 years ago

Enfamilpwr

I'm sorry that your little one had trouble with the Enfamil D-Vi-Sol Liquid Vitamin D Supplement. Another designated member of our team would like to hear more about your experience. Please contact us at 1-800-222-9123 (option 1) or by email at Enfamil.com/ContactUs and reference number 02834236. --Amilia, Enfamil Expert 10/30/18

AJ

Ohio

Review **1**

Votes **0**

★☆☆☆☆

Upset Baby!

7 years ago

I have tried a few times, even after waiting a few days in between, to give my LO these drops recommended by the pediatrician. Everytime it is the same result: he becomes gassy, fussy, and begins to cry within a hour after. He becomes almost inconsolable; nothing works. We will not be using these drops again and will let the pedia know. By trying different times; it is true and tested as the drops are the cause. I can't believe these would actually be produced and then recommended for these poor babies.

⊕ **Pros** Keeps Well, Mixes Easily

⊖ **Cons** Bad flavor, Caused Gas, Caused Stomach Aches, Causes Inconsolable Crying

⊗ No, I do not recommend this product.

Helpful? (0) (0) Report

Response from : 7 years ago

Enfamilpwr

I am sorry to learn your little on had so many issues with our Enfamil D-Vi-Sol. Our Product Information Team would like to learn more. When convenient contact them at 1-800-222-9123 (Option 1) or visit Enfamil.com/ContactUs. Thank you! ~ Cindi

lanp3

Reviews **2**

Votes **0**

★★

Upset baby's belly– Do not buy!

lanp3

a year ago

We purchased these drops after our pediatrician recommended Vitamin D drops for our newborn. Our baby is a relatively calm, easy going little guy who doesn't cry much. After purchasing these drops, though, bedtime became awful. He would wail at the top of his lungs for over an hour, and nothing that we tried would calm him down. Finally we realized that he started this when we started giving him the drops. Read the label - there are so many inactive ingredients in these drops, and at least one of them was upsetting our baby's stomach. We stopped giving him these drops, and he's been back to his usual happy self. We feel terrible that we were the ones giving him these drops that were hurting him, but at least we figured it out.

influenster Originally posted on influenster.com



Jas4

Incentivized

Wesley Chapel, Fl

Review **1**

Votes **0**

Gender **Male**

Age **18 to 24**

★★★

Not our favorite, can upset tummy

2 years ago

my son got an upset tummy though he was dairy intolerant which I'm sure contributed

Any comments about your online shopping experience? Didn't get online

✕ No, I do not recommend this product.

Helpful? (0) (0) Report

Online Shopping Experience (if you bought on Enfamil.com) 5.0

Value 3.0

Response from Enfamil: 2 years ago

Enfamil Customer Care

Hello! We are sorry your little guy had trouble while using our Enfamil D-Vi-Sol drops. We would like to speak with you directly to learn more about his experience. One of our team members will be reaching out to you via email, but if you would like to speak with us immediately please give us a call at 1-800-222-9123, select option 1 and reference #643460. We look forward to speaking with you and thank you for being a part of our Enfamil family! -Brittany

Kstring

Mobile, AL

Review **1**

Votes **0**

★★☆☆☆

Caused gas, upset tummy, and lots of crying

8 years ago

I bought this for my 6 week old after her doctor recommended I give her some drops with breastfeeding. The drops caused her to be fussy all day, caused an upset tummy with way more than normal bowel movements and made her gassy

No, I do not recommend this product.

Helpful? (0) (0) Report

Response from : 8 years ago

Enfamilpwr

Please contact our product Education Team regarding your experience. -Cindi

Megan

Dallas tx

Review **1**

Votes **0**

★

Caused tummy upset and excess gas immediately lasting 24 hrs

5 years ago

Terrible product , will not buy or recommend

⊖ **Cons** Caused Gas, Caused Stomach Aches

⊗ No, I do not recommend this product.

Helpful? (0) (0) Report

Response from : 5 years ago

Enfamilpwr

Hello. I'm sorry that your little one was unable to tolerate the D-Vi-Sol. Our Product Information Team would like to hear more about your experience. They will be reaching out to you, or you may contact them at 1-800-222-9123, option 1 or by email at Enfamil.com/ContactUs and reference number 02759171. Thank you! --Amilia, Enfamil Expert 8/7/18

Manda

Incentivized

Evansville, IN

Review **1**

Votes **0**

Gender **Female**

Age **25 to 34**

★★★★★

Belly helper

2 years ago

Helps my daughter out tremendously when her stomach gets upset

Any comments about your online shopping experience? No

✓ Yes, I recommend this product.

Helpful? (0) (0) Report

Online Shopping Experience (if you bought on Enfamil.com) 5.0

Value 5.0

Dean&AleiaMom

Review **1**

Votes **0**

Age **18 to 24**

★★★★★

Amazing!

9 months ago

This is the only vitamin that does not upset my babies tummies and I can give it directly from the dropper or mixed in their bottles with breastmilk!

Any comments about your online shopping experience? This vitamin is amazing! I can give it to my babies directly from the dropper or mixed in the bottle with breast milk! And this is the only vitamin D that does not upset their tummies!!

Online Shopping Experience (if you bought on Enfamil.com) 5.0

Value 5.0

✔ Yes, I recommend this product.

Helpful? (0) (0) Report

Response from Enfamil: 9 months ago

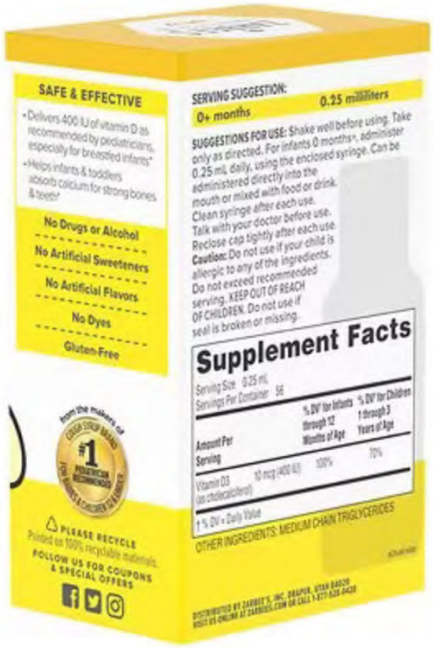
Customer Care

Thank you for telling us about your experience, Dean&AleiaMom. We are grateful for such a wonderful review of our D-Vi-Sol! — Jessica, Enfamil Expert

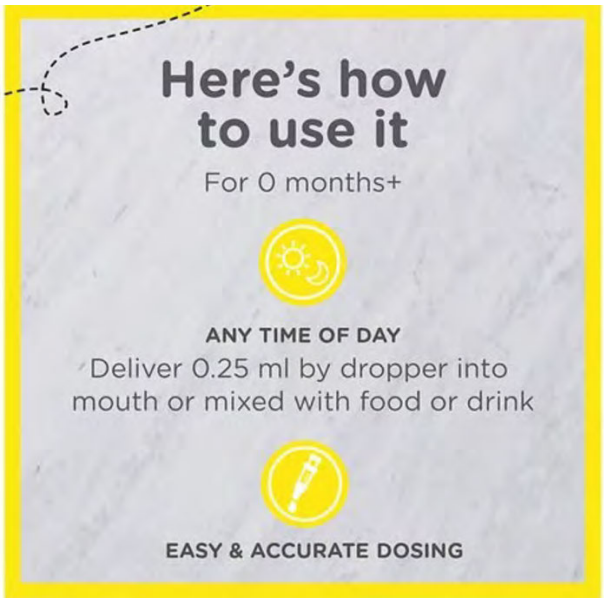
Exhibit F


ZARBEE'S
INSPIRED BY NATURE
baby
0+ MONTHS
Vitamin D
supports strong bone & teeth development*
essential for breastfed infants*
pediatrician recommended
Safe & Effective
0.47 FL. OZ. (14 mL)
Dietary Supplement

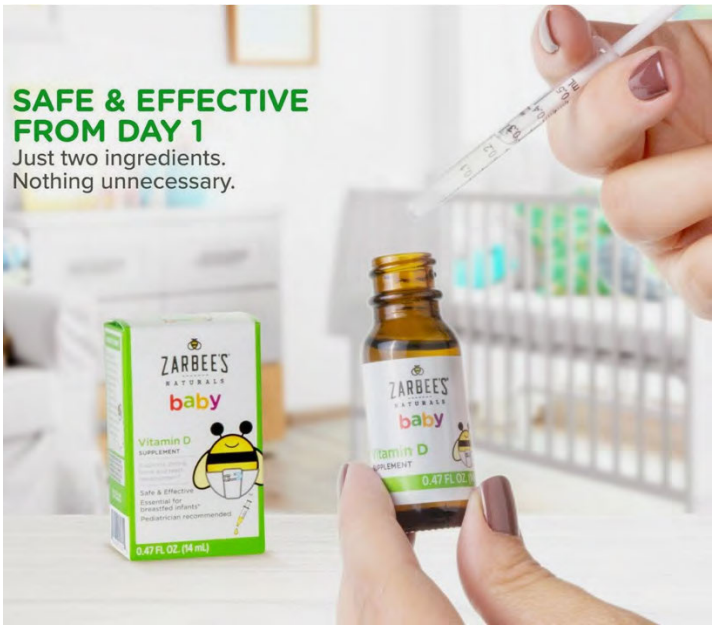

SERVING SUGGESTION:
0+ months
0.25 milliliters
SUGGESTIONS FOR USE: Shake well before using. Take only as directed. For infants 0 months+, administer 0.25 mL daily, using the enclosed syringe. Can be administered directly into the mouth or mixed with food or drink. Clean syringe after each use. Talk with your doctor before use. Reclose cap tightly after each use. Caution: Do not use if your child is allergic to any of the ingredients. Do not exceed recommended serving. KEEP OUT OF REACH OF CHILDREN. Do not use if seal is broken or missing.
Supplement Facts
Serving Size 0.25 mL
Servings Per Container 56
Amount Per Serving
% DV† for Infants through 12 Months of Age
% DV† for Children 1 through 3 Years of Age
Vitamin D3 (as cholecalciferol) 10 mcg (400 IU) 100% 70%
† % DV = Daily Value
OTHER INGREDIENTS: MEDIUM CHAIN TRIGLYCERIDES
DISTRIBUTED BY ZARBEE'S, INC. DRAPER, UTAH 84020
VISIT US ONLINE AT ZARBEES.COM OR CALL 1-877-523-6420


SAFE & EFFECTIVE
•Delivers 400 IU of vitamin D as recommended by pediatricians, especially for breastfed infants*
•Helps infants & toddlers absorb calcium for strong bones & teeth*
No Drugs or Alcohol
No Artificial Sweeteners
No Artificial Flavors
No Dyes
Gluten-Free
#1
PLEASE RECYCLE
FOLLOW US FOR COUPONS & SPECIAL OFFERS
SERVING SUGGESTION:
0+ months
0.25 milliliters
Supplement Facts
OTHER INGREDIENTS: MEDIUM CHAIN TRIGLYCERIDES


ZARBEE'S
INSPIRED BY NATURE
Vitamin D
Delivers 400 IU of liquid vitamin D as recommended by the American Academy of Pediatrics


Here's how to use it
For 0 months+
ANY TIME OF DAY
Deliver 0.25 ml by dropper into mouth or mixed with food or drink
EASY & ACCURATE DOSING


SAFE & EFFECTIVE
• Delivers 400 IU of Vitamin D as recommended by the American Academy of Pediatrics (AAP), especially for breastfed infants*
• Helps infants and toddlers absorb calcium for strong bones and teeth*
No Drugs or Alcohol
No Artificial Sweeteners
No Artificial Flavors
No Dyes
Gluten-Free
from the makers of
#1 COUGH SYRUP BRAND
PEDIATRICIAN RECOMMENDED
FOR BABIES & CHILDREN 12 & UNDER


SAFE & EFFECTIVE
FROM DAY 1
Just two ingredients.
Nothing unnecessary.
ZARBEE'S
baby
Vitamin D
0.47 FL OZ. (14 mL)

ZARBEE'S INSPIRED BY NATURE Search Products, Articl



Baby Vitamin D

Vitamin D is an essential vitamin to help baby build strong bones and teeth.*

★★★★★ 4.7 (23) Write a Review

Find a Retailer

Details

Ages
0 months+

Main Ingredients
Vitamin D

Flavor
N/A

Key Nutrients
Vitamin D

Pack Count

0.47 FL OZ. (14 mL)

How to Use

Serving Description

Deliver 0.25 mL by dropper into mouth or mixed with food or drink

Works Best

Any time of day

Consumption Tag

Oral Syringe

Ingredients

Vitamin D3, medium chain triglycerides

Facts

Supplement Facts

Serving Size 0.25 mL
Servings Per Container 56

Amount Per Serving		% DV† for Infants through 12 Months of Age	% DV† for Children 1 through 3 Years of Age
Vitamin D3 (as cholecalciferol)	10 mcg (400 IU)	100%	70%

† % DV = Daily Value

OTHER INGREDIENTS: MEDIUM CHAIN TRIGLYCERIDES

Exhibit G

From: **Ann Korman** annrk123@gmail.com
Subject: d drops
Date: January 31, 2012 at 10:20 AM
To: Susan Jankus susan.jankus@ddrops.ca

Hi There,
I am a nutritionist and I would like to be able to sell your product.
I am also launching an online store and would like to put the product there as well. I love your product!
Please lat me know how I can start a professional account with you.
Thank You,
Ann Rosen Korman
310-386-4940
ann@arkofwellness.com
450 N Greencraig Rd
Los Angeles, CA
90049

From: **Forms** forms@vitaminddrops.com
Subject: Ddrops Website Contact
Date: March 24, 2016 at 9:39 PM
To: healthcare healthcare@ddrops.ca

Name: Smriti Tuladhar
Email: smriti12345@gmail.com
Department: Consumer Enquiries
Country: US

Message:

Hi,
My pediatrician recommend this vitamin D for my little one. Got a sample from the clinic and very happy with it as there are not artificially colors or ingredients n my baby doesn\'t mind it at all. The only comment I have is you should make it available at stores like target. I usually shop baby stuff there and would be easy to get it there. Walgreens and cvs are not my usual place to shop. Anyways, also wanted to see if you can send me some coupons so I can stock up on my next trip to cvs.
Thanks!

From: **carol letourneau** ca.letourneau@gmail.com
Subject: More information
Date: September 3, 2009 at 9:47 AM
To: Susan Jankus susan.jankus@ddrops.ca

Thank you for the package that we received in the mail that was sent to Dr. Robert Moriartey here at the Paeds Clinic.
On average our clinic would see 125 new borns per month. Could you please send us another bunch of your publication as the response has been wonderful. Is there anyway to be put on an automatic mailout for samples and publications. If we were to even get 100 pamphlets per month that would be wonderful. Samples are by all means always welcome also. Thank you in advance for your attention to this. If you have anyother questions please feel free to contact me. Have a wonderful day..... on purpose.

Carol Letourneau
Paeds Clinic
219-3017-66 st
Edmonton, AB T6K 4B2

From: **Forms** forms@vitaminddrops.com
Subject: Ddrops Website Contact
Date: September 13, 2016 at 7:09 AM
To: healthcare healthcare@ddrops.ca

Name: Tiffany Thompson
Email: tiffanyt@beamishlaw.ca
Department: Consumer Enquiries
Country: Canada

Message:

Good Morning,

I would first like to say what a wonderful product you have. My whole family takes this during the winter months when there is not enough sun light to get us our daily dose of Vitamin D. My husband has Season Affective Disorder and this product helps so much. I was just wondering if you have any coupons you could send me to help with the cost of the product. It would help imseley if there were some. Please let me know and thank you for making such a wonderful product.

From: **noreply** noreply@ddropscompany.com
Subject: New submission from CA - Contact Us Form
Date: February 12, 2015 at 7:48 AM
To: healthcare healthcare@ddrops.ca

Name

Joanna Fousek

Email

Fouseks@hotmail.com

Department

Consumer Enquiries

Message

Hi, I used D drops for the first year while my baby was nursing and loved them! I was just wondering if you make a multi vitamin in a drop for toddlers? My babies 15 months and too young to take or chew a solid vitamin but the liquid vitamins just smell and taste gross so it's hard to get her to take them. I still nurse her anyway and thought a multi vitamin in a drop would be ideal! She is a picky eater so I worry that she's getting all her vitamins from food. If you don't make them do you know of anyone that does?
Thank you,
Joanna.

From: **Susan Jankus** susan.jankus@ddrops.ca
Subject: FW: Samples
Date: August 22, 2013 at 9:46 AM
To: Karin Christopherakos karin.christopherakos@ddrops.ca

Here is another US fan!

Susan Jankus
Director Sales & Marketing

Ddrops Company
126 Trowers Road
Woodbridge, Ontario
L4L 5Z4 Canada

T. +1 905 851 8898 ext 240
F. +1 905 851 8890
E. susan.jankus@ddrops.ca
www.Ddrops.ca
www.vitaminDdrops.com

Baby Ddrops™ awarded GOLD by Mom`s Choice Awards.

The Parent Tested Parent Approved

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From: "A.Giokas @ Milestones Pediatrics" <agiokas@milestonespediatrics.com>
Date: Thursday, 22 August, 2013 10:18 AM
To: Susan Jankus <susan.jankus@ddrops.ca>
Subject: Samples

Good Morning,

I would like to request more samples of your Vitamin D Drops for our patients.

Thank you in advance.

Anthula Giokas
Office Manager

Milestones Pediatrics
810 Franklin Avenue
Franklin Lakes, NJ 07417
201.485.7557 P
201.485.7556 F
Agiokas@milestonespediatrics.com
www.milestonespediatrics.com
www.facebook.com/pages/Milestones-Pediatrics

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From: **Forms** forms@vitaminddrops.com
Subject: Ddrops Website Contact
Date: September 13, 2016 at 7:09 AM
To: healthcare healthcare@ddrops.ca

Name: Tiffany Thompson
Email: tiffanyt@beamishlaw.ca
Department: Consumer Enquiries
Country: Canada

Message:

Good Morning,

I would first like to say what a wonderful product you have. My whole family takes this during the winter months when there is not enough sun light to get us our daily dose of Vitamin D. My husband has Season Affective Disorder and this product helps so much. I was just wondering if you have any coupons you could send me to help with the cost of the product. It would help imseley if there were some. Please let me know and thank you for making such a wonderful product.

From: **Sawyer, Mamie** Mamie.Sawyer1@fraserhealth.ca
Subject: Baby D-Drop Samples
Date: March 15, 2010 at 6:15 PM
To: Susan Jankus susan.jankus@ddrops.ca

I am a public health nurse in Surrey and I am finding that more and more parents are wanting to buy your product, but are having some difficulty in finding it in the stores. I wanted to know if it would be possible for you to donate some samples that I can give out to promote your product so that we can better guide clients as to what they are looking for or requesting in the store. Please send them to the following address:

ATTN:
Mamie Sawyer, RN, BScN
Guildford Public Health Unit
Suite 100- 10233 153rd Street
Surrey, BC V3R 0Z7
(604) 587-4760
Mamie.Sawyer1@fraserhealth.ca

From: **Lipman, Rachel** rlipman@pedcenter.org
Subject: RE: Do you need more Baby Ddrops samples?
Date: January 5, 2016 at 12:54 PM
To: Janet Dickieson janet.dickieson@ddrops.ca

Hello!

Yes I would LOVE to receive more samples and brochures. My parents absolutely love the product. Ddrops are just so helpful, especially for the new breastfeeding moms.

Thank you,
Rachel Lipman

From: Janet Dickieson [janet.dickieson@ddrops.ca]
Sent: Tuesday, January 05, 2016 10:10 AM
To: Lipman, Rachel
Subject: Do you need more Baby Ddrops samples?

Hello Rachel,

I hope you and your New Moms have been enjoying the Baby Ddrops samples that were sent to Eisner Pediatric some time ago. I wanted to check in with you to see if you would be interested in receiving additional samples/brochures to share with your patients at this time.

I look forward to hearing from you.

Kindest Regards,

Janet

Ddrops
The sunshine vitamin in just one drop

Janet Dickieson
Ddrops Healthcare Team

126 Trowers Road, Woodbridge, Ontario L4L 5Z4 Canada
T. +1 844-433-7677 F. +1 866-886-5786 E. healthcare@ddropscompany.com

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Elsner Pediatric & Family Medical Center
Caring for Los Angeles families since 1920
Received the 2011 Los Angeles Business Journal Healthcare Leadership Award in the community clinic category

From: **Roslyn Konter** rpkonter@gmail.com
Subject: Re: Baby Ddrops samples
Date: October 19, 2015 at 11:11 AM
To: Janet Dickieson janet.dickieson@ddrops.ca

Thanks for checking in!
Love the DDrops and yes we need more! We are completely out!!

Thanks again!
Roslyn

Sent from my iPhone

On Oct 19, 2015, at 11:19 AM, Janet Dickieson <janet.dickieson@ddrops.ca> wrote:

From: Janet Dickieson
Sent: October-19-15 11:18 AM
To: 'rpdonter@gmail.com' <rpdonter@gmail.com>
Subject: Baby Ddrops samples

Hello Roslyn,

I hope you and your New Moms have been enjoying the Baby Ddrops samples sent to you in September. I wanted to check in with you to see how you are making out with the samples and to see if you require additional samples at this time.

I look forward to hearing from you.

Kindest Regards,

Janet

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Janet Dickieson
Customer Contact Centre

126 Trowers Road, Woodbridge, Ontario L4L 5Z4 Canada
T. +1 844-433-7677 F. +1 866-886-5786 E. janet.dickieson@ddrops.ca

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From: **Forms** forms@vitaminddrops.com
Subject: Ddrops Website Contact
Date: March 24, 2016 at 9:39 PM
To: healthcare healthcare@ddrops.ca

Name: Smriti Tuladhar
Email: smriti12345@gmail.com
Department: Consumer Enquiries
Country: US

Message:

Hi,
My pediatrician recommend this vitamin D for my little one. Got a sample from the clinic and very happy with it as there are not artificially colors or ingredients n my baby doesn\'t mind it at all. The only comment I have is you should make it available at stores like target. I usually shop baby stuff there and would be easy to get it there. Walgreens and cvs are not my usual place to shop. Anyways, also wanted to see if you can send me some coupons so I can stock up on my next trip to cvs.
Thanks!

From: **noreply** noreply@ddropscompany.com
Subject: New submission from CA - Contact Us Form
Date: February 1, 2016 at 2:15 PM
To: healthcare healthcare@ddrops.ca

Name

Kimberly Kennelly

Country

USA

Email

kimkennelly@gmail.com

Department

Consumer Enquiries

Message

Hi,
My son has a liver disease which makes it hard for him to absorb Vitamin D, so he takes your drops as a supplement. They are great because it is so easy to give and he tolerates them well. He now has to take 15 drops of the 400iu a day so When I buy at a local CVS drugstore the bottle only contains 60 drops so I need to buy about 8 bottles a month and at $15 each it is getting costly. So long story short I was wondering if you offer some sort of discount or assistance at all in these matters. Thanks for your time and a great product!
Kim Kennelly 516-301-8018

From: **Summer White** swhite@panwpc.com
Subject: RE: Do you need more Baby Ddrops samples?
Date: August 8, 2016 at 11:32 AM
To: Janet Dickieson janet.dickieson@ddrops.ca

Yes please, that would be great. Our new parents love taking home your samples.
Thank you,
Summer

From: Janet Dickieson [mailto:janet.dickieson@ddrops.ca]
Sent: Monday, August 08, 2016 7:09 AM
To: Summer White <swhite@panwpc.com>
Subject: Do you need more Baby Ddrops samples?

Hello Summer,

I hope you and your New Moms have been enjoying the Baby Ddrops samples sent to Pediatric Associates of the NW at 7150 S W Dartmouth back in the spring. I wanted to check in with you to see if you require additional samples/brochures at this time.

I look forward to hearing from you.

Kindest Regards,

Janet

Janet Dickieson Customer Contact Center

T. +1 844-433-7677 F. +1 866-886-5786
126 Trowers Road, Woodbridge, Ontario L4L 5Z4 Canada
www.vitaminDdrops.com

The answers to your inquiry are for information purposes and are not intended to offer personal medical advice. You should seek the advice of your physician or other qualified health provider with any questions you may have regarding a medical condition. Never disregard professional medical advice or delay in seeking it because of a inquiry response.
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CDN Patent no. 2578881 & U.S. Patent 9066958
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From: **maparker** maparker32@verizon.net
Subject: Re: Baby Ddrops samples
Date: April 4, 2015 at 8:53 AM
To: Janet Dickieson janet.dickieson@ddrops.ca

Thanks Janet. I finally checked yesterday (sorry) and we will definitely need more. We have 2 pkgs of 6 left and most clients prefer your product.
Thanks so much,
Michele

Sent from my iPhone

On Mar 26, 2015, at 11:31 AM, Janet Dickieson <janet.dickieson@ddrops.ca> wrote:

> Hello Michelle,
>
> We hope you and your new moms have been enjoying the Baby Ddrops samples. Just checking into see how you are making out with the samples and whether you need any additional samples and brochures at this time.
> I look forward to hearing from you.
>
> Kindest Regards,
>
> Janet
>
> Ddrops The sunshine vitamin in just one drop
>
> **Janet Dickieson**
> **Ddrops Contact Center**
>
> 126 Trowers Road, Woodbridge, Ontario L4L 5Z4 Canada
> T. +1 844-433-7677 F. +1 866-886-5786 E. janet.dickieson@ddrops.ca.
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From: **Lipman, Rachel** rlipman@pedcenter.org
Subject: RE: Do you need more Baby Ddrops samples?
Date: January 5, 2016 at 12:54 PM
To: Janet Dickieson janet.dickieson@ddrops.ca

Hello!

Yes I would LOVE to receive more samples and brochures. My parents absolutely love the product. Ddrops are just so helpful, especially for the new breastfeeding moms.

Thank you,
Rachel Lipman

From: Janet Dickieson [janet.dickieson@ddrops.ca]
Sent: Tuesday, January 05, 2016 10:10 AM
To: Lipman, Rachel
Subject: Do you need more Baby Ddrops samples?

Hello Rachel,

I hope you and your New Moms have been enjoying the Baby Ddrops samples that were sent to Eisner Pediatric some time ago. I wanted to check in with you to see if you would be interested in receiving additional samples/brochures to share with your patients at this time.

I look forward to hearing from you.

Kindest Regards,

Janet

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Ddrops Healthcare Team

126 Trowers Road, Woodbridge, Ontario L4L 5Z4 Canada
T. +1 844-433-7677 F. +1 866-886-5786 E. healthcare@ddropscompany.com

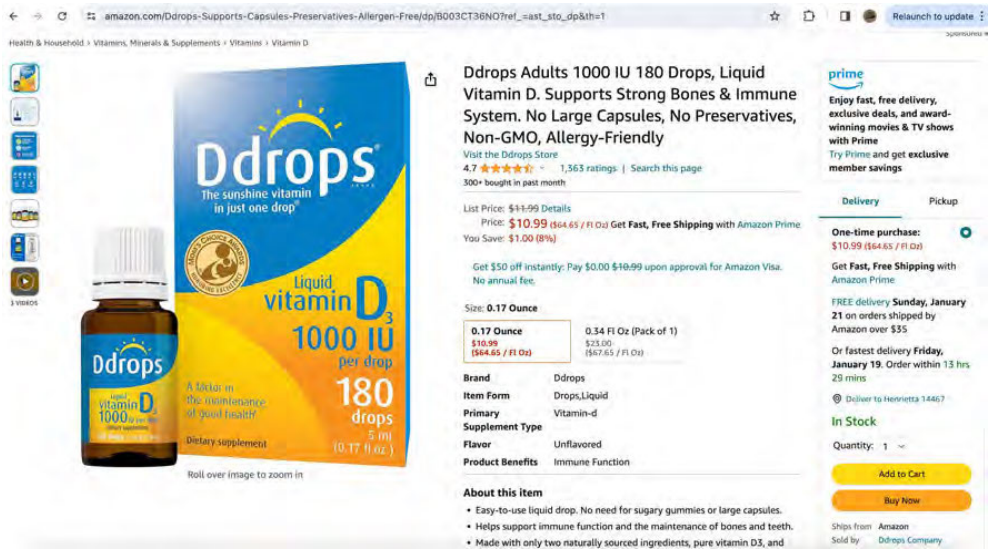
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★★★★★ **easy to use and tolerate**

By amrock in the United States on June 29, 2022

My husband and I take Vitamin D for our bone health and overall health, and for whatever benefits it may have for fighting Covid. Unfortunately, my husband can't tolerate Vitamin D tablets, and his doctor suggested trying drops. These have been great! They are easy to dispense onto a spoon, and have no taste or odor. He has not problems tolerating them. We briefly tried another, less expensive brand which tended to leak and dribble, but came back to this one as an overall better value. We'll be sticking with DDrops! see less

R

★★★★★ **Good value, good quality, very useful**

Reviewed in Canada on November 28, 2020

Size: 0.34 Fl Oz (Pack of 1) **Verified Purchase**

It's a glass bottle and all I have to do is hold it still and upside down over the back of my hand and drops come out not too fast not too slow. Better than getting it from milk which has alot of calcium and lactose, or margarine which has alot of omega 6 fats, or almond milk which has alot of calcium and alot of zinc. I take it a few times per week.

★★★★☆ **Easy to take**

By M. A. in VA in the United States on June 27, 2014

So nice not to have to swallow a pill. Easy to take, I just put a drop on a spoon. The taste is very pleasant. see less

Customer Reviews

★★★★★ **... and needs additional vitamin D and this stuff is perfect. It's tasteless and can be put on whatever ...**

By Nicole Jennifer in the United States on August 15, 2015

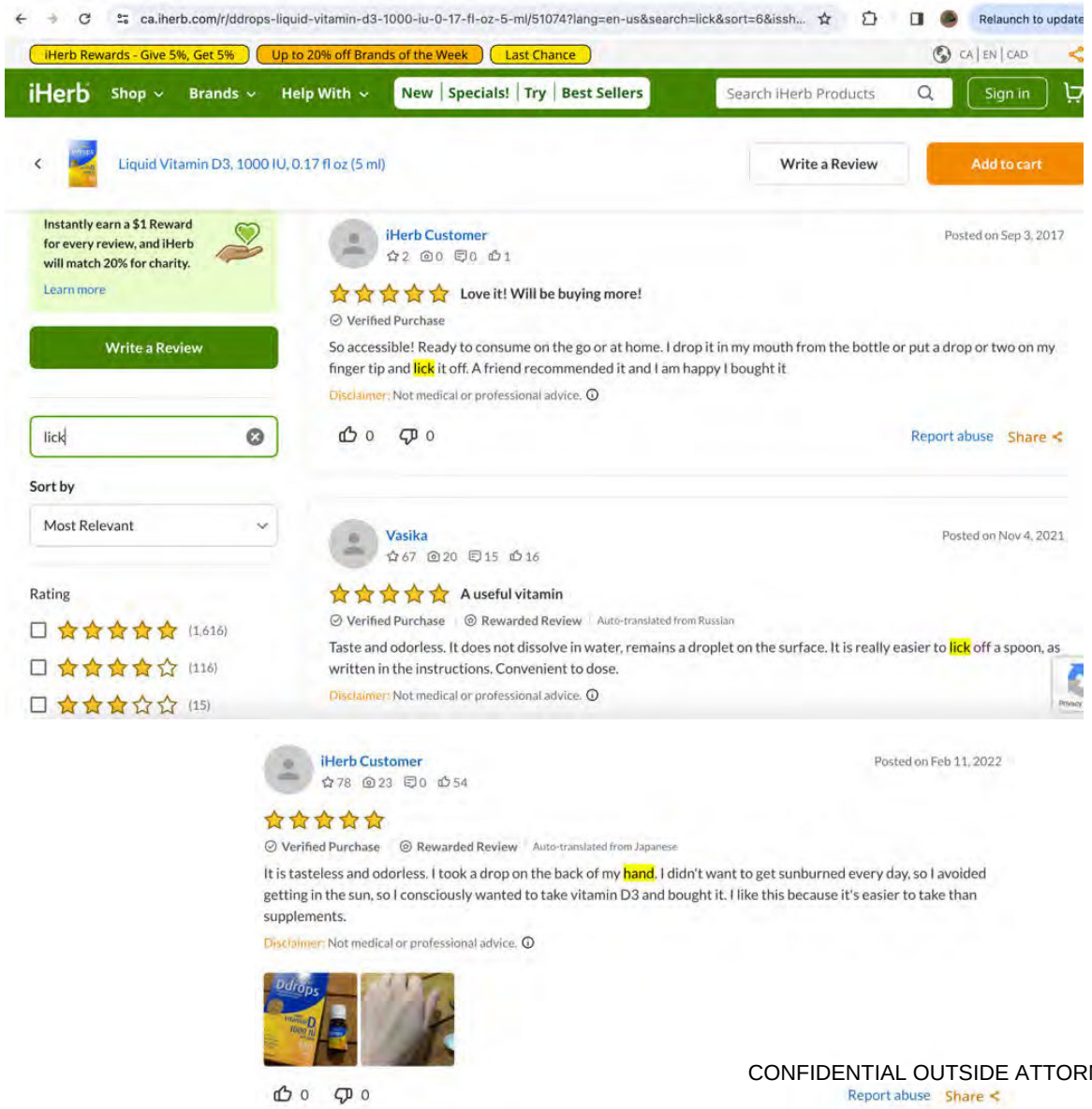
My daughter has cystic fibrosis and needs additional vitamin D and this stuff is perfect. It's tasteless and can be put on whatever she's eating or just on a spoon and she can lick it off. It's hard to find vitamin D in a liquid form, since she won't do gummies, chewables, or pills yet. I had bought a different brand but it wasn't nearly as good as Ddrops. The other brand had a bad flavor and I didn't like the way it was dispensed. Ddrops is an excellent brand. Highly recommended! see less

★★★★★ **easy to use and tasteless**

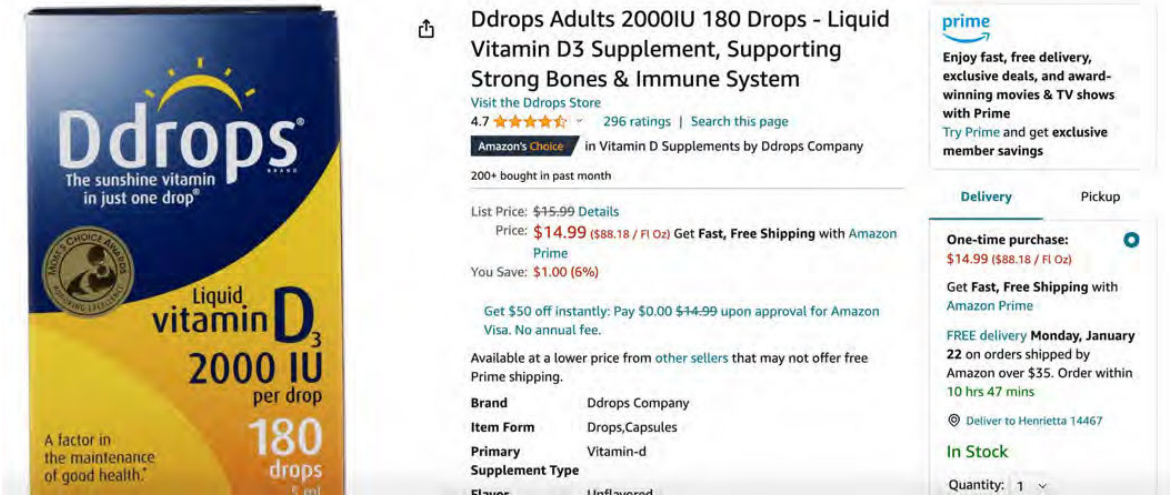
By Dan Nguyen in Canada on January 18, 2022

comes in a small glass bottle. just twist the cap. flip it upside down let one drop go on your hand. lick it off. good to go

See 2 matching customer reviews ›



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★★★★★ **First time ever my D3 level is okay taking these simple drops**

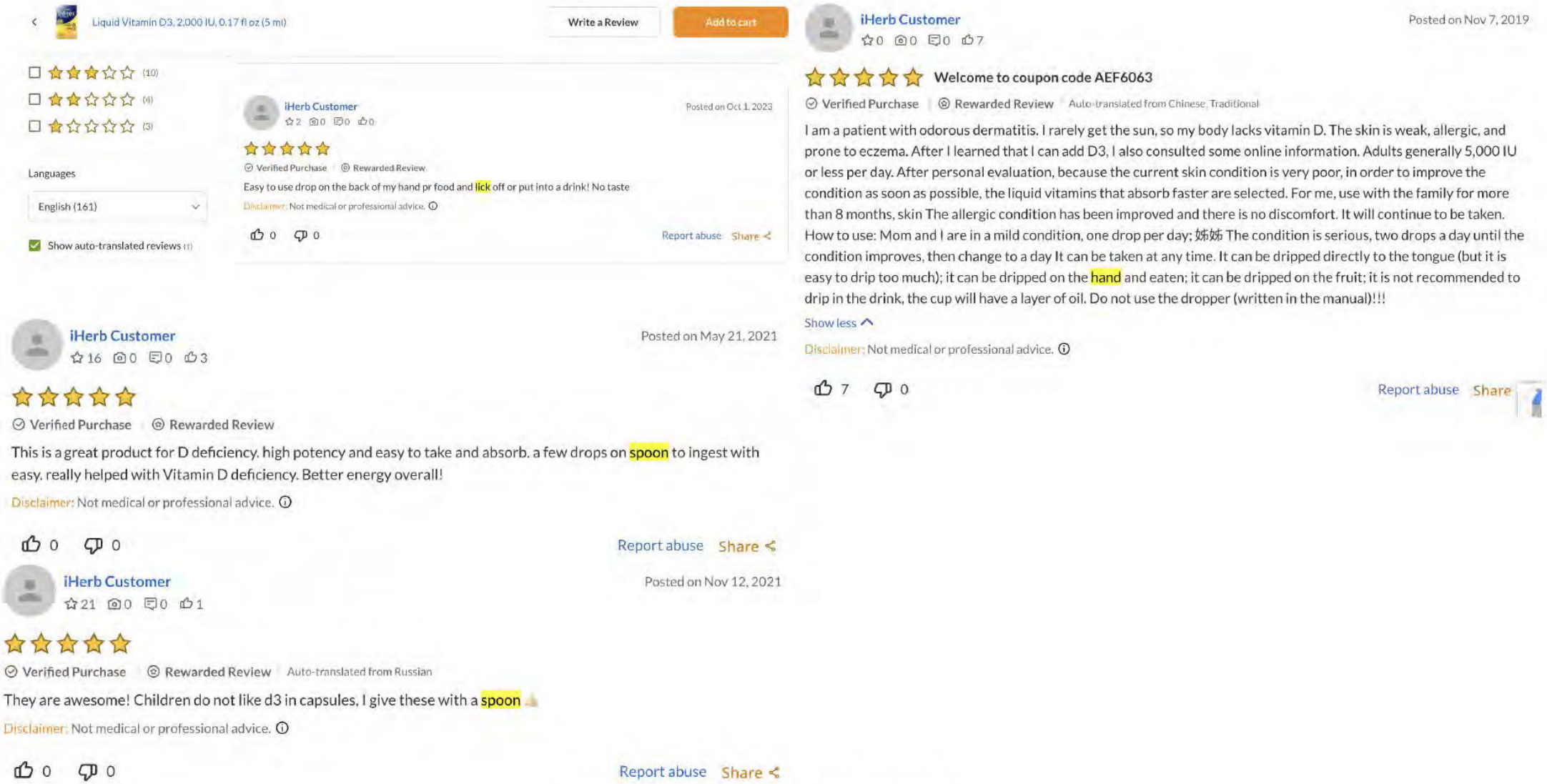
By Kindle Customer in the United States on November 1, 2021

Easy to just drop on the back of my hand and lick off or put into a drink! No taste

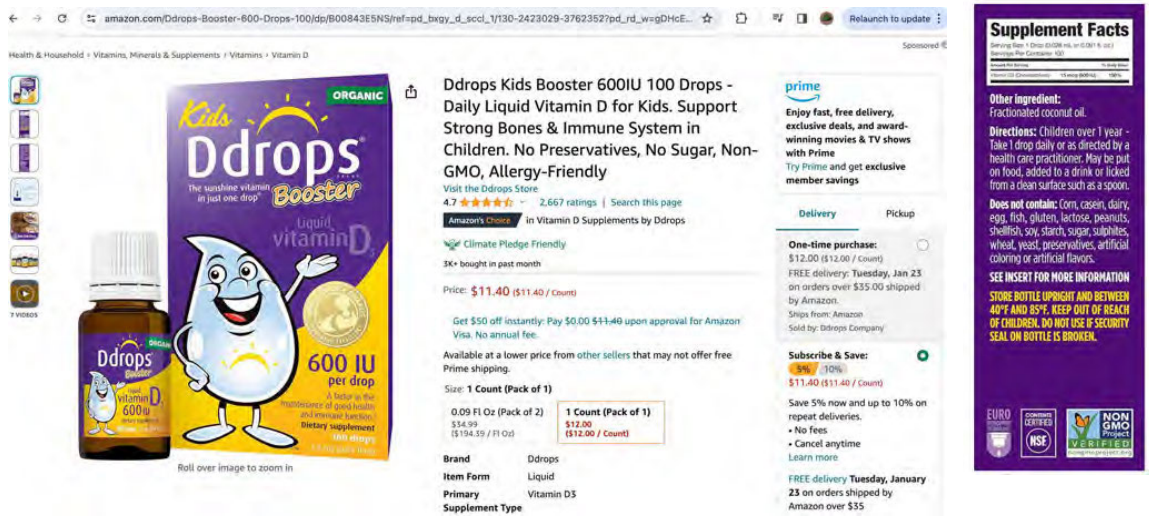
★★★★★ **Great way to get Vitamin D! Ddrops 2000 IU**

By atldsl in the United States on June 13, 2014

My doctor said I was low on Vitamin D and recommended I take 2000 IU dosage. I looked at pills and then ran across vitamin D in liquid form. Ddrops out of Canada make a liquid form which only requires one drop per day. It also comes with a unique dropper that you turn upside down and in about 3 to 5 seconds one drop will come out. So I use a spoon and then lick it after the drop dispenses. There is no taste so I don't feel compelled to have something to wash it down with though I usually do have something as I take another supplement around the same time.



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amazon.com/Ddrops-Booster-600-Drops-100/dp/B00843E5NS/ref=pd_bxgy_d_sccl_1/130-2423029-3762352?pd_rd_w=gDHcE...
Relaunch to update
Health & Household › Vitamins, Minerals & Supplements › Vitamins › Vitamin D
Sponsored
ORGANIC
Kids
Ddrops
The sunshine vitamin in just one drop*
Booster
Liquid vitamin D
600 IU per drop
Dietary supplement
100 drops
Roll over image to zoom in
Ddrops Kids Booster 600IU 100 Drops - Daily Liquid Vitamin D for Kids. Support Strong Bones & Immune System in Children. No Preservatives, No Sugar, Non-GMO, Allergy-Friendly
Visit the Ddrops Store
4.7 2,667 ratings | Search this page
Amazon's Choice in Vitamin D Supplements by Ddrops
Climate Pledge Friendly
3K+ bought in past month
Price: $11.40 ($11.40 / Count)
Get $50 off instantly: Pay $0.00 $11.40 upon approval for Amazon Visa. No annual fee.
Available at a lower price from other sellers that may not offer free Prime shipping.
Size: 1 Count (Pack of 1)
0.09 Fl Oz (Pack of 2) $34.99 ($194.39 / Fl Oz)
1 Count (Pack of 1) $12.00 ($12.00 / Count)
Brand Ddrops
Item Form Liquid
Primary Supplement Type Vitamin D3
prime
Enjoy fast, free delivery, exclusive deals, and award-winning movies & TV shows with Prime
Try Prime and get exclusive member savings
Delivery
Pickup
One-time purchase: $12.00 ($12.00 / Count)
FREE delivery: Tuesday, Jan 23 on orders over $35.00 shipped by Amazon.
Ships from: Amazon
Sold by: Ddrops Company
Subscribe & Save:
5% 10%
$11.40 ($11.40 / Count)
Save 5% now and up to 10% on repeat deliveries.
• No fees
• Cancel anytime
Learn more
FREE delivery Tuesday, January 23 on orders shipped by Amazon over $35
Supplement Facts
Other ingredient: Fractionated coconut oil.
Directions: Children over 1 year - Take 1 drop daily or as directed by a health care practitioner. May be put on food, added to a drink or licked from a clean surface such as a spoon.
Does not contain: Corn, casein, dairy, egg, fish, gluten, lactose, peanuts, shellfish, soy, starch, sugar, sulphites, wheat, yeast, preservatives, artificial coloring or artificial flavors.
SEE INSERT FOR MORE INFORMATION
STORE BOTTLE UPRIGHT AND BETWEEN 40°F AND 85°F. KEEP OUT OF REACH OF CHILDREN. DO NOT USE IF SECURITY SEAL ON BOTTLE IS BROKEN.
EURO
NSF
NON GMO Project VERIFIED

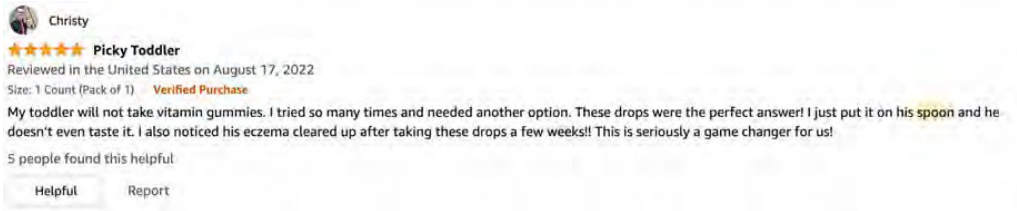

Christy
Picky Toddler
Reviewed in the United States on August 17, 2022
Size: 1 Count (Pack of 1) Verified Purchase
My toddler will not take vitamin gummies. I tried so many times and needed another option. These drops were the perfect answer! I just put it on his spoon and he doesn't even taste it. I also noticed his eczema cleared up after taking these drops a few weeks!! This is seriously a game changer for us!
5 people found this helpful
Helpful
Report

Exhibit H

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Original Article

MOLECULAR METABOLISM

Insulin-sensitizing effects of vitamin D repletion mediated by adipocyte vitamin D receptor: Studies in humans and mice

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ABSTRACT

Objective: Adipose tissue inflammation and fibrosis appear to contribute to insulin resistance in obesity. Vitamin D receptor (*Vdr*) genes are expressed by adipocytes, macrophages, and fibroblasts, all of which could potentially play a role in adipose tissue inflammation and fibrosis. As vitamin D has been shown to have direct anti-inflammatory effects on adipocytes, we determined whether specific vitamin D receptor-mediated effects on adipocytes could impact adipose tissue inflammation and fibrosis and ultimately insulin resistance.

Methods: We examined the effects of repleting vitamin D in 25(OH)D-deficient, insulin resistant, overweight-to-obese human subjects (n = 19). A comprehensive assessment of whole-body insulin action was undertaken with stepped euglycemic (~90 mg/dL) hyperinsulinemic clamp studies both before and after the administration of vitamin D or placebo. Adipose tissue fibrosis and inflammation were quantified by real-time rt-PCR and immunofluorescence in subcutaneous abdominal adipose tissue.

To determine whether vitamin D's effects are mediated through adipocytes, we conducted hyperinsulinemic clamp studies (4 mU/kg/min) and adipose tissue analysis using an adipocyte-specific vitamin D receptor knockout (VDR-KO) mouse model (adiponectin-Cre + VDR+/fl) following high-fat diet feeding for 12 weeks.

Results: 25(OH)D repletion was associated with reductions in adipose tissue expression of pro-inflammatory and pro-fibrotic genes, decreased collagen immunofluorescence, and improved hepatic insulin sensitivity in humans. Worsening trends after six months on placebo suggest progressive metabolic effects of 25(OH)D deficiency. Ad-VDR-KO mice mirrored the vitamin D-deficient humans, displaying increased adipose tissue fibrosis and inflammation and hepatic insulin resistance.

Conclusions: These complementary human and rodent studies support a beneficial role of vitamin D repletion for improving hepatic insulin resistance and reducing adipose tissue inflammation and fibrosis in targeted individuals, likely via direct effects on adipocytes. These studies have far-reaching implications for understanding the role of adipocytes in mediating adipose tissue inflammation and fibrosis and ultimately impacting insulin sensitivity.

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Keywords Vitamin D; Insulin sensitivity; Inflammation; Fibrosis; Adipocyte; Macrophage

1. INTRODUCTION

Adipose tissue regulates numerous physiological processes, and its expansion in obese humans is associated with disrupted metabolic homeostasis, insulin resistance, and type 2 diabetes mellitus (T2D) [1]. Adipose tissue expansion in obesity is accompanied by adipose tissue dysfunction characterized by adipocyte hypertrophy and hyperplasia, increased inflammation, impaired extracellular matrix remodeling, and upregulation of pro-fibrotic signaling pathways mediated by fibroblasts together with altered secretion of adipokines [2–6]. Obesity is also characterized by increased macrophage infiltration into fat tissue, which is another important source of adipose-derived pro-inflammatory factors [7–10]. An inter-relationship among adipose tissue inflammation, adipose fibrosis, and insulin resistance has been established in rodents [11] and may also be relevant in humans [9,12–15].

Low 25-hydroxyvitamin D [25(OH)D] levels are associated with glucose intolerance, insulin resistance, metabolic syndrome, and increased risk

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of T2D development [16–18], although the causal effects of vitamin D deficiency on these conditions have yet to be clearly established. The prevalence of vitamin D deficiency has increased globally, with an estimated 1 billion people worldwide qualifying as vitamin D deficient (defined as a 25(OH)D level of less than 20 ng/ml or 50 nmol/L [19,20]) or insufficient (defined as a 25(OH)D level of 21–29 ng/ml or 52–72 nmol/L). Of note, a recent large clinical trial demonstrated that vitamin D repletion reduced the risk of developing T2D by 62% in individuals with initial vitamin D levels <12 ng/ml [21]. Adipose tissue is a reservoir for vitamin D in humans, and adipose 25(OH)D content appears to correlate to 25(OH)D plasma levels [22–24]. Vitamin D receptor (*Vdr*) genes are expressed by adipocytes, macrophages, and fibroblasts [25–31], all of which could potentially play a role in adipose tissue inflammation and fibrosis. Vitamin D has inhibitory effects on adipose tissue inflammation and on leukocyte infiltration both in vivo and in vitro, in part by upregulating the expression of anti-microbial peptides such as LL-37 [32]. $1,25(OH)_2D$ inhibits the nuclear factor kappa light-chain-enhancer of activated B cells (NF-κB) signaling pathway, which in turn leads to decreased release of interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), and IL-6 by human preadipocytes and MCP-1 by human adipocytes [33,34]. Recent studies have shown that vitamin D reduces hepatic and renal fibrosis by suppressing TGF-β-SMAD signal transduction [3,31,35–37]. As vitamin D has been shown to have direct anti-inflammatory effects on adipocytes [28,38], we hypothesized that vitamin D could mediate beneficial effects on adipose tissue inflammation and fibrosis by working through adipocyte vitamin D receptors. Therefore, studying an adipocyte-specific *Vdr* knockout model, we determined whether specific vitamin D receptor-mediated effects on adipocytes could impact adipose tissue inflammation and fibrosis and ultimately insulin resistance.

The objectives of the present study were to evaluate the effects of two levels of vitamin D repletion (to 30 ng/ml and greater than 50 ng/ml, respectively) on insulin sensitivity and adipose tissue biology in overweight or obese insulin-resistant humans with vitamin D deficiency and conduct parallel rodent studies establishing the impact of adipocyte vitamin D signaling on systemic glucose metabolism. Distinct from prior research into vitamin D repletion in humans, the current studies employed a placebo-controlled dose- and duration-dependent study design at different vitamin D repletion levels and state-of-the art stepped hyperinsulinemic clamps to separately quantify hepatic vs peripheral insulin sensitivity.

2. MATERIALS AND METHODS

2.1. Experimental design: human studies

This randomized, double-blind, placebo-controlled study examining the dose-dependent effects of vitamin D repletion on insulin sensitivity and adipose tissue biology in individuals at risk of T2D was conducted at the Clinical Research Center of the Albert Einstein College of Medicine (Einstein) in the Bronx, New York. The study was approved by Einstein's Institutional Review Board, and all of the participants provided written informed consent. The inclusion criteria included 19 ($N = 19$) overweight or obese (BMI range 25–39 kg/m^2) participants with insulin resistance by homeostasis model assessment-estimated insulin resistance (HOMA-IR $\geq$ 3.0) and 25-hydroxyvitamin D levels less than 20 ng/ml who were randomly assigned to receive vitamin D3 or a placebo matched in taste and appearance once a week for up to 6–8 months. The exclusion criteria included a history of diabetes, major psychiatric disorders on medication, HIV/AIDS, cancer, sarcoidosis, alcohol or substance abuse, Cushing's syndrome, primary hyperparathyroidism, nephrolithiasis, pregnancy or breast-feeding, regular visits to a tanning salon, hypercalcemia or hypocalcemia, uncontrolled hypertension, any chronic illness requiring medication other than arthritis, hypertension and hyperlipidemia, and a positive urine toxicology screen at any of the visits.

The participants were recruited through print advertisements beginning in March 2009. Potential participants underwent screening first by telephone and then via an in-person clinical visit. The study flow diagram is shown in Figure 1A, which indicates the number of subjects who needed to be screened to identify the study population based on BMI, HOMA-IR, and 25(OH)D criteria. A detailed medical history and physical examination, routine clinical biochemistry, and electrocardiogram were performed at the clinical visit. The participants were screened for vitamin D deficiency (25(OH)D $<$ 20 ng/ml). Serum 25(OH)D levels were measured using a Diasorin chemiluminescent assay (Diasorin, Stillwater, MN, USA). Measuring 25(OH)D levels is more clinically applicable as it is stable with a longer half-life (15–50 days) than 1,25(OH)D with a half-life of 15 h [39,40]. The subjects' baseline characteristics are included in Supplementary Table 1.

The enrolled participants were instructed to maintain their body weight and usual diet and exercise lifestyle for the study duration. The participants were assigned an intervention (either vitamin D or placebo) that was logged and dispensed by the hospital pharmacy in a manner that kept the research team and participants blinded. The randomization sequence used in this study was created following a biostatistics consultation and was kept in a secure, password-protected file that was only accessed by the study coordinator and research pharmacist. The subjects in the treatment arm were given weekly doses of vitamin D3 (Ddrops Company, Woodbridge, ON, Canada) to reach a target level of 30 ng/ml (second visit) during the first three months of treatment and thereafter continued on weekly vitamin D3 doses until a level of at least 50 ng/ml (third visit) was achieved. The vitamin D3 dose was calculated based on the formula "change in serum 25(OH)D levels = 44.5 x vitamin D dose (in μg/kg/day)" as reported by Barger-Lux et al. [41]. The last study visit and subject follow-up was concluded by September 2015. Four patients in the vitamin D group and one patient in the placebo group were not analyzed during the third clamp due to loss of intravenous access during the study, inability to achieve target vitamin D levels, weight loss, and unsuccessful attempts to reach the patient.

2.1.1. Euglycemic stepped hyperinsulinemic pancreatic clamp studies

Euglycemic stepped hyperinsulinemic clamp studies were conducted in the vitamin D-deficient participants at baseline [25(OH)D level $<$ 20 ng/ml] and after repletion to two different 25(OH)D levels [level I $\simeq$ 30 ng/ml (second visit), and level II $\simeq$ 50 ng/ml (third visit)] (Figure 1B,C). Euglycemic stepped hyperinsulinemic clamp studies were also performed in the placebo group at baseline and two different time points based on the expected time necessary for vitamin D repletion, and monthly screening visits were conducted to check their vitamin D levels. On the day of the study, the participants presented to the Clinical Research Center (CRC) at 8:00 a.m. after an overnight fast. Two intravenous cannulas were established, one for infusions and another for blood sampling in a dorsal vein of the opposite arm. A primed continuous infusion of [6,6-2H2] glucose (bolus 200 mg/m^2 for 3 min) was started at $t = 0$ min, followed by continuous infusion of 2 $mg/min/m^2$ for the entire study. From 0 to 120 min, basal insulin infusion rates were maintained at 15 $mU/m^2/min$. From 120 to 240 min, insulin infusion rates were increased to 30 $mU/m^2/min$ to mimic physiologic hyperinsulinemia (the clamp's "low-insulin" step),

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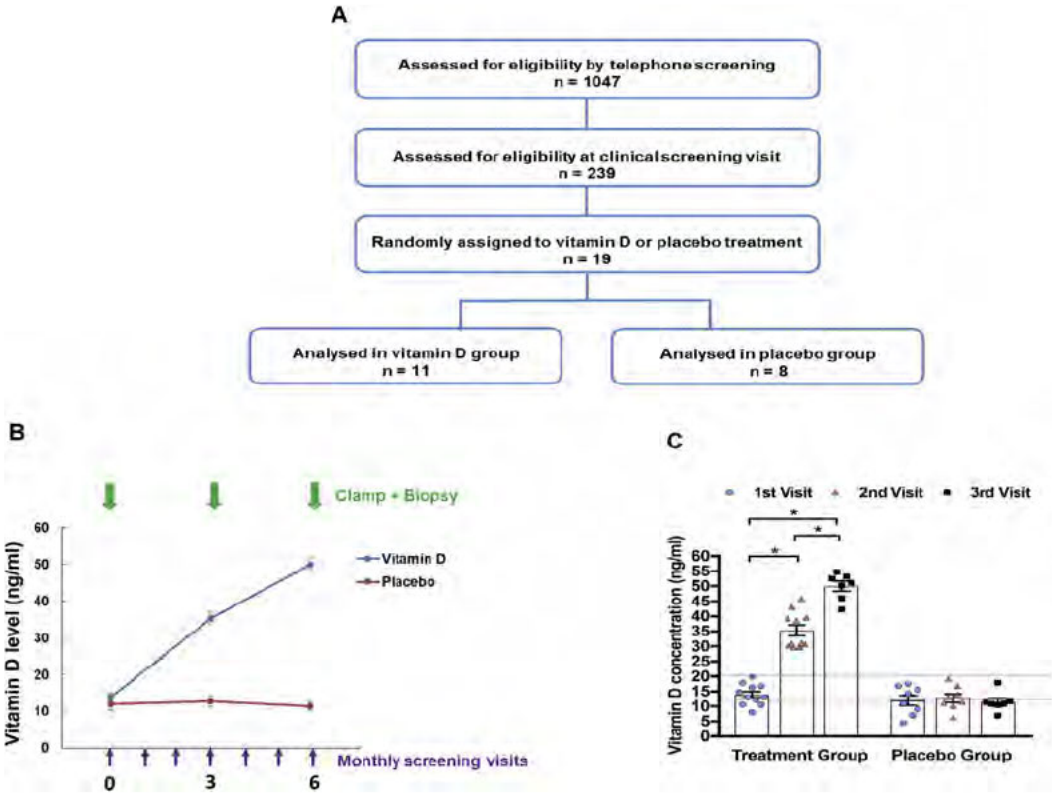


Figure 1: Identification and treatment of humans with vitamin D deficiency and insulin resistance. (A) Recruitment process. (B) Schematic Vitamin D repletion protocol for both vitamin D and placebo groups. (C) Vitamin D concentrations at the three clamp study visits in both groups.

and insulin infusion rates were then further increased to 80 mU/m^2/min for 240–360 min (the clamp's "high-insulin" step) as previously described [9,42]. Fixed levels of glucoregulatory hormones were maintained throughout the 360 min clamp studies using somatostatin infusion (250 μg/h) and replacement of glucagon (0.6 ng/kg/min) and growth hormone (3 ng/kg/min). Euglycemia (~90 mg/dl) was maintained by a variable infusion of 20% dextrose (the specific activity of infused dextrose was maintained equivalent to the plasma glucose-specific activity by adding D-[6,6-2H2] glucose to the infusate), and plasma glucose levels were measured every 5 min for the study duration. Blood samples were obtained at hourly intervals to determine the plasma levels of insulin, C-peptide, glucagon, cortisol, lactate, free fatty acids (FFA), and glycerol. Samples for D-[6,6-2H2] glucose determinations were obtained every 15 min during the steady-state periods (t = 180–240 and 300–360 min). At the end of the study, all of the infusions except dextrose were stopped at t = 360 min. To avoid hypoglycemia, dextrose infusion was continued for ~30 min after the completion of the study. The participants were given a standard meal and had their plasma glucose concentrations checked for an additional ~1 h before being discharged home.

2.1.2. Analytical procedures

2.1.2.1. Plasma hormones and substrates. Plasma glucose levels were measured using a Beckman glucose analyzer (glucose oxidase method; Fullerton, CA, USA). Plasma insulin, C-peptide, glucagon, and cortisol concentrations were measured by radioimmunoassay at the Diabetes Research Center Hormone Assay Core as previously reported [42]. Plasma lactate, free fatty acids, and glycerol were measured using spectrophotometric techniques.

2.1.2.2. Glucose turnover. 6,6-2H2 glucose concentrations were measured by gas chromatography mass spectrometry (GCMS) as previously described [43]. Rates of glucose appearance (R_a) and glucose disappearance (R_d, or glucose uptake) were calculated using Steele's steady–state equation [44]. Rates of endogenous glucose production (EGP) were determined by subtracting the rates of glucose infusion from the tracer-determined R_a. Data for glucose turnover, plasma hormones, and substrate concentrations represent the mean values during the final 60 min of the clamp's low-insulin phase (t = 180–240 min) and the final 60 min of the clamp's high-insulin phase (t = 300–360 min).

2.1.2.3. Adipose tissue studies. At t = 60 min of the clamp studies, adipose tissue biopsies were obtained from the periumbilical region. A small 0.25-cm periumbilical cutaneous incision was made under local anesthesia (1% lidocaine), and 1–2 g of adipose tissue was obtained by aspiration [45]. Biopsy specimens (~300 mg) were homogenized immediately in TRIzol reagent (Invitrogen Technologies) on ice at the bedside to inhibit any RNAase activity and subsequently stored at −80 °C for future real-time RT-PCR analysis.

2.1.2.4. Adipose tissue separation. Adipose tissue samples from all the participants were immediately washed at least three times with saline. The samples were then digested with collagenase type 1

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(0.05 g per 30 mL of Hanks Balanced Salt solution with 4% BSA; Worthington Biochemical) for 30 min at 37 °C with intermittent shaking, followed by extensive washing with Dulbecco's phosphate-buffered solution depleted of magnesium and calcium (Mediatech). The adipocytes were separated from the stromal-vascular fraction (SVF) by centrifugation at 3,000 rpm for 10 min. Macrophages were isolated from the SVF using CD14+ antibody-coated magnetic Dynabeads (Dynal Biotech) following the manufacturer's protocol. The adipose tissue samples and macrophages were washed with PBS, stored in TRIzol, and analyzed by real-time RT-PCR.

2.1.2.5. Quantitative real-time RT-PCR. Total RNA was extracted from the adipose tissue samples and macrophages following the protocol of a Qiagen RNeasy Mini kit (Cat# 74104). A SuperScript III First-Strand Synthesis System (Life Technologies, Cat# 18080051) was used to synthesize first-strand cDNA, the real-time RT-PCR template. As previously described [15], to verify that the relative expression value of the housekeeping gene provided an accurate reflection of cDNA loading, we first correlated the relative expression values of three housekeeping genes [glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and ribosomal proteins 19 (RPL19) and 18S], B2M, and beta-actin with each other and the geometric mean of the three values. Pearson's correlation coefficients were consistently $\geq$0.92, indicating that the expression of the five genes provided a reasonably accurate reflection of cDNA loading. GAPDH was chosen as the housekeeping gene to be included in every RT-PCR reaction because it was consistently expressed among the various cell types and study conditions [15]. Gene expression of inflammatory factors (*TNF* = tumor necrosis factor alpha, IL-6 = interleukin-6, iNOS = inducible nitric, and *PAI-1* = plasminogen activator inhibitor-1), pro-fibrotic factors (*TGFB1* = transforming growth factor beta 1, *HIF1A* = hypoxia-inducible factor-1 alpha, *COL1,5,6* = collagen I, V, and VI, respectively, *MMP7* = matrix metalloproteinase-7, and *TSP1* = thrombospondin 1), and VDR target genes in the adipose tissue samples (DUSP10 = dual-specific phosphatase 10, TRAK1 = trafficking protein, kinesin binding 1, NRIP1 = nuclear receptor interacting protein 1, and THBD = thrombomodulin) were determined by quantitative real-time RT-PCR using the specific protocol for LightCycler (Roche Diagnostics, Indianapolis, IN, USA) as previously described [42]. Primers used for the VDR target genes [46] and pro-fibrotic genes were as follows: (DUSP10: forward GAGGCTTTTGAGTTCATTGAG, reverse ATCCGAGTGTGCTTCATC; TRAK1: forward AGATCACACACCTGCTATCGC, reverse GTATTGGGCATGGTTTTGTTCC; NRIP1: forward TGGAATGCAGTCATCCATGT, reverse CTGGTTCAGGACCTGTTGGT; THBD: forward GACCTTCCTCAATGCCAGTC, reverse GCCGTCGCCGTTCAGTAG; TSP-1: forward TCAGGACCCATCTATGATAAAACCTA, reverse TCAGGTCAGAGAAGAAGAACACATTTC; MMP-7: forward AGCCAAACTCAAGGAGATGC, reverse ACTCCACATCTGGGCTTCTG; collagen 6a: forward GACCTCGGACCTGTTGGGT, reverse TACCCCATCTCCCCCTTCAC; collagen 5a: forward GATTGAGCAGATGAAACGG, reverse CCTTGGTTAGGATCGACCC; collagen 1a: forward TACAGCGTCACTGTCGATG, reverse TCAATCACTGTCTTGCCCCAG; HIF1A: forward TGCATCTCCATCTCCTACCC, reverse CGTTAGGGCTTCTTGGATA; and TGF-b1: forward AACCGGCCTTTCCTGCTTCTCA, reverse CGCCCGGGTTATGCTGGTTGTA). Reaction conditions were as follows: 40 cycles of denaturation at 95 °C for 2 s, annealing at 60 °C for 5 s, and elongation at 74 °C for 12 s and 82 °C for 3 s. Results are expressed as the relative change by determining the ratio of the genes of interest a given individual 2 and 5 h after IL infusion vs SAL infusion corrected for the relative expression of GAPDH in the same pair of samples. To calculate the mRNA copy number, we generated standard curves for GAPDH and the genes of interest using plasmid standards with known concentrations. Melting curves were used to determine the specificity of the gene products, which were subsequently confirmed by running the PCR products on agarose gels. All the reactions were performed at least three times [15].

2.1.2.6. Endotrophin immunofluorescence staining. Adipose tissue was prepared for immunofluorescence staining in the following manner. Formalin-fixed, paraffin-embedded adipose tissue samples were cut into 5 μm sections, deparaffinized in xylene, and rehydrated through a series of decreasing concentrations of ethanol. After regular antigen retrieval, the sections were incubated with rabbit polyclonal anti-endotrophin antibody (kindly provided by Professor Philipp Scherer, UT Southwestern Medical Center) [47] diluted at 1:500 (v/v) for 1 h at room temperature. After incubation with primary antibodies, the sections were repeatedly rinsed with PBS and incubated for 1 h at room temperature with Alexa Fluor 568 donkey anti-rabbit IgG (Molecular Probes, Cat# A10042, dilution 1:250 (v/v)) as the secondary antibody. All of the antibodies were diluted in Dako Ready-to-use Antibody Diluent (Cat# S080981-2). Incubation steps were carried out with Dako Auto Stainer Plus. Negative control slides were run in parallel with positive slides by applying rabbit IgG isotype control antibody (Cat# 02-6120) diluted at the same protein concentration as the antibody. The sections were sealed with ProLong Gold Antifade Mountant (Cat# 36935) with 4' 6-diamidino-2-phenylindole (DAPI).
The slides were digitally scanned on a 3DHISTECH Panoramic 250 Whole Slide Scanner (3DHISTECH, Budapest, Hungary). They were taken with a multi-color LED source Lumencor Spectra with a 20X/0.8 air objective. A low-noise high-speed 5.5 megapixel scientific CMOS camera (PCO, edge 4.2 m, PCO Inc.) was used for image acquisition. The exposure time of each channel was selected to avoid saturation and maintained constant for all of the slides. The acquisition software Panoramic Scanner automatically stitched the individual field of view of the tissues, creating one image. The HistoQuant module from QuantCenter software (3DHISTECH, Budapest, Hungary) was used for the analysis. The criteria for segmentation of positive signals were extensively tested and selected based on user inspection. After the criteria were established, the analysis parameters were maintained constant for all of the slides.

2.1.2.7. Collagen VI immunofluorescence staining. Adipose tissue was prepared for immunofluorescence staining in the following manner. Formalin-fixed, paraffin-embedded adipose tissue samples were cut into 5 μm sections, deparaffinized in xylene, and rehydrated through a series of decreasing concentrations of ethanol. The sections were pretreated with Dako Cytomation Target Retrieval Solution pH 9.0 buffer (Cat# S236884-2). The sections were incubated with rabbit polyclonal anti-collagen VI antibody (Abcam, Cat# 6588, dilution 1:250 (v/v)) for 1 h at room temperature. After incubation with primary antibodies, the sections were repeatedly rinsed with PBS and incubated for 1 h at room temperature with Alexa Fluor 568 donkey anti-rabbit IgG (Molecular Probes, Cat# A10042, dilution 1:250 (v/v)) as the secondary antibody. All the antibodies were diluted in Dako Ready-to-use Antibody Diluent (Cat# S080981-2). The incubation steps were carried out with Dako Auto Stainer Plus. Negative control slides were run in parallel with positive slides by applying rabbit IgG isotype control antibody (Cat# 02-6120) diluted at the same protein concentration as the antibody. The sections were sealed with ProLong Gold Antifade

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Mountant (Cat# 36935) with 4' 6-diamidino-2-phenylindole (DAPI). Subsequent scanning, imaging, and analysis of the slides were performed as previously described.

2.1.2.8. Hydroxyproline assay. A hydroxyproline kit (Catalog #K226-100, BioVision, Milpitas, CA, USA) was used to measure the hydroxyproline levels in the human adipose tissue following the protocol provided by BioVision. The plates were read using an Enspire Multilabel Reader from the Einstein Biomarker Analytic Research Core Facility. Then 100 μl of distilled H_2O was added for every 10 mg of tissue, which was homogenized with an ultrasonic probe homogenizer. Then 100 μl of 10 N concentrated NaOH was added to 100 μl of the sample, which was heated at 120 °C for 1 h. Following alkaline hydrolysis, the vials were placed on ice to cool briefly, then 100 μl of 10 N concentrated HCl was added to neutralize the residual NaOH. The vials were vortexed and centrifuged at 10,000×*g* for 5 min to pellet any insoluble debris following hydrolysis. The samples were placed on a heating plate at 65 °C for evaporation, then chloramine-T concentrate was added to the wells and they were incubated at room temperature for 20 min. Then 50 μl of developer solution was added to each well and incubated at 37 °C for 5 min, and 50 μl of DMAB concentrate solution was added to each well and incubated at 65 °C for 45 min. The absorbance of all the samples was measured at 560 nm (OD_{560}) in endpoint mode. The plates were read using the Enspire Multilabel Reader from the Einstein Biomarker Analytic Research Core Facility.

2.2. Experimental design: adipose VDRKO mice

Adipocyte-specific VDR knockout (Ad-VDRKO) mice were generated by breeding mice with the VDR gene flanked by loxP sites (gift of David Gardner, UCSF) with mice expressing the Cre recombinase under the control of the adiponectin promoter [48]. The resulting Adipoq-Cre:$Vdr^{fl/fl}$ mice were intercrossed to generate mice with loxP-flanked VDR alleles with and without Cre recombinase, and the latter served as littermate controls. The mice were maintained on a C57BL/6 background and housed in a pathogen-free facility with a 12 h light—dark cycle and free access to water and irradiated rodent chow.

Seven wild-type (WT) and six adipose-specific vitamin D receptor (VDR) knockout (KO) mice were fed a high-fat diet (HFD) (58 kcal% fat w/ sucrose, D12331; Research Diets, New Brunswick, NJ, USA) for 12 weeks from 6 weeks of age. The mice were housed in a temperature- and humidity-controlled environment (light cycle 6:00 a.m. to 6:00 p.m.). All of the procedures were approved by the Beth Israel Deaconess Medical Center and Vanderbilt University Institutional Animal Care and Use Committee. Body composition was determined using ECHO-MRI.

2.2.1. Hyperinsulinemic-euglycemic clamps (mice)

One week before performing the hyperinsulinemic-euglycemic clamps, carotid artery and jugular vein catheters were surgically placed for sampling and infusions. The mice were fasted for 5 h and then clamped as previously described [49]. The clamp was initiated at $t = 0$ min with a continuous insulin infusion (4 mU/kg/min) for 2 h. Primed continuous infusions of [3-^{3}H]glucose (1.5 μ Ci bolus, 50 n Ci/min infusion) were administered to assess glucose uptake and production. [3-^{3}H]glucose (0.06 μ Ci/μL) was added to the glucose infusate to clamp both arterial glucose and glucose-specific activity. At 120 min, a 5 μ Ci bolus of ^{14}C-2-deoxyglucose was administered to assess the tissue-specific glucose metabolic rate (Rg). Arterial glucose levels were monitored every 10 min to provide feedback for adjusting the glucose infusion rate (GIR) (50% dextrose + [3-^{3}H]glucose). Erythrocytes were replaced to prevent a decline in hematocrits with repeated blood sampling. [3-^{3}H]glucose kinetics were determined at −10 min and from 80 to 120 min. Blood was collected at 2, 5, 15, 25, and 35 min after injection to measure the disappearance of [14C]2-DG from the plasma. At t = 155 min, the mice were anesthetized with pentobarbital and the tissues were freeze-clamped for subsequent analyses. Whole-body glucose appearance (Ra) and endogenous glucose production (EGP), a measure of hepatic glucose production, were calculated as described using non-steady-state equations [49]. Radioactivity of [3-^{3}H]glucose, [14C]2DG, and [14C]2DG-6-phosphate were determined by liquid scintillation counting. EGP was determined by subtracting the GIR from the total Ra. The glucose metabolic index (Rg) was calculated as previously described [50]. Plasma insulin was determined by ELISA. Plasma non-esterified fatty acids (FFAs) and tissue triglycerides were measured spectrophotometrically.

2.2.2. Hydroxyproline assay in rodents

Tissue hydroxyproline content was measured by assessing 4-hydroxyproline levels using a Hydroxyproline Calorimetric Assay kit from BioVision (Cat# K555-100, BioVision). One hundred milligrams of epididymal WATs were homogenized in distilled water and then mixed with 12 N of HCl at 120 °C for 3 h. After centrifugation at 10,000×*g* for 3 min, the supernatants were dried, incubated with chloramine-T at room temperature for 5 min, and then incubated with p-dimethylaminobenzaldehyde (DMAB) for 90 min at 60 °C. The absorbance was measured at 560 nm using a microplate reader (SpectraMax i3, Molecular Devices) as suggested by the manufacturer. Hydroxyproline content was normalized to the total protein measured by a BCA Assay (Cat# 23227, Pierce BCA Protein Assay kit, Thermo Fisher Scientific).

2.3. Statistical analysis

Several glucose metabolism parameters were measured in each subject, with the primary endpoint being the percent change in endogenous glucose production (EGP) during the final hour of the clamp's "low-insulin" (30 mU/m^2/min) step as a measure of the insulin-sensitizing effects of vitamin D. Peripheral glucose uptake (Rd) was examined as a secondary endpoint. The results are expressed as mean ± standard error of the mean. The data were tested for normal distribution using the Shapiro—Wilk test. Differences between the study groups (vitamin D and placebo groups) were assessed using a two-sample t-test. Paired Student's t-tests were used to compare the baseline and endpoint measures within the groups. For the mouse clamps, Student's t-tests or two-way ANOVA followed by Tukey's post hoc tests were conducted. All the human and rodent data are presented as mean ± standard error of the mean. $P < 0.05$ was considered significant for all of the analyses. Statistical analysis of the data over time was performed using the Statistical Package for the Social Sciences 11.5 software (SPSS Inc., Chicago, IL, USA).

2.4. Sample size

Our main endpoint measurement was the percent change in endogenous glucose production (EGP) during the final hour of the clamp's low-insulin phase. The null hypothesis was that the percent change in EGP would be the same in both groups (vitamin D and placebo). The EGP rates were 1.64 ± 0.6 and 1.03 ± 0.5 mg/kg/min at baseline and following vitamin D repletion, respectively, in our preliminary studies. The sample size calculation was based on a 2-sided, paired t-test assuming a mean difference in EGP of 0.61, a standard deviation of 0.5, and no change with placebo. This resulted in a sample size of 8 per group to reach a power of 80%.

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3. RESULTS

3.1. Identification and treatment of humans with vitamin D deficiency and insulin resistance

Following a recruitment process, 19 obese non-diabetic, insulin-resistant (HOMA-IR $>$ 3) subjects with 25(OH)D levels $<$ 20 ng/ml were enrolled in the study (Figure 1A). Of these, 11 subjects were randomized to the treatment group (vitamin D) and eight received placebo in a double-blinded study design (Figure 1B). The baseline subject characteristics, including age, weight, height, body mass index (BMI), fasting plasma glucose and insulin levels, HOMA-IR, and 25(OH)D levels were similar in the two groups at the first (baseline) visit (Supplementary Table 1). In the group that received vitamin D treatment, the average weekly vitamin D dose was 38,107 $\pm$ 3,185 IU. Vitamin D repletion significantly increased serum 25(OH)D levels, successfully attaining target levels within $\sim$3 months for the second visit and $\sim$6 months for the third visit (Figure 1C). All the participants in both study groups were initially vitamin D deficient. Serum 25(OH)D levels remained consistently reduced over the same six-month period in the placebo group (Figure 1C).

3.2. Vitamin D improves hepatic insulin sensitivity in vitamin D-deficient insulin-resistant humans

To determine whether vitamin D repletion would improve insulin action in insulin-resistant individuals, a comprehensive study of whole-body insulin action was undertaken with stepped euglycemic ($\sim$90 mg/dL) hyperinsulinemic clamp studies (Figure 2A) both before and after the administration of vitamin D or placebo (Figure 1B). Specifically, a low-insulin phase ($\sim$60 uU/ml) was used to optimally study hepatic insulin sensitivity, while peripheral glucose uptake was maximized during a high-insulin phase ($\sim$400 uU/ml). Steady-state dynamics were attained during these two phases in all of the studies as demonstrated by comparable atom percent excess (APE) during these phases of the vitamin D (Figure 2B) and placebo studies (Figure 2C) and by comparable glucose levels during these two phases for both the vitamin D (Figure 2D) and placebo studies (Figure 2E). There was a trend toward a small increase in GIR in the treatment group, consistent with the decrease in EGP (Supplementary Fig. 1A). Given the high variability, this did not achieve significance. There were no changes in the placebo group (Supplementary Fig. 1B and C).

Comparable plasma levels of insulin (Figure 2F), C-peptide, glucagon, cortisol, lactate, free fatty acids, and glycerol were attained at the three phases of the euglycemic stepped hyperinsulinemic clamp studies: basal, during the "low-insulin" phase (120–240 min), and during the "high-insulin" phase (240–360 min) (Supplementary Table 2A and B). Repletion of vitamin D levels to the therapeutic range resulted in a significant improvement in hepatic insulin sensitivity. Endogenous glucose production (EGP) during the clamp studies' low-insulin phase decreased at both the second and third visits compared to the first visit baseline, with no significant difference in EGP between the second and

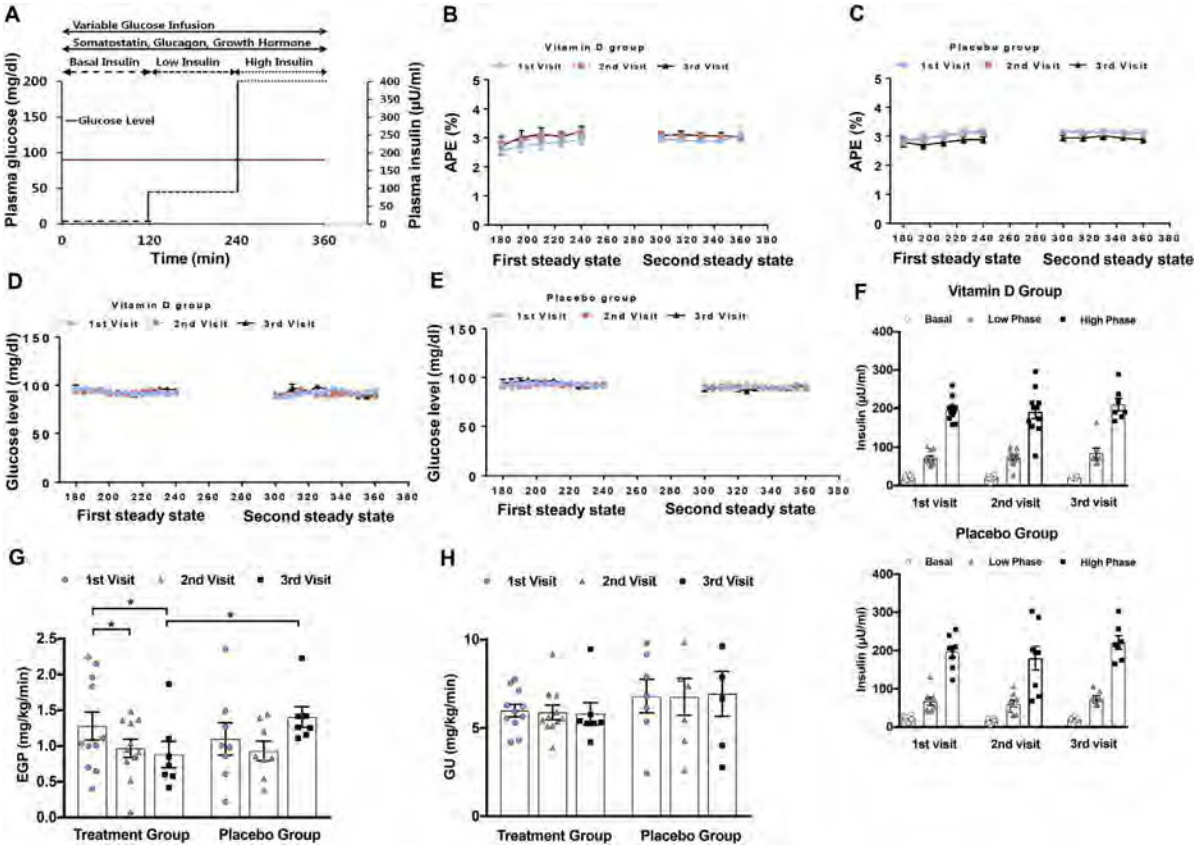


Figure 2: Clamp studies in humans. (A) Stepped hyperinsulinemic euglycemic clamp protocol. (B) Average atom percent excess (APE) during two steady states for vitamin D group and (C) placebo group per each visit. (D) Average glucose level during two steady states for vitamin D group and (E) placebo group per each visit. (F) Insulin levels during each clamp per each visit for vitamin D and placebo group. (G) Endogenous glucose production (EGP) and (H) glucose uptake (GU) during the three clamp studies. Data are represented as means $\pm$ SEM. *p $<$ 0.05; * = significantly different.

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third visits (Figure 2G). Intriguingly, in the placebo group, there were upward trends in EGP from the baseline (first visit) to the third visits (Figure 2G), such that EGP was 37% higher following 6 months of placebo administration than with optimal vitamin D replacement ($p = 0.04$). However, neither level of vitamin D repletion affected peripheral glucose uptake (GU) (Figure 2H). Taken together, these findings may suggest that vitamin D deficiency over a longer period of time could be associated with worsening hepatic insulin resistance, and that this hepatic insulin resistance can be improved by vitamin D repletion.

3.3. The expression of pro-inflammatory genes (whole fat and adipose tissue macrophages) is reduced after vitamin D repletion

We next investigated the effects of vitamin D administration on adipose tissue inflammation. The expression of the pro-inflammatory genes *TNF*, *IL-6*, *iNOS*, and *PAI-1* was significantly reduced in the peri-umbilical subcutaneous adipose tissue following vitamin D repletion into the normal range (Figure 3A). Interestingly, the expression of the same pro-inflammatory factors in the adipose tissue macrophages also decreased to a similar degree (Figure 3B). Only a few pro-inflammatory gene markers in either whole fat or adipose tissue macrophages were significantly reduced when the vitamin D levels increased from the second to third visits (Figure 3A,B). In the placebo group, no significant differences were observed from the first (baseline) visit, although there was a trend toward the elevated expression of some pro-inflammatory genes at the end of the 6-month study period (Figure 3C,D). These results suggest that vitamin D administration has significant modulatory effects on the gene expression of inflammatory proteins within adipose tissue.

3.4. Vitamin D repletion reduces the expression of pro-fibrotic genes in adipose tissue

Given the metabolic importance of adipose fibrosis, the gene expression of various pro-fibrotic factors was examined to determine the effects of vitamin D on adipose tissue fibrosis. After vitamin D repletion to ~30 ng/ml, the expression of the pro-fibrotic genes *TGFB1*, *HIF1A*, *COL1,5,6* (collagen I, V, and VI), and *MMP7* decreased in the whole fat (Figure 4A). Vitamin D repletion to ~50 ng/ml was associated with reduced gene expression of only some of these pro-fibrotic factors (Figure 4A). In the placebo group, no significant differences were observed except for *HIF1A* from the first to second visits, although there was a trend toward worsening adipose tissue fibrosis at the end of the 6-month study period (Figure 4B). These results suggest that vitamin D repletion has modulatory effects on the expression of pro-fibrotic genes in adipose tissue.

As a quantitative indicator of the overall collagen tissue content, we assayed the hydroxyproline content of whole adipose tissue and found that it significantly decreased after vitamin D repletion [51] (Figure 4C,D). To further determine the effect of vitamin D repletion on adipose tissue fibrosis, we assessed immunofluorescence staining for endotrophin and collagen VI. Endotrophin is a cleavage product of collagen VI in adipose tissue that is involved in fibrosis and

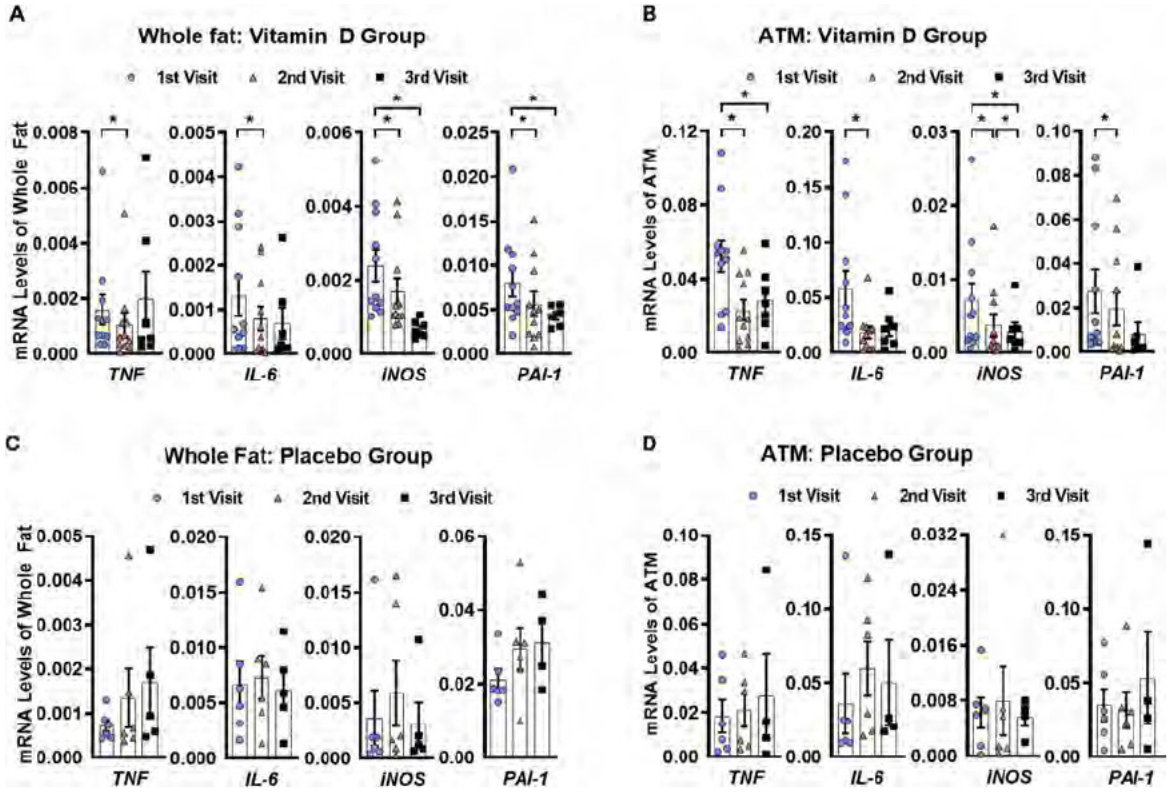


Figure 3: Adipose tissue gene expression of pro-inflammatory markers in humans. (A) Fold decreases in gene expression of various pro-inflammatory markers in whole fat and (B) adipose tissue macrophages for vitamin D group. (C) Fold decreases in gene expression of various pro-inflammatory markers in whole fat and (D) adipose tissue macrophages for placebo group. Data are represented as means ± SEM. *$p < 0.05$; * significantly different. *TNF* tumor necrosis factor alpha, *IL-6* interleukin-6, *iNOS* inducible nitric, *PAI-1* plasminogen activator inhibitor-1.

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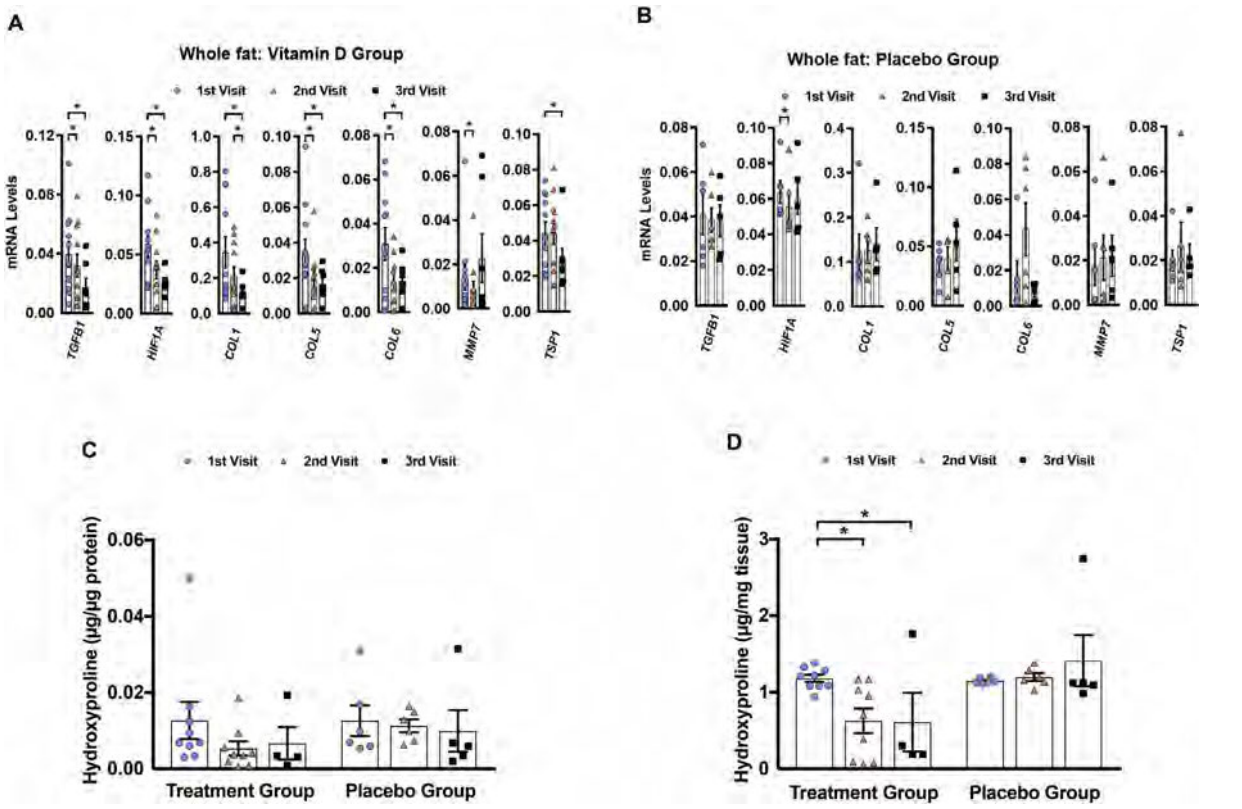


Figure 4: Adipose tissue gene expression of pro-fibrotic markers in humans. (A) Fold decreases in gene expression of various pro-fibrotic markers in whole fat samples from vitamin D group and (B) placebo group. (C) Adipose tissue hydroxyproline concentration in µg per µg of protein and in (D) µg per mg of tissue. Data are represented as means ± SEM. *p < 0.05; * = significantly different. *TGFB1* = transforming growth factor beta 1, *HIF1A* = hypoxia-inducible factor-1 alpha, *COL1,5,6* = *Collagen I, V, VI respectively*, *MMP7* = matrix metalloproteinase-7, *TSP1* = thrombospondin1.

inflammation; human studies revealed a strong upregulation of endotrophin in the adipose tissue of diabetic human patients [52–54]. Endotrophin intensity was found to be significantly decreased by approximately 50% in the vitamin D group at the second visit with no further decrease at the third visit, whereas in the placebo group, there was no significant difference between the three visits (Figure 5A,B). In the vitamin D group, there was also a significant reduction in collagen VI immunofluorescence by 19% from the first (baseline) visit to the second visit, with no further reduction from the second to third visits (Figure 5C,D), consistent with the change in gene expression. In the placebo group, collagen VI immunofluorescence did not significantly change throughout the study period, although there was a trend toward worsening of adipose tissue fibrosis at the third visit compared to the baseline when the subjects remained vitamin D deficient for 6 months (Figure 5C,D).

3.5. Expression profiling of primary vitamin D receptor target genes

We examined the expression of four primary VDR target genes in the adipose tissue samples: nuclear receptor interacting protein 1 (NRIP1), thrombomodulin (THBD), dual-specific phosphatase 10 (DUSP10), and trafficking protein kinesin binding 1 (TRAK1) as representative examples. NRIP1, THBD, and DUSP10 gene expression significantly increased from the first to second visits ($p = 0.003$, $p = 0.03$, and $p = 0.04$, respectively) (Supplementary Fig. 2). There was also a trend toward the increased expression of TRAK1 from the first to the second visits ($p = 0.06$) (Supplementary Fig. 2). These findings are consistent with the results of a study in which vitamin D was repleted over a similar time course, indicating that these genes can serve as biomarkers for vitamin D3 response in adipose tissue of human subjects [46].

3.6. Generation and characterization of an adipocyte-specific vitamin D receptor-deficient mouse model (Ad-VDRKO mice)

Since adipocytes express the vitamin D receptor (*Vdr*) and may modulate adipose tissue inflammation and fibrosis [55,56], we hypothesized that the observed actions of vitamin D were mediated through the VDR in adipocytes. Therefore, we next examined the impact of adipose-specific vitamin D receptor knockout on hepatic insulin resistance and adipose tissue inflammation and fibrosis. Confirming the efficiency of adipocyte-specific VDR deletion, *Vdr* mRNA levels decreased by approximately 81% in the epididymal white adipose tissue (eWAT) and 84% in the inguinal white adipose tissue (iWAT) of the Ad-VDRKO compared with WT control mice (Figure 6A) and trended lower in the brown adipose tissue ($p = 0.067$). In contrast, *Vdr* expression was not reduced in the kidney, intestine, heart, or liver (Figure 6A). Following 12 weeks of high-fat diet (HFD) feeding, there were no differences in body weight, percentage lean mass, or adiposity between the wild-type (WT) and adipose-specific vitamin D receptor (VDR) knockout (KO) mice (Figure 6B,C, and D). The WT and Ad-VDRKO mice also did not differ with respect to fasting plasma triglycerides (Figure 6E).

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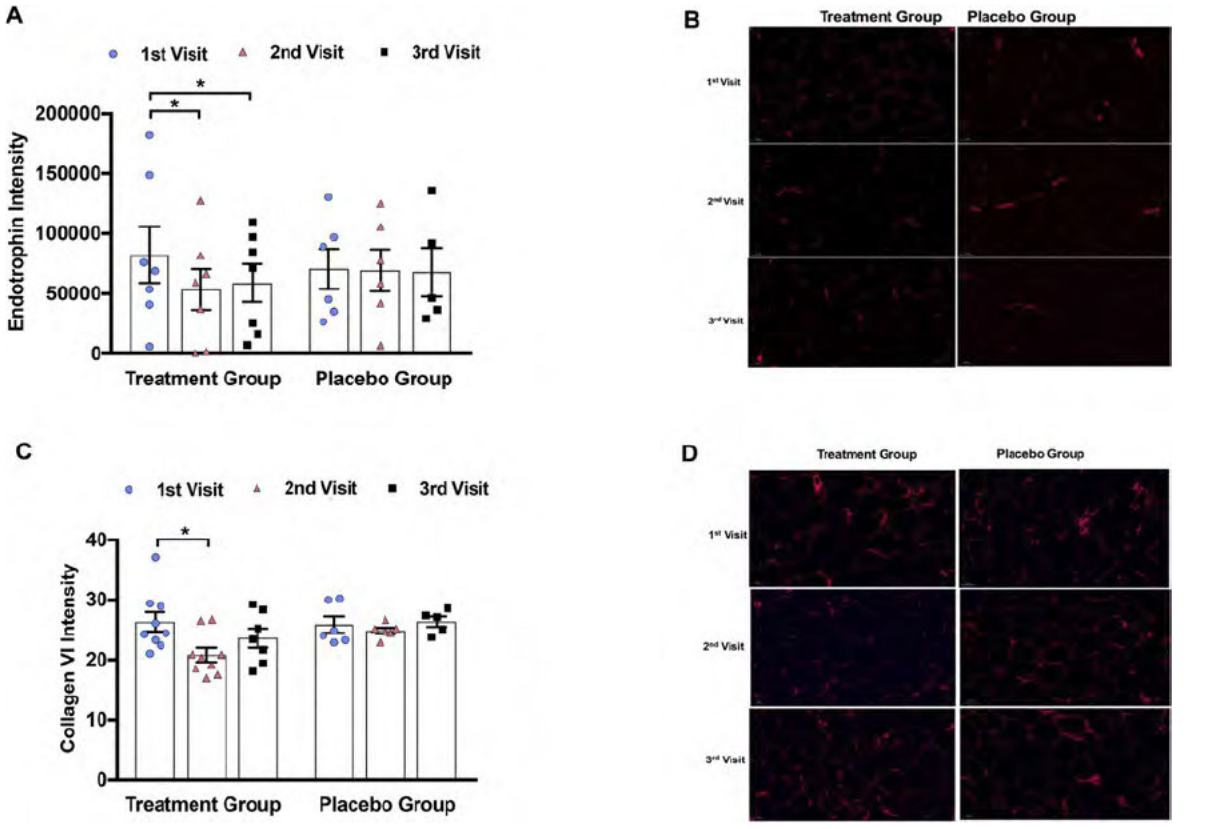


Figure 5: Adipose tissue immunofluorescence staining in humans. (A) Endotrophin immunofluorescence relative to baseline at each clamp visit in both vitamin D treatment group and placebo group. (B) Endotrophin immunofluorescence: representative images at each clamp visit in both vitamin D treatment group and placebo group. (C) Collagen VI immunofluorescence relative to baseline at each clamp visit in both vitamin D treatment group and placebo group. (D) Collagen VI immunofluorescence in vitamin D and placebo group. Data are represented as means ± SEM. *$p < 0.05$; * significantly different from the first visit (1st visit).

3.7. Ad-VDRKO mice on a high-fat diet develop adipose tissue inflammation and fibrosis

We next determined whether and how Ad-VDRKO would impact adipose tissue inflammation and fibrosis. As expected, the expression of several pro-inflammatory genes, including *Tnf*, *iNOS*, *Serpine1* (*PAI-1*), *Mcp*-1 and *Adgre1* (*F4/80*) was significantly higher in adipose tissue of Ad-VDRKO mice compared to WT mice, with a trend toward higher expression of *Il6* in the former (Figure 6F). Additionally, the expression of pro-fibrotic genes *TGFB1*, *Col6A* (*collagen VI*), and *THBS1* (*TSP1*) in fat was significantly higher in the adipose tissue of the Ad-VDRKO mice compared to the WT mice, with an upward trend in collagen I in the former (Figure 6G). These data suggest that modulating the VDR within the adipocytes may regulate adipose tissue inflammation and fibrosis. As observed in the human studies, adipose tissue hydroxyproline content increased in the Ad-VDRKO mice compared to the WT mice [57] (Figure 6H,I).

3.8. Ad-VDRKO mice on a high-fat diet exhibit increased hepatic insulin resistance

The impact of adipocyte VDR ablation on systemic glucose metabolism was then examined. To delineate the contributions of individual tissues to insulin action in this model, hyperinsulinemic-euglycemic clamps were performed on WT and Ad-VDRKO mice. Euglycemia was maintained (~120 mg/dl) throughout the clamp studies, and the insulin concentrations increased to comparable levels in both groups. The WT and Ad-VDRKO mice did not differ with respect to fasting plasma insulin, FFA concentrations, and arterial glucose levels (Figure 7A,B, and C). Additionally, circulating FFAs were suppressed in all of the groups, suggesting equivalent insulin-induced suppression of lipolysis.

The glucose infusion rate (GIR) necessary to maintain euglycemia trended lower in the Ad-VDRKO mice for most of the time points during the clamp (Figure 7D), suggestive of insulin resistance. Endogenous glucose production (EGP) and rates of glucose disappearance (Rd) did not differ between the WT and Ad-VDRKO mice under basal conditions (Figure 7E,F). EGP was markedly higher in the Ad-VDRKO mice compared to the WT mice during the insulin clamp (Figure 7E). This indicates diminished suppression of EGP by insulin in the Ad-VDRKO mice, that is, increased hepatic insulin resistance. However, there was no difference in Rd between the WT and Ad-VDRKO mice under clamped conditions (Figure 7F).

In addition to hepatic insulin resistance, there were trends toward reduced skeletal muscle and adipose tissue glucose uptake (decreased Rg) in the Ad-VDRKO mice, with small but significant reductions in perigonadal adipose glucose uptake (Figure 7G). In contrast, tissue-specific Rg was no different between the Ad-VDRKO

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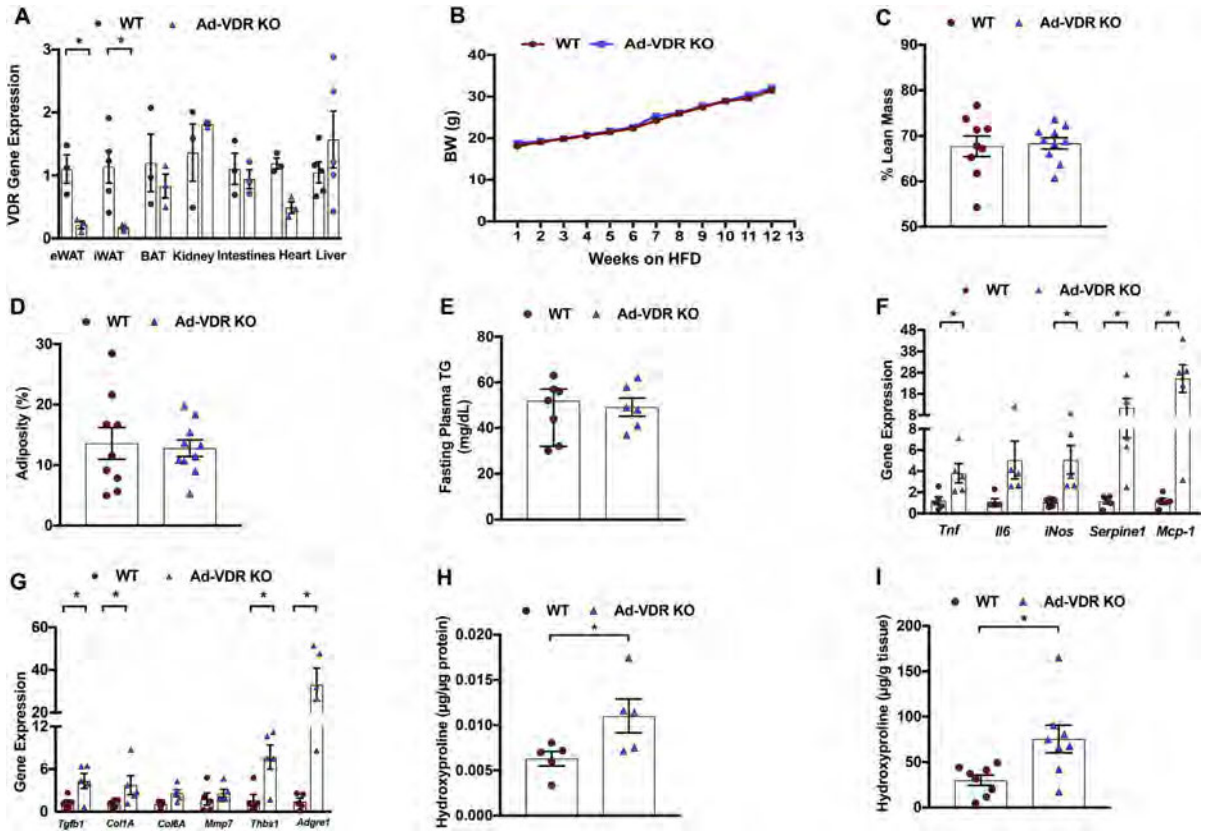


Figure 6: Adipose tissue gene expression of pro-inflammatory and pro-fibrotic markers in adipose-specific vitamin D receptor knockout mice. Seven wild type (WT) and six adipocyte-specific vitamin D receptor knockout (Ad-VDR KO) mice were fed a high-fat diet (HFD) for twelve weeks from 3 weeks of age. (A) VDR gene expression was significantly decreased in white adipose tissue (eWAT) and inguinal white adipose tissue (iWAT) of Ad-VDR KO compared to WT mice but VDR expression was not reduced in brown adipose tissue (BAT), kidney, intestines, heart or liver. (B) Body weight (BW) of WT and Ad-VDR KO mice fed with HFD for 12 weeks. (C) After 12 weeks on the HFD, percentage lean mass, and (D) adiposity did not differ between WT and Ad-VDR KO mice. (E) WT and Ad-VDR KO mice were not different with respect to basal fasting plasma triglycerides. (F) Adipose tissue expression of pro-inflammatory and (G) pro-fibrotic genes in WT and Ad-VDR KO mice. (H) Adipose tissue hydroxyproline concentration in µg per µg of protein tissue and in (I) µg per gram of tissue. *Tnf* = tumor necrosis factor alpha, *Il6* = interleukin-6, *iNos* = inducible nitric oxide synthase, *Serpine1* (*PAI-1*) = plasminogen activator inhibitor-1, *Mcp*-1 = monocyte chemoattractant protein-1. *Tgfb1* = transforming growth factor beta 1, *Col1A* = *Collagen I, Col6A* = *Collagen VI, Mmp7* = matrix metalloproteinase-7, *Thbs1* (*Tsp1*) = thrombospondin1, *Adgre1* = *F4/80*. Data are represented as means ± SEM. *p < 0.05.

and WT mice in gastrocnemius, vastus lateralis, subcutaneous adipose tissue, soleus, brown adipose tissue, heart tissue, and brain, suggesting that adipocyte VDR is not a critical determinant of insulin action in these tissues (Figure 7G,H). Taken together, these results indicate that vitamin D exerts effects on hepatic insulin sensitivity through its effects on adipose tissue.

4. DISCUSSION

Using a parallel design in rodents and humans, these studies are the first to comprehensively delineate the effects of vitamin D on systemic insulin sensitivity and adipose tissue inflammation and fibrosis. This prospective, randomized, double-blinded placebo-controlled study showed significant improvement in hepatic insulin sensitivity following vitamin D repletion in vitamin D-deficient, insulin-resistant humans in concert with reduced adipose tissue expression of pro-inflammatory and pro-fibrotic genes as well as direct adipose tissue markers of fibrosis. Further elevations of serum vitamin D levels above the normal target range were not associated with additional improvements. Of note, there was a worsening trend in hepatic insulin sensitivity and adipose tissue fibrosis and inflammation in the placebo group over the course of 6 months, suggesting that vitamin D deficiency over a long period of time could be associated with worsening hepatic insulin resistance. Importantly, adipocyte-specific VDRKO mice on a high-fat diet phenocopy the vitamin D deficient humans, exhibiting increased adipose tissue fibrosis, inflammation, and hepatic insulin resistance, suggesting that vitamin D modulates insulin sensitivity via specific effects on adipocytes.

The VDR is expressed in several insulin-responsive metabolic tissues, including the liver, skeletal muscle, and adipose tissue. Adipose tissue-derived inflammatory factors, which increase in proportion to the degree of adiposity, are believed to contribute to insulin resistance [6]. Adipocytes have been shown to initiate and/or sustain a pro-inflammatory response within adipose tissue [15,56]. This is consistent with the demonstrated regulatory role of the adipocytes, potentially through peroxisome proliferator-activated receptor-γ (PPAR-γ) to modulate adipose tissue inflammation and systemic insulin sensitivity [55]. It was recently determined that certain adipocyte progenitors modulate obesity-induced white adipose tissue fibrogenesis and are therefore associated with loss of metabolic fitness [58].

As inflammation impairs insulin sensitivity, it was likely that reduced adipose tissue inflammation and fibrosis observed in our studies

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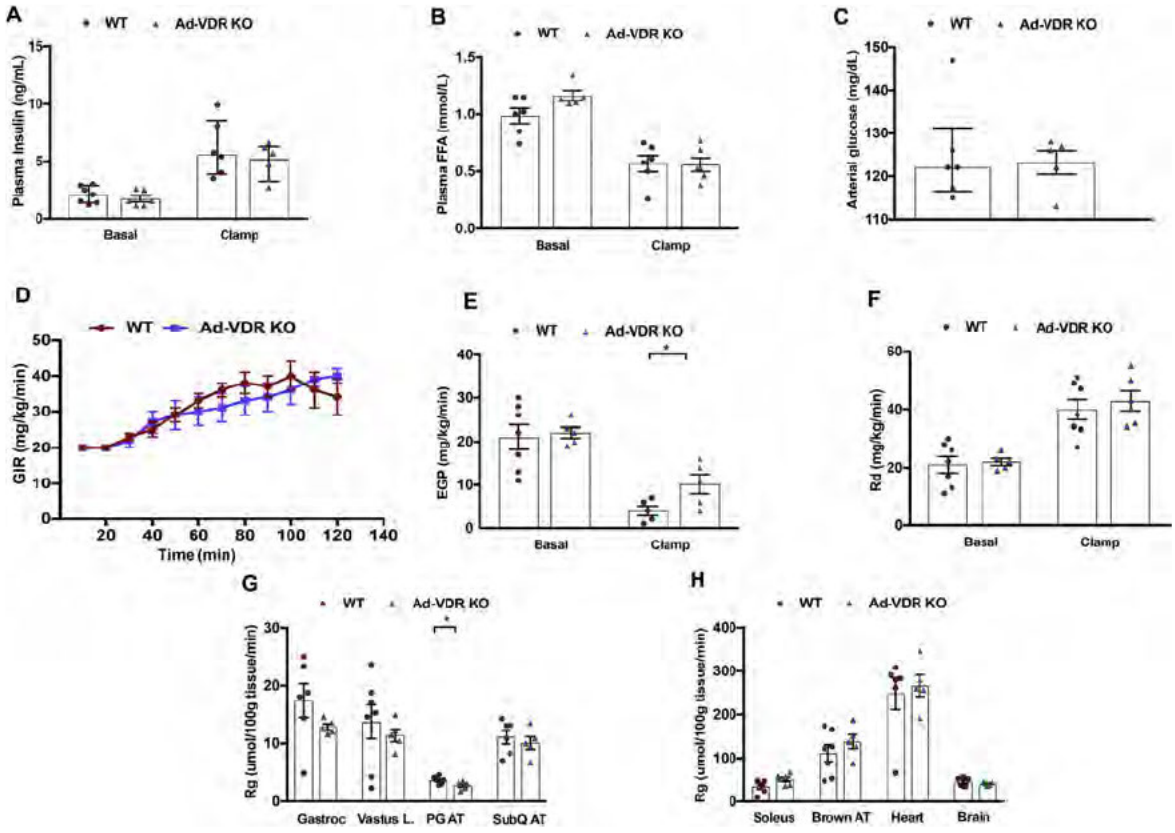


Figure 7: Clamp studies in mice. At 12 weeks of age wild type (WT) and adipocyte-specific vitamin D receptor knockout (Ad-VDR KO) mice underwent hyperinsulinemic-euglycemic clamps. Mice were fasted 5 h before the onset of the hyperinsulinemic-euglycemic clamps. WT and Ad-VDR KO mice were not different with respect to (A) basal insulin, and (B) basal plasma free fatty acids (FFA). (A) Insulin concentrations was raised to comparable levels in both WT and Ad-VDR KO mice during the clamps. (B) Circulating FFA were suppressed in all groups during the clamps. (C) Baseline arterial glucose levels. (D) The glucose infusion rates (GIR) in the venous catheter needed to maintain euglycemia in WT and Ad-VDR KO mice. (E) Endogenous glucose production (EGP), a marker of hepatic glucose production, in WT and Ad-VDR KO mice, determined by administration of [6-6 H] glucose during the steady-state period of the insulin clamp. (F) Whole-body disappearance (Rd) were determined during the steady-state period of the insulin clamp. (G, H) Tissue-specific 2-DG uptake (Rg) after the insulin clamp in the gastrocnemius (Gastroc), vastus lateralis (Vastus L.), paragonadal adipose tissue (PG AT), subcutaneous adipose tissue (SubQ AT), soleus, brown adipose tissue (Brown AT), heart tissue and brain in WT and Ad-VDR KO mice. Data are represented as means ± SEM. *$p < 0.05$.

contributed to improving hepatic insulin sensitivity. Consistent with the current research, there is a substantial body of rodent studies linking adipose tissue inflammation with insulin resistance, particularly of the liver [59]. Activation of hepatic inflammatory pathways involving IKK-β and NF-κB by adipocyte-derived cytokines has been shown to cause hepatic insulin resistance, suggesting a connection between adipose (particularly visceral) inflammation and hepatic insulin resistance [31,60].

Despite the improvements in adipose tissue inflammation and fibrosis in the current studies, peripheral insulin sensitivity measured by GU did not change. Of note, increasing adiposity does not appear to activate inflammatory cascades in skeletal muscle as it does in adipose tissue and the liver [59]. This may suggest why hepatic, but not peripheral, insulin sensitivity was associated with adipose tissue inflammation and fibrosis in both the human and rodent studies. Vitamin D deficiency is associated with increased circulating matrix metalloproteinase (MMP-2 and MMP-9) and C-reactive protein (CRP), which were shown in a few studies to be reduced by vitamin D supplementation [61–63]. Additionally, the active form of vitamin D [1,25$(OH)_2$D] may restrain macrophage cytokine production by suppressing the NF-κB inflammatory pathway [64]. Vitamin D is a fat-soluble vitamin that is stored in adipose tissue and therefore could exert important paracrine effects within this tissue [65,66]. Thus, these studies in adipocyte-specific VDRKO mice point to important effects of vitamin D on adipose tissue, with systemic metabolic sequelae.

Along with our observation of significant reductions in adipose tissue inflammation, normalizing vitamin D levels led to a significant reduction in adipose tissue fibrotic gene expression and collagen (by immunofluorescence), while the placebo group trended toward a worsening of these parameters over six months of continued vitamin D deficiency. Complementary findings of increased adipose tissue fibrosis markers were observed in the Ad-VDRKO mice. Pathological expansion of adipose tissue in obesity results in inflammation, fibrosis, and insulin resistance [67]. To the best of our knowledge, this is the first study establishing a connection between vitamin D deficiency and adipose tissue fibrosis and among the first to make a connection between adipose tissue fibrosis and insulin resistance in humans [12–14,68]. There is substantial evidence in rodents that adipose tissue fibrosis is linked to metabolic dysfunction, further supported by the current findings in Ad-VDRKO mice [13,14,69,70]. PPARγ agonists and adiponectin both reduce adipose tissue fibrosis, inflammation, and insulin resistance [4]. With the activation of vitamin D receptors and

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inhibition of the TGF-β/SMAD signaling pathway [3,35–37], it is likely that vitamin D repletion can prevent the development of adipose tissue fibrosis and offer additional metabolic benefits.

Discrepancies in the results of previous human studies investigating the relationship between vitamin D and insulin resistance highlight the need for carefully designed research with measurement of serum 25(OH)D levels before and after adequate dosing. This is particularly noteworthy given a recent clinical trial in which administering vitamin D to 2,423 pre-diabetic participants, the vast majority of whom were vitamin D replete, did not impact progression to T2D [21]. Of note, in those subjects with initial vitamin D levels <12 ng/ml, vitamin D repletion reduced the hazard ratio of developing T2D to 0.38 (CI: 0.18 to 0.80) [21]. The lack of benefit in the Pittas study from increasing mean vitamin D levels from 27.7 to 54.3 ng/ml is consistent with the lack of further benefit of raising vitamin D levels to ~50 ng/ml in the current study. Furthermore, there is a clear need for more sensitive metabolic measures [71–73] as used in the current research. Some studies have shown no effect of vitamin D supplementation on insulin sensitivity [74–77]. This includes a meta-analysis of prospective randomized clinical trials [77] employing HOMA-IR, which is recognized to have limitations as a longitudinal measure of insulin sensitivity [78]. Conversely, several studies showed positive associations between serum vitamin D levels and peripheral insulin sensitivity in both pre-diabetic and T2D subjects [79,80]. Four out of six cross-sectional studies using euglycemic stepped hyperinsulinemic clamps, considered the gold standard for measuring insulin sensitivity, showed positive associations between vitamin D levels and insulin sensitivity in non-diabetic subjects [81–86]. Prospective studies also revealed beneficial effects of vitamin D supplementation on insulin sensitivity [87,88], including a meta-analysis of prospective cohort studies [17,89,90]. Intriguingly, prospective studies comparing euglycemic-hyperinsulinemic clamps at baseline and after 12 and 24 weeks of vitamin D supplementation noted improved insulin sensitivity in obese insulin-resistant subjects [91,92] but not in T2D subjects [74], suggesting that the established metabolic defects of T2D are not easily reversed by vitamin D repletion.

Further elevations in serum vitamin D levels above the normal range were not associated with additional improvements in inflammatory and fibrotic markers nor with further improvement in EGP or increases in VDR-related gene expression. Our findings are consistent with those of Pittas et al. in which increasing vitamin D levels from 27.7 to 54.3 ng/ml failed to impact the progression to type 2 diabetes [21]. This suggests that increasing vitamin D levels above normal/replete levels is not associated with further metabolic benefits. Additionally, in our study, not all of the subjects included in the first visit completed the third visit; hence, separate analyses were conducted to compare the first vs second and first vs third visits. The reduced sample number at the third visit might explain instances in which there was significant improvement at the second but not third visit.

Our results may appear to be at odds with our prior work demonstrating that the VDR promotes insulin resistance in cultured adipocytes [93]. In those studies, the VDR was shown to be induced by both dexamethasone and TNF-α, and knockdown of the VDR protected against the development of insulin resistance induced by those agents. Conversely, the overexpression of the VDR in adipocytes caused reduced insulin-stimulated glucose uptake, yet the addition of vitamin D did not affect insulin action [93]. Although the reasons for the discrepancy between those in vitro studies and the in vivo work presented herein are not entirely clear, we speculate that the effects of vitamin D/VDR on fibrosis and inflammation, not modeled in the culture system, would predominate over any direct effects of the VDR on adipocyte insulin action. An additional potential limitation is that our study lacked sufficient power to detect potential sex- or ethnicity-specific 25(OH)D thresholds or dose–response relationships. It is unclear how long the corrective effects of vitamin D would last post-treatment. Finally, these results cannot be extrapolated to patients with diabetes, because such subjects were excluded.

5. CONCLUSIONS

In summary, increasing vitamin D concentrations in the normal range improved hepatic insulin resistance and reduced adipose expression of pro-inflammatory and pro-fibrotic genes in concert with diminished tissue fibrosis markers. These findings were accentuated by the worsening trends in insulin resistance and adipose tissue fibrosis observed following 6 months of uncorrected vitamin D deficiency. Further elevations in vitamin D concentrations had no additional impact on metabolism or adipose tissue characteristics. Importantly, parallel rodent studies demonstrated that adipocyte-specific depletion of the vitamin D receptor induced adipose tissue inflammation and fibrosis as well as worsened hepatic insulin resistance, suggesting that the latter could be attributed to the effects of vitamin D on adipose tissue. Thus, correcting vitamin D deficiency in targeted individuals may have favorable metabolic effects and could potentially offer substantial public health benefits given the magnitude of the global obesity epidemic. These studies have far-reaching implications for understanding the role of adipocytes in mediating adipose tissue inflammation and fibrosis, ultimately impacting insulin sensitivity.

DATA ACCESS AND RESPONSIBILITY

The principal investigator, Dr. Meredith Hawkins, had full access to all of the data in this study and takes responsibility for the data integrity and the data analysis accuracy.

AUTHOR CONTRIBUTIONS

E.R., P.K., and M.K. conceived the study, designed and conducted the experiments, analyzed the results, and drafted the manuscript. E.L-Y., S.K. A.G., and K.Z. conducted the experiments, analyzed the results, and drafted the manuscript. J.Y.Y., M.C., S.J., and S.K. analyzed the results and drafted the manuscript. S.B. and P.G. conducted the experiments. All of the authors edited and approved the final manuscript.

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conducted at the Analytical Imaging Facility, which is funded by NCI Cancer Grant P30CA013330. The mouse phenotyping studies were conducted at Beth Israel Deaconess Medical Center except for the clamp studies, which were performed at the Vanderbilt University Medical Center Mouse Metabolic Phenotyping Center (DK059637). The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views or policies of the FDA, NCRR, or NIH.

CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2020.101095.

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Exhibit I

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Original research

Effect of a vitamin and mineral supplementation on glycemic status: Results from a community-based program

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ABSTRACT

Aims: Diet is a major risk factor for type 2 diabetes mellitus. As cofactors necessary for enzyme function of all metabolic pathways, vitamins and minerals have the potential to improve glucose metabolism. We investigated the effects of a nutrient intervention program on glycemic status.

Methods: We used a form of natural experiment to compare Pure North program participants (n = 1018) that received vitamin D alone (Vital 1) or vitamin D in combination with other nutrients (Vital 2) during two different time periods. Changes in 25-hydroxyvitamin D [25(OH)D], high-sensitivity C reactive protein (hs-CRP), glycated hemoglobin (HbA1c) and glycemic status were characterized over one and two years.

Results: Serum 25(OH)D concentrations increased significantly in both Vital 1 (to 111 ± 49 nmol/L) and Vital 2 (to 119 ± 52 nmol/L) over one year. HbA1c and hs-CRP were significantly reduced over time in Vital 2. Higher 25(OH)D levels after one year were associated with larger decreases in HbA1c and hs-CRP in Vital 2. At one year, 8% of Vital 2 and 16% of Vital 1 participants progressed from normoglycemia to prediabetes/diabetes, whereas 44% of Vital 2 and 8% of Vital prediabetes/diabetes subjects regressed to normoglycemia.

Conclusions: Vitamin D combined with other nutrients was associated with a reduced risk of progression to diabetes and with an increased rate of reversion to normoglycemia in high risk participants. The results suggest that nutrient supplementation regimes may provide a safe, economical and effective means for lowering diabetes risk. Further examination of this potential via randomized controlled trials is warranted.

Introduction

World-wide, 347 million people have diabetes mellitus, with type 2 diabetes constituting 90% of cases [1,2]. Diabetes is one of the most common chronic diseases in Canada, with a 70% increase in prevalence between 1999 and 2009 [3]. The prevalence of type 2 diabetes is highest in older persons, but over 50% of the affected population are of working age [3]. The economic burden associated with direct and indirect medical costs is estimated at $12.2 billion annually [4]. There are known modifiable lifestyle risk factors for type 2 diabetes that provide the opportunity for intervention and prevention [5]. The possibility of modifying the risk of diabetes by improving nutrient levels may offer a simple, safe and scalable strategy to reduce the burden of this prevalent chronic disease.

While lifestyle interventions tend to be the primary focus of diabetes prevention strategies, other strategies are also used, such as bariatric surgery or the use of pharmacological agents [6]. High intensity programs, such as the US Diabetes Prevention Program, have demonstrated up to a 58% reduction in relative risk of diabetes [7]. However, such studies often have protocols that are labour intensive, expensive and severely limited in their capacity to be implemented in a community setting [8].

The development of an inexpensive and more easily implemented intervention program for the prevention of diabetes could play a role in improving the health of individuals worldwide. The use of nutritional supplements as part of such an intervention strategy is an area that merits investigation. The development of diabetes is preceded by abnormalities in glucose homeostasis leading to insulin resistance, glucose intolerance and eventually the development of type 2 diabetes [9]. It has been postulated that daily variation in glucose homeostasis may be aggravated by inadequate nutrient composition as many micronutrients are necessary cofactors for the proper function of enzymes involved in energy metabolism. The role of dietary supplements in glucose control has been investigated in basic research and observational studies as a

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means to address inadequate nutrition and chronic disease [10,11]. Nutrients with proposed benefit in glucose homeostasis include vitamin D, vitamin K, calcium, magnesium, zinc, chromium, and omega-3 fatty acids [12,13]. Vitamin D, in particular, appears to play a significant role in the progression of diabetes, with studies linking low serum 25(OH)D levels with both insulin resistance and β-cell dysfunction [14,15]. However, randomized clinical trial evidence for individual nutrients, including vitamin D, is inconsistent; some trials show benefit while others report null results which may only reflect the differences in study design [16,17]. It is also possible that a combination of nutrients is required to derive benefit. In this study we characterize the effect of a nutritional intervention program, utilizing a natural experiment in which two supplement groups occur, on glycemic status over one and two years.

Patients and materials and methods

Study design

This study is a form of a "natural experiment" [18] where two groups were retrospectively identified, Vital 1 (Dec. 2008 to Mar 2010) and Vital 2 (Mar. 2010 to May 2012). We compare the effect of the different interventions received by the two groups on glycemic status and development of type 2 diabetes mellitus.

This was a retrospective database analysis. This study focused on participants who joined the program between December 2008 and May 2012 and was approved by the research ethics board at the University of Calgary (E-24890). Participants provided written informed consent for the use of their data for research.

The Pure North community-based program (intervention)

Pure North S'Energy Foundation is a not-for-profit wellness program based in Calgary, Alberta, Canada, that focuses on the prevention of chronic disease. The Pure North program offers lifestyle advice, education and nutritional supplements to participants. There is no inclusion/exclusion criteria for entering the program and the program does not substitute for conventional health care. The nutritional supplements provided by the Pure North program are selected to address common problems such as vitamin D deficiency.

The core tenant of the program is to achieve optimal nutritional status with a focus on physiological levels of vitamin D. All participants are encouraged to achieve a 25(OH)D level above 100 nmol/L (< 250 nmol/L); levels that can be naturally attained through regular sun exposure [19], are safe [20,21], and associated with a reduced risk of many chronic diseases including bone disease [22,23], depression [24], autoimmune disease and cancers [25,26].

Because of large inter-individual response differences to a given dose of vitamin D3, dosages were adjusted accordingly for the individual to achieve the target serum 25(OH)D level by the treating health care professional. Vitamin D3 doses were often above the upper level of intake, 4000 IU/d, to achieve the target. Vitamin D3 intake recommendations ranged from 1000 to 20,000 IU/d [26] under medical supervision.

In the program, each participant meets with a health care professional (Medical Doctor, Naturopathic Doctor or Nurse Practitioner) who provides lifestyle advice appropriate for the individual participants' health goals and current diagnoses. Dietary advice is provided as deemed appropriate, such as the DASH diet for patients with cardiovascular disease or risk factors like hypertension or hyperlipidemia. There is generally a focus on increasing vegetable and fruit intake and reducing processed foods as only 50% of the Canadian population is consuming more 5 servings of vegetables and fruit daily and the recommendation is for 7–10 servings daily. Exercise that is appropriate for the participants' health is recommended to ensure cardiovascular health and muscle strength. This applied to both the Vital 1 and Vital 2 groups.

Intervention groups

We exploit changes in the program to compare two groups that differed in their supplement intake: Vital 1 and Vital 2. The group construction was based on dates when the program difference occurred. Supplement composition was the only major difference in the program experienced by Vital 1 and Vital 2; other aspects of the program remained consistent between the two groups.

Vital 1

The Vital 1 multivitamin supplement (Table S1) was introduced to the program in December 2008 and was given in combination with a liquid vitamin D_3 supplement (1000 IU/drop; Ddrops®, Toronto, ON). Vital 1 contained 200 mg of niacin, twice daily, for a total of 400 mg/d of niacin. An informal survey of participants in the Vital 1 cohort suggested a very low compliance with the multivitamin as a result of the flushing produced by the niacin. The lack of change in serum vitamin B12 levels despite an increase in serum 25-hydroxyvitamin D [25(OH)D] levels was consistent with the low level of compliance with Vital 1 (Fig. 1), but supported compliance with vitamin D_3 supplements. Thus, the Vital 1 cohort is referred to as the "Vitamin D_3 only group."

Vital 2

Over time the Vital 1 multivitamin was reformulated, niacin was reduced to 15 mg, and the new multivitamin was named Vital 2. The Vital 2 formulation (Table S1) was introduced in March 2010. At the same time other supplements were incorporated creating the "full program." The core supplements of the full program were vitamin D_3 (1000–20,000 IU/d as needed to achieve target levels), Vital 2 (multivitamin), and omega-3 fatty acids (400 mg EPA and 200 mg DHA). Additional supplements were recommended on a discretionary basis, in response to deficiency or clinical indication, including vitamin C, magnesium, caprylic acid, PGX (PolyGlycopleX®) and probiotics. These were reported by participants and used for covariates in statistical models where appropriate.

When building the dataset, not all of the participants included in the Vital 1 and Vital 2 groups at one year are included at two years (lost to follow-up). As such, this results in slightly different baseline values and we have presented baseline versus one year and baseline versus two years for each Vital cohort separately to allow a direct comparison

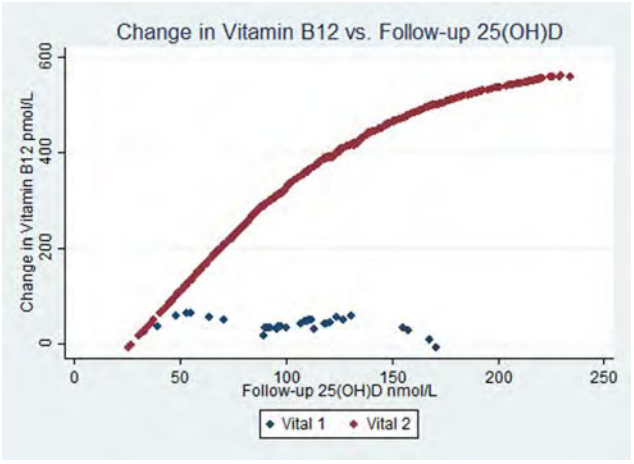


Fig. 1. Establishing Groups Vital 1 versus Vital 2. Comparison of Vitamin B12 and 25(OH)D changes at one year in each multivitamin cohort. In Vital 1 participants only vitamin D increases over one year suggesting that they are not taking the multivitamin containing vitamin B12. The proportional increase in vitamin D and B12 in Vital 2 suggests that Vital 2 participants are consuming both vitamin D and the multivitamin (containing vitamin B12) and supports the assumption of two groups.

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Table 1
Baseline Characteristics of Pure North Program Participants for Vital Cohorts that Remained in the Program for each Time to Follow up.

Characteristic	One year follow up		Two year follow up	
	Vital 1	Vital 2	Vital 1	Vital 2
	N = 223	N = 790	N = 101	N = 269
Male No. (%)	135 (61%)	393 (50%)*	65 (64%)	123 (46%)*
Age yr.	40.1 ± 8.2	41.1 ± 8.9	40.2 ± 7.8	41.8 ± 9.1
BMI	27.8 ± 5.3	27.1 ± 5.4	29 ± 5.9	26.4 ± 4.8*
HbA1C %	5.51 ± 0.39	5.64 ± 0.35*	5.55 ± 0.43	5.68 ± 0.51*
Hs CRP (mg/L)	2.08 ± 2.0	1.60 ± 1.78	2.13 ± 2.0	1.53 ± 1.7*
25(OH)D (nmol/L)	79 ± 35	87 ± 45*	77.5 ± 33	91.8 ± 48*

Values are mean ± standard deviation or mean (standard error).
Please note: Baselines differ for each year of follow up due to discontinuation in the program.
*Indicates significant difference between Vital cohorts (two sample *t* test, $p < .05$).

between baseline and follow-up (Tables 1-3).

Dataset construction and measurements

Participants included in the dataset analyzed were 25–54 years of age at entry and had baseline and follow-up measures for the assessments listed below. The sample was restricted based on hs-CRP values to include measures < 10 mg/L to avoid the influence of persons with acute inflammation. One year measures were considered at 365 ± 185 days and two year at 730 ± 185 days. Participants who reported a diagnosis of diabetes or reported taking medications for diabetes were excluded from the study.

Participants were interviewed and assessed by health care professionals at each program visit to collect demographic information, lifestyle information (including fruit/vegetable and fish consumption, exercise, tobacco and alcohol use) medical history, medication use (including diabetic medications), biometric measurements were obtained (including waist circumference and BMI) and non-fasting blood work was collected.

Biochemical assessments included HbA1c, 25(OH)D, vitamin B12, and hs-CRP. Measurements were made at Calgary Laboratory Services (Calgary, Alberta) from the start of our sample period until April 2013, after which time samples were sent to Doctor's Data (St. Charles, IL). During the transition between labs, samples were sent to both labs simultaneously, correlations were: HbA1c $r = 0.96$ ($n = 242$, $p < .001$); hs-CRP $r = 0.99$ ($n = 241$, $p < .001$) by the same method. Samples for 25(OH)D measurement were compared between labs, where CLS used the Liaison (chemiluminescent reaction) and Doctor's Data employed a liquid chromatography, tandem mass spectrometry (LC/MS-MS) method, giving a correlation of $r = 0.801$ ($n = 3015$, $p < .001$), a typical correlation between 25(OH)D assay methods.

Categorical glycemic status was defined according to HbA1c values: normoglycemic ≤ 5.8%, prediabetic 5.8–6.4%, and diabetic ≥ 6.4% [27,28]. Prediabetic participants were considered at risk for developing diabetes.

Statistical analyses

All statistical analyses were performed using Stata v10 (StatCorp LP, TX) and included descriptive statistics and t-tests for significance. Ordinary least squares (multiple) regression modeling and Pearson correlation analyses were performed for continuous measures of biomarker changes, and Probit regression techniques for changes in categorical diabetic statuses. Marginal effects from a Probit model are interpreted the same way as coefficients from most regressions.

Multiple regression analysis was used to determine differences

Table 2
Changes in Metabolic Variables and Diabetic Status Over One and Two Years.

Variable	Vital 1			Vital 2			Vital 1			Vital 2		
	N	Base-line	One Year	N	Base-line	One Year	N	Base-line	One Year	Two Years	N	Base-line	One Year	Two Years
HbA1C (%)	223	5.51 ± 0.39	5.65 ± 0.4[a]	790	5.64 ± 0.35	5.56 ± 0.38[a]	101	5.55 ± 0.43	5.66 ± 0.45	5.74 ± 0.66[a]	269	5.68 ± 0.51	5.66 ± 0.74	5.51 ± 0.53[a]
25(OH)D (nmol/L)	223	79 ± 35	111 ± 49[a]	790	87 ± 45	119 ± 52[a]	101	77 ± 33	109 ± 50[a]	123 ± 57[a]	269	92 ± 48	132 ± 52[a]	117 ± 51[a]
Hs-CRP (mg/L)	223	2.08 ± 2.0	1.91 ± 1.89	790	1.60 ± 1.78	1.52 ± 1.73	101	2.14 ± 2.00	1.72 ± 1.74[a]	1.61 ± 1.71[a]	269	1.53 ± 1.70	1.44 ± 1.58[a]	1.46 ± 1.66[a]
Normoglycemic to Prediabetes or Diabetes	185	–	0.16 (0.37)	549	–	0.078 (0.27)	81	–	0.09 (0.28)	0.23 (0.43)	184	–	0.10 (0.30)	0.02 (0.15)
Prediabetic or Diabetic to Normoglycemic	39	–	0.08 (0.27)	245	–	0.44 (0.5)	20	–	0.05 (0.22)	0.15 (0.37)	85	–	0.39 (0.49)	0.48 (0.5)

Values are mean ± standard deviation or mean (standard error).
Please note: Baselines differ for each year of follow-up due to discontinuation in the program.
[a] Indicates significant difference from baseline.

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Table 3
Estimation for Changes in HbA1C and hs CRP after one year and two years in the Pure North program (Ordinary Least Squares).

	Δ HbA1C (%)			Δ hs CRP (mg/L)		
	(1)	(2)	(3)	(4)	(5)	(6)
Variables	One Year	One year	Two Years	One Year	One year	Two Years
Vital1	0.185[a]	0.123[b]	0.345[a]	0.134	0.061	0.050
	(0.018)	(0.055)	(0.050)	(0.114)	(0.161)	(0.179)
25(OH)D[¥] (nmol/L)	−0.001[a]	−0.002	−0.001	−0.004[a]	−0.003[b]	−0.003[b]
	(0.001)	(0.001)	(0.001)	(0.001)	(0.001)	(0.001)
$25(OH)D^2$	5.14e 06[a]	6.25e 06	2.28e 06			
	(1.66e 06)	(5.00e 06)	(4.02e 06)			
Age[¥]	0.002	−0.002	0.006[b]	0.011[b]	0.019[b]	0.009
	(0.001)	(0.003)	(0.003)	(0.005)	(0.008)	(0.009)
Male	0.034[b]	−0.012	0.017	−0.023	−0.068	−0.120
	(0.015)	(0.047)	(0.044)	(0.094)	(0.141)	(0.158)
Centered Value of Biomarker	−0.167[a]	0.050	−0.233[a]	−0.500[a]	−0.494[a]	−0.580[a]
	(0.022)	(0.049)	(0.047)	(0.026)	(0.039)	(0.044)
Constant	−0.071[a]	0.072	−0.195[a]	0.010	−0.150	−0.011
	(0.022)	(0.075)	(0.062)	(0.118)	(0.185)	(0.190)
Observations	1013	370	370	1013	370	370
R squared	0.173	0.026	0.191	0.276	0.322	0.341

Please note: Baselines differ for each year of follow up due to discontinuation in the program.
Constant terms in all models refer to the mean value of the dependent variable for the Vital 2 cohort.
[a] (Standard error); Indicates p < .01.
[b] (Standard error); Indicates p < .05.
[¥] Baseline value. The dependent variable is change in the biomarker. In columns (2), (3), (5) and (6), the constant term is for 25(OH)D of 50 nmol/L at follow up, for a female with mean age and mean value of the biomarker at baseline for the entire sample.

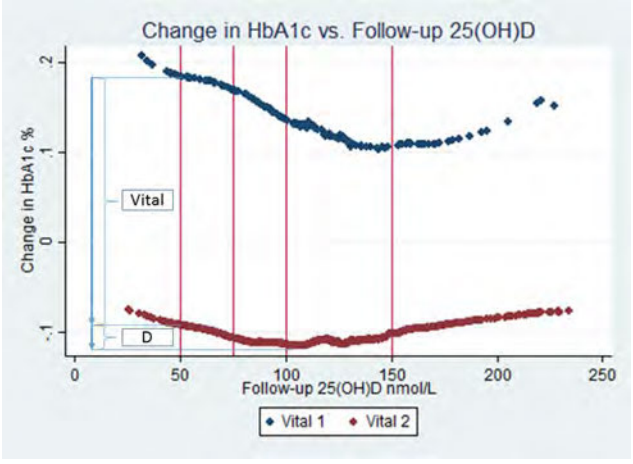


Fig. 2. The effect of vitamin D3 (Vital 1) in comparison to vitamin D3 with other supplements (Vital 2) on change in glycosylated hemoglobin. LOWESS plot of ΔHbA1c versus 25(OH)D level at follow up for the Vital 1 and Vital 2.

between Vital 1 and Vital 2 interventions and what could be attributed to vitamin D supplementation (Vital 1) and the full program including vitamin D (Vital 2). The dependent variable in all models was the change in the biomarker over one year regressed on a constant and on an indicator variable for Vital 1 (Fig. 2). Further models controlled for age, sex, use of PGX® and changes in BMI, waist circumference, fruit and vegetable consumption, exercise, tobacco and alcohol use, and the baseline value of the biomarker (to account for any tendencies in the data toward mean reversion). Models are based on the observed quadratic function of serum 25(OH)D concentrations. To confirm this functional form implication of including baseline bio-marker values in a model of biomarker changes we regressed the value of the biomarker at one year on the baseline value and its baseline value squared along with other control variables to confirm that we get the same coefficient estimates for the Vital 1 indicator variable and the 25(OH)D controls. We also used the strategy of limiting the sample to participants with baseline hs-CRP greater than two, greater than three and greater than five. This approach confirmed the results in the models including baseline hs-CRP where < 10 limit was used.

Probit models are equivalent to Logistic regression but are based on the standard normal distribution rather than the Logistic for modeling the probability for the outcome of interest. Probit models are useful for estimating absolute risk and reported marginal effects were interpreted as the change in the probability of the outcome of interest occurring. Odds ratios in Logistic regression are measures of relative risk and are not clearly interpretable when explanatory variables are continuous rather than dichotomous.

Changes in biomarker levels were used in modeling rather than absolute values to account for any fixed, but unobserved, characteristics of participants or potential confounding influences. Consequently participants who had both a baseline measure within 30 days of beginning the Pure North program and a one-year follow-up measure of all variables were included in the statistical model. BMI, waist circumference, fruit and vegetable consumption, exercise, alcohol and tobacco use were not reported consistently so models incorporating these covariates had smaller sample sizes.

Statistical modeling equations and explanation

A simple statistical model for explaining changes in HbA1C over a year was used:

$$\Delta HbA1\ c = f(vitD, multivitamin) + \varepsilon$$

The model suggests that the change in HbA1c is a function of changes in 25(OH)D, changes in other micronutrients status and random variation in HbA1c. Given two cohorts, one of which only received vitamin D3 (Vital 1 (V1)) and one of which received vitamin D3 in combination with other supplements (Vital 2 (V2)).

$$\Delta HbA1\ c = f_{V2}(vitD, multivitamin) + f_{V1}(vitD) + \varepsilon$$

If we assume that fV2 and fV1 are linear functions, then we can re-write the equation as:

$$\Delta HbA1\ c = \gamma_{V2} + \gamma_{V1}V1 + \beta_{V2}(vitD) + \beta_{V1}(vitD{*}V1) + \varepsilon$$

V1 is an indicator variable equal to one for an observation of a member of Vital 1 and 0 otherwise (Vital 2) and epsilon is the error term of the model. Comparison of γV2 and γV1 identified the effect of vitamin D3

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in combination with other supplements (Vital 2) but not Vital 1 for a given 25(OH)D serum level. If those coefficients were equal then the parts of the Vital 2 program absent for the Vital 1 cohort would have no effect on HbA1c.

The regression model specifies that the value of the outcome of interest is equal to the mean value plus some random difference. In a regression model, the mean of the distribution varies with observables like a vitamin D level. The error term of the model is a random variable assumed to have a normal distribution with mean 0 and constant standard deviation sigma. The purpose of the estimation method is to choose values of the model's constant term and slope coefficient(s) that best describe the relationship between the explanatory variable of interest and the outcome variable (the line of best fit).

If vitamin D had a direct effect on HbA1c, then $\beta V1 = 0$ and $\beta V2 < 0$, whereas increased 25(OH)D measured broader program adherence only, then $\beta V1 + \beta V2 = 0$ and $\beta V2 < 0$.

Fig. 2 illustrates how these effects were identified and shows LOWESS smooth plots of *D*HbA1C against the follow-up value of 25(OH)D for the Vital 1 and Vital 2 cohorts. These non-parametric curves suggest that higher 25(OH)D is associated with larger reductions in HbA1C. It is also clear that the relationship is U- or J-shaped. The curve for Vital 2 indicates that the cohort on average had reductions in HbA1C over the year whereas Vital 1 participants had increases in HbA1C. The vertical distance between the two curves would be interpreted as the effect of the full program whereas the change along a given curve from 50 nmol/L to a point further to the right would be the change in HbA1C attributable to higher 25(OH)D.

Results

There were 236 participants, 25–54 years, in Vital 1 from which 13 were excluded due to a self-reported diagnosis of diabetes and taking diabetic medication. Of the 223 participants in Vital 1 at one year, 101 participants returned for follow-up at two years. There were 814 participants in Vital 2 but 40 were excluded because of diabetes. Of 790 in Vital 2, 269 had a follow-up at two years. In both cohorts, of those who did not return for follow-up, approximately half were lost to follow-up and the other half attended the clinic outside the specified time frame. Fig. 3 is flow diagram of participant inclusion in the dataset.

Because some participants were lost to follow-up, the cohorts for comparison at baseline differ between one year of follow-up and those that continue in the program for two years of follow-up (Table 1). At baseline Vital 2 participants at one year in the program (n = 790) and at two years (n = 269) had higher HbA1c values (by 0.13% for both baseline comparisons), higher 25(OH)D concentrations (8 nmol/L and 14 nmol/L higher for one and two-year cohorts, respectively), and had more females (11% and 18% for one and two-year cohorts, respectively). Age, BMI and hs-CRP did not differ between Vital 1 and Vital 2 at baseline for the cohort with one year of follow-up. For the two-year cohort, Vital 2 participants at baseline had a statistically significantly lower BMI (5.68 ± 0.51 vs. 5.64 ± 0.35 for Vital 2 and 1, respectively) and lower baseline hs-CRP levels (1.53 ± 1.7 vs. 1.60 ± 1.78 for Vital 2 and 1, respectively).

To confirm our presumption, that the Vital 1 cohort had not been taking the multivitamin, we compared baseline and one-year values of serum vitamin B12 (from the multivitamin) and 25(OH)D (vitamin D_3 taken separately as drops). Fig. 1 indicates that serum 25(OH)D concentrations coincide with increases in B12 in the Vital 2 cohort, whereas vitamin B12 concentrations do not increase in Vital 1 even at higher 25(OH)D levels. There was no correlation between changes in serum vitamin B12 and 25(OH)D for Vital 1 whereas higher 25(OH)D concentrations were significantly correlated with increases in vitamin B12 in the Vital 2 cohort, suggesting that both groups were taking vitamin D supplementation but only Vital 2 was compliant with the multivitamin. This supports the natural experiment.

BMI did not change significantly at one year in either group, nor did BMI change differ between groups at one year (−0.16 ± 1.6 for Vital 1 and −0.11 ± 2.5 for Vital 2). There was no change in BMI over two years in either cohort. At one and two years, HbA1c values were significantly lower in comparison to baseline in Vital 2 whereas in Vital 1 values increased ($p < .001$) (Table 2). Serum 25(OH)D concentrations were significantly higher in both groups at one and two years versus baseline, with mean increases that did not differ between groups (32 ± 48 nmol/L for Vital 1 and 32 ± 55 nmol/L for Vital 2) (Table 2).

Glycemic status is presented in Table 2. Despite having lower baseline HbA1c than the Vital 2 cohort, the proportion of Vital 1 participants progressing from normoglycemic to prediabetic or diabetic, and prediabetic to diabetic was double that for Vital 2. Conversely, improvement in diabetic status at one year (returning to normoglycemic) was 44% for Vital 2 and 8% for Vital 1 at one year (Table 2). The probability of a normoglycemic participant progressing to prediabetes/diabetes after one year is 8% higher for Vital 1 than for Vital 2 (Table 2).

Probit Model (1) confirmed that HbA1c values increased at one year in Vital 1 participants and decreased in Vital 2 participants (Table 3). Higher 25(OH)D was associated with larger decreases in HbA1c for Vital 2 and lower increases for Vital 1. The effect of 25(OH)D levels on HbA1c change (inflection point) was greatest at a value of 125 nmol/L. All models were assessed for confounding variables that were theoretically important to the relationship under study.

Given the comparable magnitudes of the coefficient estimates for 25(OH)D controls for changes in HbA1c across the models, it is likely that the lower power of the smaller sample is the reason for this difference. Models (2) and (3) show that the changes in HbA1c at two years (0.345) were more than double the changes at one year (0.123), suggesting that the beneficial effects of the program continue to at least two years. The results do not differ when BMI, waist circumference, fruit and vegetable consumption, exercise, alcohol and tobacco use are included in the model.

Models for hs-CRP (Table 3) demonstrate a linear effect of 25(OH)D. Models (5) and (6) show decreases in hs-CRP at one year (−0.061) and two years (−0.050). In Vital 1, at 50 nmol/L of 25(OH)D there was an increase in hs-CRP over one year, but not in participants with a mean 25(OH)D serum concentration of 111 nmol/L. For Vital 2, there is decrease in hs-CRP values of 0.26 mg/L when mean 25(OH)D concentrations increase from 50 to 119 nmol/L. For hs-CRP, reductions were sustained to two years and almost entirely explained by 25(OH)D levels based on the similarities of the estimated coefficients. To investigate a "program" effect, changes in HbA1c and follow-up 25(OH)D levels were compared between Vital 1 and Vital 2 (Fig. 2). The portion of change statistically attributable to the other supplements was one-third to three-quarters of the total change whereas the effect of vitamin D varied between one quarter to two thirds of the total effect. These non-parametric curves suggest that higher 25(OH)D was associated with larger reductions in HbA1c. The vertical distance between the two curves was interpreted as the effect of the multivitamin and other supplements whereas the change along a given curve from 50 nmol/L to a point further to the right was interpreted as the change in HbA1c attributed to higher 25(OH)D.

Discussion

Our results suggest that a relatively straightforward preventative health program that provides nutritional supplementation may have the potential to reduce the risk of diabetes and improve glycemic status in individuals at risk. The combination of nutritional supplements taken by the Vital 2 participants was associated with a reduced risk of progressing from normoglycemia to prediabetes and an increased likelihood of a prediabetic participant improving to normoglycemia, changes that were sustained at two years after program entry.

Furthermore, the results in Table 3 suggest that while increasing

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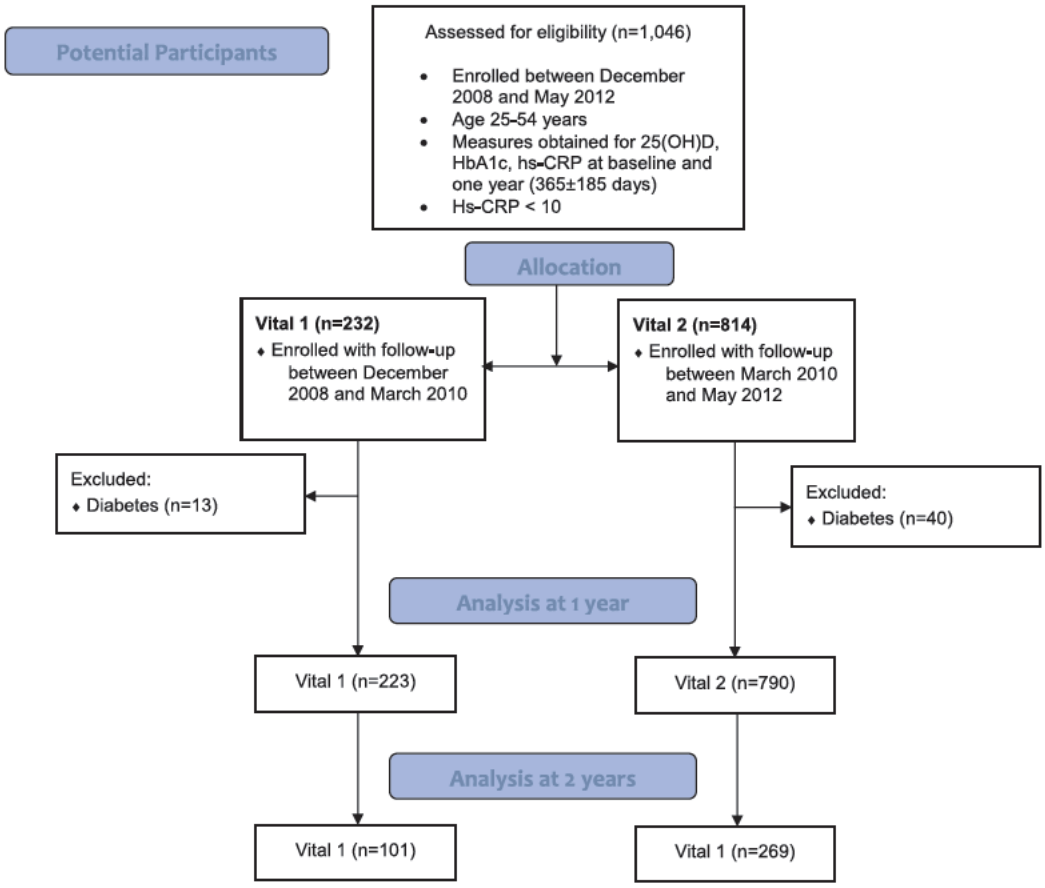


Fig. 3. Flowchart of participants included in the dataset.

25(OH)D concentrations through vitamin D_3 supplementation contributed to a reduced rate of increase in both HbA1c and hs-CRP, vitamin D in combination with other supplements was needed to decrease them. Further, the lack of change in BMI in both groups, the lack of effect of lifestyle measures (waist circumference, fruits and vegetables, etc.) on regression models and the reduction in Vital 2 but not Vital 1 suggest that the reductions in HbA1c and hs-CRP are due to the supplementation program.

With stable 25(OH)D concentrations at 50 nmol/L, the portion attributable to a change in 25(OH)D from 50 nmol/L to 119 nmol/L was one quarter to two thirds of the total effect, but neither HbA1c nor hs-CRP would have been reduced with vitamin D_3 supplementation alone. This may explain why the results of our study conflict with results of other studies examining the relationship between vitamin D_3 supplementation and diabetes risk. For example, a study of adults at risk for type 2 diabetes who received 2000 IU/d of vitamin D_3 did not find an effect on HbA1c [29]. Similarly, prediabetic patients with low 25(OH)D levels who were supplemented with 12,700 IU/d of vitamin D_3 did not demonstrate changes in HbA1c [30]. Based on our findings we posit that poor overall nutrition may be the reason for the discrepancy. The combination of achieving physiological levels of 25(OH)D with other nutrients (a multivitamin and omega-3 fatty acids) was required to observe reductions in HbA1c. Several lines of evidence suggest that vitamins D, C and E play roles in reducing oxidative stress and inflammation resulting in improved insulin sensitivity [31]. It is therefore plausible that overall nutritional support, and not just vitamin D alone, may be required to reverse the likely multifactorial mechanisms responsible for the progression to diabetes.

The relative risk of progression from prediabetes to diabetes in the general population has been estimated to be 12% compared to those with normoglycemia [32]. In contrast, regression from prediabetes to normoglycemia occurred in approximately 8% of participants in the Diabetes Prevention Study [33]. At one year 8% of Vital 1 progressed to diabetes with 11% at two years, rates comparable to that expected in the general population. In contrast, Vital 2 had a significantly lower rate of progression than expected, with 3% progression from prediabetes to diabetes at one year and 1% at two years. Of note, 44% of Vital 2 participants regression from prediabetes to normoglycemia whereas only 8% of Vital 1 regressed. These results suggest that the Pure North program may be helpful in reducing the risk of, or even reversing, prediabetes and diabetes in a population.

Vital 2 reduced HbA1c from baseline, while Vital 1 (vitamin D_3 only) had slightly increased HbA1c levels. If we expect an increase in HbA1c levels, from 5.5% at baseline, a large majority of Vital 1 with normoglycemia would be in the prediabetic range at 2 years. In contrast, the mean baseline HbA1c was 5.65% for Vital 2 and if the same changes occurred in this cohort as were seen for Vital 1, they would have been in the prediabetic range on average after one year. Instead, Vital 2 was observed to have decreased HbA1c levels and 44% were normoglycemic at one year. This is in line with findings from the Diabetes Prevention Program, and intensive and expensive lifestyle intervention program, produced a 58% reduced risk of diabetes. Fig. 3 highlights the association of vitamin D with progression and regression rates in Vital 2. Higher levels of 25(OH)D were associated with better outcomes, with the effect being greatest at a value of 125 nmol/L.

Table 3 suggests that around 14% fewer normoglycemic persons would have progressed to prediabetes after one year. Applied to 1000 normoglycemic individuals this would result in ~ 140 persons who do not progress to diabetes. Given the mean increase in HbA1c for Vital 1 was 0.13 in one year, with mean baseline HbA1c of 5.7%, without the program these individuals would have been diabetic in about 5 years. Similarly, the effect sizes for returning from prediabetic to

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normoglycemic, if the Vital 2 intervention were applied to 1000 persons with prediabetes, would see 510 persons returned to normoglycemic status.

The results of this study are of particular interest because of the scalable nature of this simple, relatively inexpensive, program. The supplements provided could be implemented at an annual cost of $350. If the results observed in this natural experiment could be replicated across a larger population, it could represent a highly cost effective option for reducing diabetes risk. From an economic perspective, Vital 1 demonstrates rates of transitions between glycemic statuses that are similar to those for the Canadian population [28]; Vital 1 rates remain steady whereas Vital 2 rates of prediabetes decline with a corresponding increase in normoglycemia. Accordingly, by comparing Vital 1 with Vital 2 we can estimate the cost implications possible for a cohort of 1000 people. Assuming Vital 2 rates remain consistent over 5 years, an average health care utilization cost of $2053 [28], a 5% discount factor and a net lifetime cost from the mean age of Vital 2 (41–74 yr), the Net Present Value of costs for diabetes is $34,906 and for prediabetes is $27,262. For a cohort of 1000 people there would be 133 more normoglycemics, 130 fewer prediabetics ($3.6 million) and 10 fewer people with diabetes ($363,000). This results in a total avoidance of $4,000,000 in health care spending for 1000 individuals, or on average $4000 per person. At an annual cost of $350 for vitamin D_3 plus the other nutrients provided, the net present value is approximately $6000 per person, not including gains from improved quality of life or potential reductions in other chronic disease outcomes.

There are several limitations to the current retrospective study. To address selection bias we controlled for several variables (age, sex, BMI, etc.). There were slight differences between cohorts as outlined. Lifestyle counselling is a part of the Pure North program and may have been a confounder in the analyses, but it does not appear that weight loss was a contributing factor as there was no difference found in BMI between entry to the program and follow-up for either group. The lack of effect of waist circumference, BMI, fruit and vegetable intake, exercise, tobacco and alcohol on regression models use also suggests that lifestyle did not change significantly in either group.

There is also no way to elucidate which nutrients exactly were responsible for the effect on HbA1c and hs-CRP improvement. With modeling we were able to estimate the effect of vitamin D_3 to be between one quarter and one third with the other nutrients contributing the rest. However, we are unable to determine exactly which components of the multivitamin or omega-3 fatty acids were responsible. Biologically speaking, interactions between various nutrients make it plausible that more than one nutrient is responsible. Energy metabolism alone requires five different B vitamins, magnesium and manganese. Further, as in any population, participants may have been taking other nutrients to address their own deficiencies and symptoms. As such, the analysis presented assesses the success of a “real-life” prevention program that focused on optimizing nutritional status.

Although the design of this study as a retrospective, natural experiment limits the extent to which these results can be generalized to a larger population outside of the study group [30], the results suggest a real, positive effect of the Pure North intervention program on glycemic status in the participants. Diabetes is one of the fastest growing chronic diseases worldwide, and the role of nutritional supplementation in the prevention of this disease warrants further research. Well-designed clinical studies are required to ascertain if improving 25(OH)D levels in combination with multivitamin supplementation and/or omega-3 fatty acids could provide a safe, effective and economical means of reducing diabetes risk in the general population.

There are important implications of this study for the ongoing controversies over nutrient supplementation in healthy populations and the position on vitamin D adequacy. Our outcomes across two biomarkers, HbA1c and hs-CRP, are longitudinal outcomes in healthy subjects. We observed that the combination of vitamin D and multivitamin supplementation resulted in clinically important health improvements in our study's participants. We suggest that further examination of the role of vitamin D supplementation along with other nutrients through randomized controlled trials is required to further evaluate the potential of such nutritional intervention programs on diabetes risk.

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Author contributions

SMK, JCHE and RZL designed the study and had primary responsibility for final content, JCHE directed the statistical analysis, and SMK and RZL wrote the paper. All authors have read and approved the final manuscript

Conflicts of interest

RZL and JCE declare no conflict of interest. SMK is employed by the Pure North S’Energy Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcte.2017.11.002.

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Exhibit J

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Article

In "High-Risk" Infants with Sufficient Vitamin D Status at Birth, Infant Vitamin D Supplementation Had No Effect on Allergy Outcomes: A Randomized Controlled Trial

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Abstract: Lower vitamin D status at birth and during infancy has been associated with increased incidence of eczema and food allergies. The aim of this study was to investigate the effect of early infancy vitamin D supplementation on allergic disease outcomes in infants at "hereditary risk" of allergic disease, but who had sufficient vitamin D levels at birth. Here, we report the early childhood follow-up to 2.5 years of age of "high-risk" infants who participated in a double-blinded, randomized controlled trial. For inclusion in this trial, late gestation (36–40 weeks) maternal 25-hydroxyvitamin D levels needed to be ≥50 nmol/L. Infants were randomized to either oral vitamin D supplementation of 400 IU/day (n = 97) or a placebo (n = 98) for the first six months of life. Vitamin D levels and allergic disease outcomes were followed up. There were no statistically significant differences in incidence of any medically diagnosed allergic disease outcomes or allergen sensitization rates between the vitamin D-supplemented and placebo groups at either 1 year or at 2.5 years of age. In conclusion, for "allergy high-risk" infants who had sufficient vitamin D status at birth, early infancy oral vitamin D supplementation does not appear to reduce the development of early childhood allergic disease.

Keywords: allergen sensitization; allergic disease; eczema; hereditary risk; infant; prevention; randomized controlled trial; vitamin D; wheeze

1. Introduction

Allergic disease prevalence has dramatically increased over recent decades at the same time as environmental and lifestyle changes have occurred, suggesting that these are linked [1,2]. One factor that must be considered is a global change from a more outdoor to an increasing indoor lifestyle.

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Increased reliance on digital technology and associated time indoors for work and relaxation, and subsequently a lack of sunlight exposure, is a global phenomenon applying to all age groups, including pregnant women and young children [3]. It is known that sunlight exposure plays a crucial role in vitamin D synthesis, with all vitamin D requirements able to be provided by cutaneous synthesis under the influence of ultraviolet (UV) light [4]. Yet despite the potential for adequate endogenous production, vitamin D deficiency is endemic in many parts of the world, even in sun-rich regions, affecting all age groups including the early stages of life [5].

Substantial evidence supports a role for vitamin D in the normal functioning of innate and adaptive immunity in addition to mucosal and epidermal barrier function—factors relevant to the development of immune tolerance and allergic outcomes [6]. Further, notably, the prevalence of allergic disease has been associated with increasing distance from the equator where higher rates of vitamin D deficiency generally occur [7–9]. Lower vitamin D status at birth and during early childhood have been associated with increased incidence of allergic disease outcomes [10–14]. However, four randomized controlled trials (RCTs) [15–18] have investigated the effects of maternal vitamin D supplementation during pregnancy on offspring allergy outcomes and a meta-analysis of these RCTs by Garcia-Larsen et al. [19] found no overall effect on the risk of early childhood recurrent wheeze (4 trials, n = 1781 participants, with 358 cases of recurrent wheeze), with a relative risk of 0.76 (95% confidence intervals 0.54; 1.08), nor eczema (3 trials, n = 1538 participants, with 357 cases of eczema), with a relative risk of 0.92 (95% confidence intervals 0.77; 1.11). These results suggested that rather than the antenatal period, the early post-natal period in early infancy may be potentially the most critical time to ensure adequate vitamin D status. This is particularly important in breastfed infants as the vitamin D content of breast milk is low and reliant on good maternal vitamin D status during lactation [20]. However, in contrast, one RCT examining maternal vitamin D supplementation found increased food allergy by two years of age in children whose mothers had vitamin D supplementation within the first few months of lactation [21].

In this context, we conducted a double-blinded, randomized controlled trial to investigate early infancy vitamin D supplementation in term infants at "hereditary risk" of allergy development, but who had sufficient vitamin D levels at birth. Here, we report the early childhood allergic disease follow-up outcomes of these children to 2.5 years of age.

2. Materials and Methods

2.1. Study Population and Intervention

Full details of participant screening, randomization, allocation concealment, intervention and placebo products, and infant outcomes to the end of the intervention period at 6 months of age have been previously published [22]. In brief, inclusion criteria included only infants whose mothers had a sufficient maternal 25-hydroxyvitamin D (25(OH)D) serum concentration of ≥50 nmol/L between 36 and 40 weeks' gestation. All infant participants were "high-risk" for allergic disease development due to a positive family history of at least one parent or sibling diagnosed with allergic disease (asthma, eczema, allergic rhinitis). Enrolment began on 9 October 2012 and ended on 23 January 2017. A total of 195 infants were randomized into the trial, 97 infants allocated to the intervention vitamin D group and 98 infants to the placebo group.

The intervention group received 400 IU (10 µg) of vitamin D3 per day orally (Ddrops Company, Woodbridge, ON, Canada), while the placebo control group received an identical product of palm kernel and coconut oil without vitamin D3 (Ddrops Company, Woodbridge, ON, Canada). These were given to the infants as 1 drop of liquid (0.03 mL) per day. The randomized controlled trial (RCT) was a parallel design with a 1:1 ratio allocation, and double-blinded with participants and investigators masked to group allocations. Randomization was stratified according to a history of maternal allergic disease and the infant's sex. The intervention period was from birth until six months of age. For compliance monitoring, caregivers completed a diary card to record each day they gave their infant the study product. Following recommendations for infant vitamin D supplementation [23], caregivers

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were advised to cease administration of the trial product if the infant was consuming >1000 mL/day of vitamin D-fortified infant formula.

Written informed consent was obtained before trial participation and included consent to participate in both the 1 year and 2.5 years of age clinical allergy follow-up assessments. Human Research Ethics Committee approvals were granted by the Princess Margaret Hospital for Children (approval number 1959/EP) and the University of Western Australia (approval number RA/4/1/5566). The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12612000787886).

2.2. Allergic Disease Clinical Outcomes Assessments

At 1 and 2.5 years of age, the participating children were assessed by a pediatric clinical immunologist at the Princess Margaret Hospital in Perth, Australia, who was blinded to the intervention group allocation. A structured history and a standardized clinical examination to diagnose any allergic disease were performed. Skin prick testing was undertaken to common food and environmental allergens, including cow's milk, hen's egg, peanut, cashew nut, wheat, cod fish, house dust mite (*Dermatophagoides pteronyssinus*), cat and rye grass pollen on the day of assessment. Sensitization was defined as a positive skin prick test (wheal ≥ 3 mm above negative control) to at least one of the allergens assessed. The children were assessed for eczema, wheeze/asthma and food allergy at 1 year and 2.5 years of age. At the 2.5-year appointment only, the participants were also assessed for a medical diagnosis of allergic rhinoconjunctivitis.

Eczema was defined according to the criteria of Hanifin et al. [24] on medical review or when a typical history was taken of an itchy rash distributed to the facial, extensor or flexural surface of the skin following a chronic or fluctuating course. The use of medically prescribed steroids for eczema treatment was also recorded. IgE-mediated food allergy was defined by a history of an immediate (within 90 min) skin rash (urticaria, erythema), angioedema, gastrointestinal symptoms (abdominal pain, vomiting, diarrhea) and/or irritability associated with or without cardiovascular symptoms (collapse) and/or respiratory symptoms (wheeze, stridor, persistent cough, hoarse voice) following the ingestion of a specific food trigger combined with a matching food allergen sensitization. Wheeze was defined by a history of an audible wheeze responding to inhaled beta2-agonists. Asthma was defined in children over two years of age only, with a history of recurrent wheeze (triggered by upper respiratory tract infections, exercise, cold air or allergen exposure) responding to inhaled beta2-agonists and/or use of a preventer for symptom control. In recognition of the difficulties of asthma diagnosis in young children, at 2.5 years of age, the outcomes of wheeze and/or asthma were combined. Allergic rhinoconjunctivitis was defined as a history of sneezing or blocked/runny nose accompanied by itch-watery/red eyes unrelated to an upper respiratory tract infection. When wheeze/asthma or allergic rhinoconjunctivitis was combined with a sensitization to at least one aeroallergen, it was defined as IgE-associated.

2.3. Blood Collection and Measurement of 25-Hydroxyvitamin D (25(OH)D) Levels

Cord blood was collected at birth and peripheral blood samples were collected at 3 months, 6 months, 1 year and 2.5 years of age by venipuncture into lithium heparin tubes (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria). The 25(OH)D levels were quantified by a competitive Chemiluminescent Immunoassay, automated on the Abbott Architect i2000 (Abbott Laboratories, Abbott Park, Illinois, operated by PathWest Laboratory Medicine, Western Australia). The Abbott Architect i2000 is accredited by the National Association of Testing Authorities for the measurement of 25(OH)D using a two-step incubation process with human serum calibrators. Internal quality control data indicate the coefficient of variation for 25(OH)D as follows: 11.4% at 22 nmol/L; 5.2% at 48 nmol/L; 4.5% at 68 nmol/L; and 4.0% at 90 nmol/L.

To ensure the infant participants did not develop vitamin D deficiency or toxicity during the trial, blood samples collected at 3 months of age were analyzed for 25(OH)D and the results were sent to a pediatric endocrinologist (AS). Caregivers and the participants' nominated general practitioner were

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informed if the 3 month of age level was out of the expected normal range, and the caregivers were asked to cease trial product use and consult their general practitioner for advice. The researchers involved in data collection and analysis remained blinded to these test results throughout the trial.

2.4. Statistical Methods

Normality of the continuous variables was determined with the Kolmogorov–Smirnov test of normality. For between-group differences of continuous variables, independent samples t-test or Mann–Whitney U test were used. Mean (standard deviation (SD)) and median (interquartile range (IQR)) were reported for parametric and non-parametric data, respectively. Pearson's Chi-square test or a Fisher exact test were used for between-intervention/placebo group comparisons and clinical outcomes (categorical variables). To evaluate the effect of the intervention/placebo group on clinical allergy outcomes, logistic regression was applied. All tests were two-sided with a $p < 0.05$ considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Study Population Characteristics

Data collection for the follow-up assessments was completed on 31 January 2019. Participant baseline characteristics have been previously published [22]. Briefly, 53.3% were male infants, 81.5% infants were Caucasian and 9.2% were Asian ethnicity, 61.5% had a vaginal delivery and mean gestational age at birth was 39.2 weeks. During pregnancy, 34.4% of their mothers took vitamin D supplementation. Allergy outcome data were collected on 175/195 (89.7%; 87 from vitamin D group) infants at 1 year of age, and 162/195 (83.1%; 83 from vitamin D group) children at 2.5 years of age, see Figure 1 for full study participant flow details. Nine infants (six in the vitamin D group) were withdrawn by their caregiver(s) during the intervention period, three infants who developed health issues (not allergic disease-related), two infants due to parents becoming too busy and four infants due to parents not wanting to proceed with the blood collections. No infants were withdrawn in the follow-up period from 6 months to 2.5 years of age. One placebo group infant who was thought to be lost to follow-up at 6 months of age could be contacted again and attended their 1 year of age appointment. At 1 year of age, five infants did not attend their appointment due to moving away from the study city, and six infants were lost to follow-up. At 2.5 years of age, eight infants did not attend their appointment (five had moved away and three had both parents now full-time working) and 16 infants were lost to follow-up. No infants experienced any serious adverse events during the intervention or follow-up periods.

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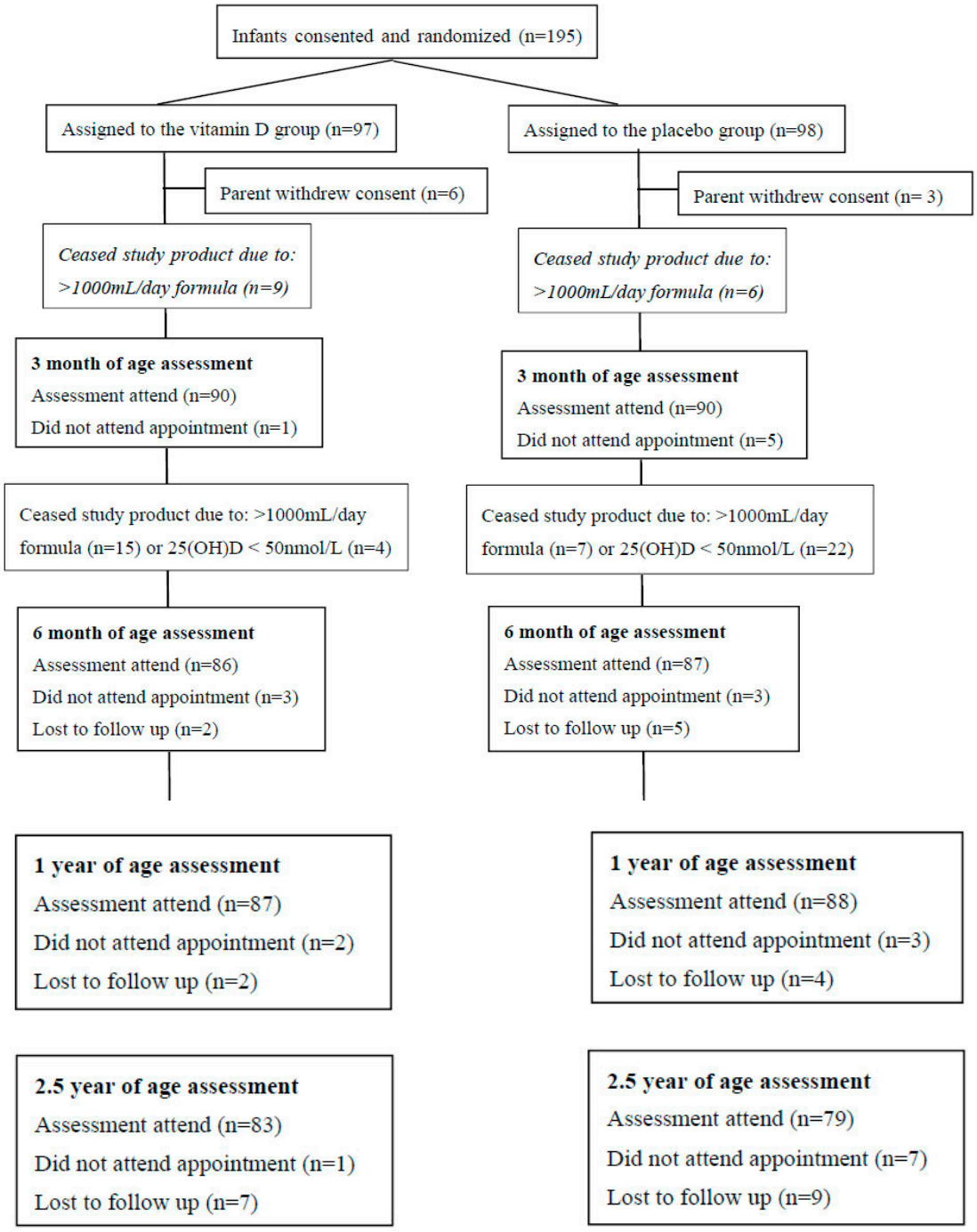


Figure 1. Study flow of participants.

3.2. Intervention Period Trial Product Compliance and Infant Feeding

During the intervention period from birth until 6 months of age, there were no differences between the two groups regarding [22] blinded caregivers taking the trial product drops ($n = 68$ vitamin D group) to 3 months of age, 14 infants ($n = 70$ vitamin D group) to 68 (59–76) days in the placebo group, $p = 0.92$. From 3 months to 6 months of age, the median (IQR) compliance = 75 (58–84) days in the vitamin D group compared to 75 (50–89) days in the placebo group, $p = 0.84$. From randomization to three months of age, in the vitamin D supplementation intervention group, 83.5% (76/91) of infants were given their trial product drops on ≥5 days per week, compared to 79.2% (76/96) infants in the control group, $p = 0.57$. Between three and six months of age, in the vitamin

3.3. Longitudinal 25(OH)D Levels over the Course of the Study

As per previously published, baseline cord blood 25(OH)D levels were not significantly different between the two groups [22]. Blood sampling collected from 140 infants ($n = 68$ vitamin D group) to 154 children ($n = 79$ vitamin D group) at 1 year and 125 children ($n = 63$ vitamin D group) at 2.5 years of age. As previously published [22], infants in the vitamin D group had significantly higher 25(OH)D levels at 3 and 6 months of age relative to the placebo group [22], however, these differences were not observed to persist beyond the intervention period (Table 1).

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D supplementation intervention group, 76.9% (70/91) of infants were given their trial product drops on ≥5 days per week, compared to 74.5% (70/94) infants in the control group, $p = 0.83$. As illustrated in Figure 1, there were 9 infants in the vitamin D group and 6 infants in the control group between zero and three months, and 15 infants in the vitamin D group and 7 control group infants between three and six months, who were no longer taking the study product due to consuming >1000 mL/day of vitamin D-fortified infant formula.

After being notified that an infant had an insufficient 25(OH)D level of < 50 nmol/L at 3 months of age, general practitioners recommended either no supplementation or varied dosages of vitamin D supplementation. As illustrated in Figure 1, in the control group, 22 infants ceased the study product use due to a 25(OH)D level of < 50 nmol/L at 3 months of age, but to our knowledge only eight infants commenced supplemental vitamin D use (two infants were supplemented with 200 IU/day for three months, four infants had 400 IU/day for one month, one infant had 400 IU/day for two months and one infant was recommended 1000 IU/day for three months). In the vitamin D-supplemented intervention group, four infants ceased the study product use due to a 25(OH)D level of < 50 nmol/L at 3 months of age, but only one infant was supplemented with 400 IU/day for two months.

Breastfeeding rates were high (98.5% at randomization, 85.2% at 3 months and 70.5% at 6 months of age) and infant formula use was low in our trial participants. There were no differences between the two groups regarding consumption of infant formula. From birth to three months of age, in the vitamin D supplementation intervention group, the median intake of infant formula was 0 mL per day (interquartile range (IQR) 0–250 mL per day), compared to 0 mL per day (IQR 0–235 mL per day) in the control group, $p = 0.53$. Between three to six months of age, in the vitamin D supplementation intervention group, the median intake of infant formula was 3 mL per day (IQR 0–565 mL per day), compared with 17 mL per day (IQR 0–589 mL per day) in the control group, $p = 0.84$. The ingestion of daily infant formula volumes over the preceding three months were also not correlated with vitamin D levels at 3 months of age ($r = 0.117$, $p = 0.17$) nor at 6 months of age ($r = 0.044$, $p = 0.61$).

There were no differences between the intervention and placebo groups regarding the age of introduction to solid foods. In the vitamin D group, the median age of commencing solid foods was 5.0 months (IQR 4.3–5.5 months), compared to the control group who had a median age of commencing solid foods of 5.0 months (IQR 4.5–5.5 months), $p = 0.49$.

3.3. Longitudinal 25(OH)D Levels over the Course of the Study

As per previously published, baseline cord blood 25(OH)D levels were not significantly different between the two groups [22]. Blood samples were collected from 140 infants ($n = 68$ vitamin D group) at 3 months, 141 infants ($n = 73$ vitamin D group) at 6 months, 154 children ($n = 79$ vitamin D group) at 1 year and 125 children ($n = 63$ vitamin D group) at 2.5 years of age. As previously published, infants in the vitamin D group had significantly higher 25(OH)D levels at 3 and 6 months of age relative to the placebo group [22], however, these differences were not observed to persist beyond the intervention period (Table 1).

Table 1. Longitudinal 25-hydroxyvitamin D (nmol/L) levels over the course of the study. The randomized controlled trial intervention phase was 0–6 months of age. Values are mean (standard deviation) unless otherwise stated. Bold number means significant.

Time Point	Vitamin D Group	Placebo Group	*p*-Value
Cord Blood	67.8 (17.5)	61.1 (14.2)	0.17
3 months of age	83.2 (27.8)	59.2 (22.7)	**<0.01**
6 months of age	93.1 (28.7)	82.0 (27.9)	**0.02**
1 year of age	78.4 (21.9)	81.2 (23.5)	0.45
2.5 years of age [1]	79.0 (69.0–110.0)	74.0 (60.0–86.5)	0.19

[1] median (inter-quartile range).

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3.4. Clinical Allergic Disease Outcomes

As expected, the most common medically diagnosed allergic disease in this "high-risk" population was eczema, with an overall incidence of 51/175 (29.1%) infants at 1 year of age and 59/161 (36.6%) children at 2.5 years of age. By 1 year of age, food allergy affected 11/175 (6.3%) infants and wheeze symptoms were diagnosed in 37/175 (21.1%) infants. Positive allergen sensitization to food or environmental allergens was found in 26/167 (15.6%) infants at 1 year and in 31/136 (22.8%) children at 2.5 years of age using skin prick testing. As summarized in Table 2, there were no statistically significant differences in incidence for any of the medically diagnosed allergic disease outcomes or allergen sensitization rates between the vitamin D-supplemented and placebo groups at either 1 year or at 2.5 years of age. The use of prescription steroids for eczema treatment (as a marker for eczema severity) also did not differ between the groups at either 1 year of age with 9.2% (8/87) in the vitamin D group compared to 10.2% (9/88) in the placebo group (p = 1.00), or at 2.5 years of age with 12.0% (10/83) in the vitamin D group compared to 12.7% (10/79) in the placebo group (p = 1.00).

Table 2. Comparison between vitamin D-supplemented and placebo groups for medically diagnosed allergic disease outcomes and allergen sensitization rates at 1 and 2.5 years of age. Values are participant numbers (percentages). Abbreviations: CI = confidence interval.

Allergic Disease	Vitamin D Group	Placebo Group	Relative Risk (95% CI)	p-Value
Eczema at 1 year	30/87 (34.5)	21/88 (23.9)	1.45 (0.90–2.32)	0.17
Eczema at 2.5 years	33/83 (39.8)	26/78 (33.3)	1.19 (0.79–1.80)	0.50
Food allergy at 1 year	6/87 (6.9)	5/88 (5.7)	1.21 (0.39–3.83)	0.98
Food allergy at 2.5 years	3/83 (3.6)	6/79 (7.6)	0.48 (0.12–1.84)	0.45
Wheeze at 1 year	23/87 (26.4)	14/88 (15.9)	1.66 (0.92–3.01)	0.13
Asthma and/or wheeze at 2.5 years	25/83 (30.1)	18/79 (22.8)	1.32 (0.79–2.23)	0.38
Allergic rhinitis at 2.5 years	16/83(19.3)	11/78 (14.1)	1.37 (0.68–2.76)	0.51
Sensitization at 1 year	10/85 (11.8)	16/82 (19.5)	0.60 (0.29–1.25)	0.24
Sensitization at 2.5 years	17/69 (24.6)	14/67 (20.9)	1.18 (0.63–2.20)	0.75

4. Discussion

In this RCT designed to investigate the effects of vitamin D supplementation during early infancy in those infants "at-risk" of allergy development, but with sufficient vitamin D status at birth, we found no statistically significant differences in the incidence of allergic diseases over the first 2.5 years of life between the vitamin D intervention and placebo control groups.

Our finding of no statistically significant effect between vitamin D supplementation and medically diagnosed allergic disease over the first 2.5 years of age is consistent with other intervention trials which have been performed investigating the effect of maternal vitamin D supplementation during pregnancy and offspring allergic disease outcomes [15–18]. Even more importantly, our findings are also consistent with those in the recent infant RCT by Rosendahl et al. [25], where they also found no statistically significant differences in allergic disease outcomes at 1 year between two groups of infants supplemented daily with either 10 (400 IU) or 30 µg (1200 IU) vitamin D from the age of 2 weeks. While our trial investigated the effect of the Australian recommended dose (400 IU) [26] for vitamin D supplementation in infancy versus placebo on allergy outcomes in early childhood, the study by

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Rosendahl et al. was based on a secondary analysis comparing two different supplemental doses of vitamin D on allergy outcomes in the first year of life. Rosendahl et al. [25] found no statistically significant differences between the vitamin D supplementation groups for any food allergy (OR 1.35; 95% CI 0.75–2.46), atopic eczema (OR 0.72; 95% CI 0.49–1.01) or wheeze (OR 0.94; 95% CI 0.58–1.50) outcomes at 1 year of age. Furthermore, and of concern, Rosendahl et al. [25] found a higher incidence of cow's milk allergy in infants administered 30 µg (1200 IU) vitamin D compared with the 10 µg (400 IU) dose (OR 2.23; 95% CI 1.00–4.96), indicating a possible adverse effect of high doses of infant vitamin D supplementation. This has also been identified in the Japanese RCT by Norizoe et al. [21], where maternal vitamin D supplementation (800 IU/day) during early lactation was found to increase the risk of food allergy at 2 years of age.

Due to the exclusion criteria for our RCT of infants with maternal 25(OH)D levels of <50 nmol/L in late pregnancy, it is unclear whether the null findings of this trial would also apply to infants born at higher risk of vitamin D deficiency. However, following international guidelines [27,28], we, along with our Institutional Ethical Review Committee, did not think that it is ethical to allow infants to be knowingly vitamin D-deficient without treatment. Thus, the inclusion of only infants whose mothers were vitamin D-sufficient in late gestation, and the regular three-month testing of vitamin D status within the intervention period of this trial minimized the number and period of time any infant had a deficient vitamin D status. This may have influenced the outcome of our results and explain why we found no differences between the vitamin D intervention group compared to the placebo group. We chose to supplement only the first six months of infancy, but acknowledge that a longer intervention period throughout infancy, and even into early childhood, may have resulted in differences in vitamin D status and in allergy outcomes between the groups at 1 and 2.5 years of age. We also acknowledge as a limitation of this trial, that clinical allergic disease outcomes are a secondary analysis and that our trial sample size was not computed for these clinical allergy outcomes, but rather our sample size was powered to detect potential differences in immune function outcomes at 6 months of age, as per previously published [22].

In our RCT and in that of Rosendahl et al. [25], both involving infants born at term gestation, we did not find any significant benefit of infant vitamin D supplementation on reducing the incidence of medically diagnosed wheeze at 1 year of age. This contrasts with the results at 1 year of age from a recently published paper by Hibbs et al. [29], where preterm black infants (mean gestational age of 33 weeks) were randomized to vitamin D supplementation of 400 IU/day until 6 months of age, and were found to have reduced recurrent wheezing (relative risk 0.66; 95% CI 0.47 to 0.94) compared with a placebo. It needs to be considered that these results were in preterm infants of a different ethnic group and based on parental report by questionnaire rather than medical diagnosis which can lead to misclassification. Hibbs et al. [26] excluded infants who had a 25(OH)D concentration less than 37.5 nmol/L initially, and then lowered this to 25 nmol/L during the trial recruitment period to improve generalizability of the sample and to improve accrual, as it was noted that the prior level of 37.5 nmol/L was excluding a high number of otherwise healthy asymptomatic neonates. In agreement with our RCT results, Hibbs et al. [26] found no significant differences for the most commonly seen allergic diseases in early childhood, eczema nor food allergy between their intervention and control groups. The effect of preterm infant vitamin D supplementation on reduced wheeze outcomes reported by Hibbs et al. [29] appears to relate to the concept previously suggested that vitamin D may have a role in fetal lung development [30], rather than on the development of allergic disease outcomes. This effect may have been found due to the inclusion of some vitamin D-insufficient infants. Furthermore, other antecedents of preterm birth including inflammation, maternal smoking, metabolic derangement, T-cell development and airway reactivity could potentially have had an impact on wheeze outcomes in premature children [31] and were not considered in the study by Hibbs et al. [29].

A limitation of our RCT was that the sample size was powered for immunological outcomes, rather than allergy clinical outcomes. Hence, we eagerly await with interest the clinical allergic disease outcomes from current RCTs investigating infant vitamin D supplementation with the same

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intervention dose (400 IU/day) in Melbourne, Australia (ANZCTR12614000334606) and in Chiba, Japan (UMIN 000034864). In addition, as these current trials are not only including infants who had sufficient vitamin D status at birth, the potential influence of antenatal vitamin D deficiency may also be able to be examined in these larger current RCTs.

It is interesting that multiple observational studies have previously found that lower vitamin D status at birth, and during infancy, has been associated with increased incidence of early childhood allergic disease [10–14]. This may have been a result of antenatal and/or postnatal vitamin D deficiency, which has not been able to have been investigated in RCTs like our trial reported here, where maternal vitamin D deficiency was an exclusion criterion, and for safety and ethical reasons, any identified infant vitamin D deficiency was treated.

Another potential explanation, related to the associations found in many observational studies between increasing distance from the equator and increasing incidence of allergic diseases is that vitamin D status could be a coincidental marker of ultraviolet (UV) light exposure. UV light may be having a direct effect on the development of allergic disease as well as having a role in vitamin D synthesis. This theory is supported by animal study data showing that UV radiation can cause antigen-specific regulatory T-cell and dendritic cell expansion, causing systemic immunosuppression irrespective of vitamin D status [32–35]. Moreover, mast cells [36] and regulatory B-cells affecting dendritic cell-mediated T-cell activation are also involved in UV exposure-triggered immunosuppression [37]. A study in mice by Gorman et al. [38] demonstrated that 25(OH)D3 was not essential in mediating the immunosuppressive effects of erythemal UV radiation on contact hypersensitivity responses. Bioactive molecules, such as urocanic acid [39,40] and nitric oxide [41], which are released after sunlight exposure from the skin into the circulation, have been proposed to mediate long-lasting effects on immune function. We suggest that future research closely monitor UV light exposure, both maternal during pregnancy and throughout infancy, to further advance our knowledge regarding our understanding and future recommendations in this field.

5. Conclusions

For "high-risk" infants who had sufficient vitamin D status at birth, early infancy oral vitamin D supplementation does not appear to reduce the development of early childhood allergic diseases, however, larger RCTs are required to confirm this. We also do however recommend more research on the possible effects of early life UV light exposure on the development of allergic disease outcomes.

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ORIGINAL ARTICLE

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Adverse Effects of High-Dose Vitamin D Supplementation on Volumetric Bone Density Are Greater in Females than Males

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ABSTRACT

Three years of high-dose vitamin D supplementation (400 IU, 4000 IU, 10,000 IU) in healthy vitamin D–sufficient individuals aged 55 to 70 years (serum 25(OH)D 30–125 nmol/L at baseline), resulted in a negative dose-response relationship for bone density and strength. This study examined whether response differed between males and females. A total of 311 participants (53% male) were randomized to 400 IU (male = 61, female = 48), 4000 IU (male = 51, female = 49), or 10,000 IU (male = 53, female = 49) daily vitamin D_3. Participants were scanned with high-resolution peripheral quantitative computed tomography (HR-pQCT) to measure total volumetric BMD (TtBMD) at baseline, 6, 12, 24, and 36 months. Finite element analysis estimated bone strength. Balance, physical function, and clinical biochemistry parameters were also assessed. Constrained linear mixed effects models determined time-by-treatment group-by-sex interactions. Baseline, 3-month, and 3-year levels of 25(OH)D were 76.3, 76.7, and 77.4 nmol/L (400 IU); 81.3, 115.3, and 132.2 (4000 IU); and 78.4, 188.0, and 144.4 (10,000 IU), respectively. There were significant time-by-treatment group-by-sex interactions for TtBMD at the radius ($p = .002$) and tibia ($p = .005$). Treatment with 4000 IU or 10,000 IU compared to 400 IU resulted in TtBMD losses in females, but this was not observed with males. After 3 years, females lost 1.8% (400 IU), 3.8% (4000 IU), and 5.5% (10,000 IU), whereas males lost 0.9% (400 IU), 1.3% (4000 IU), and 1.9% (10,000 IU) at the radius. At the tibia, losses in TtBMD were smaller, but followed a similar trend. There were no significant bone strength interactions. Vitamin D supplementation with 4000 IU or 10,000 IU, compared with 400 IU daily, resulted in greater losses of TtBMD over 3 years in healthy vitamin D–sufficient females, but not males. These results are clinically relevant, because vitamin D supplementation is widely administered to postmenopausal females for osteoporosis prevention. Our findings do not support a benefit of high-dose vitamin D supplementation for bone health, and raise the possibility of harm for females. © 2020 American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: BONE DENSITY; DUAL X-RAY ABSORPTIOMETRY; HIGH-RESOLUTION PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY; MICROARCHITECTURE; SEX DIFFERENCES; VITAMIN D; BONE QCT/μCT; PTH/VITAMIN D/FGF23; OSTEOPOROSIS; NUTRITION; AGING; DXA

Introduction

Age- and sex-related differences in bone mineral density (BMD) and bone quality are well documented. Typically, healthy adult males have a larger bone size, higher BMD, and higher bone strength than females. Females lose bone more rapidly than males as they age,[1–3] particularly around the time of menopause.[4,5] Most studies have used dual X-ray absorptiometry (DXA) to assess sex- and age-related differences in BMD; however, because DXA estimates BMD based on a two-dimensional measurement, results may be influenced by differences in bone size.

High-resolution peripheral quantitative computed tomography (HR-pQCT) is an alternative imaging modality to DXA, measuring BMD in three dimensions, assessing bone microarchitecture and geometry with higher resolution and sensitivity, and thereby decreasing some of the known DXA confounders. Few longitudinal studies exist using HR-pQCT to assess normal sex- and age-related differences.[6–8]

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Public clinical trial registration: http://clinicaltrials.gov/show/NCT01900860. Randomized Double-blind Study Investigating Dose-dependent Longitudinal Effects of Vitamin D Supplementation on Bone Health.

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Recently, HR-pQCT has been used to explore changes in volumetric BMD and strength associated with 3 years of high-dose vitamin D supplementation.[9] In this study of healthy male and female participants who were vitamin D–sufficient at baseline, it was found that higher doses of vitamin D supplementation (4000 IU or 10,000 IU daily) were associated with larger decreases in BMD. Although between-sex differences in bone response to vitamin D supplementation was not a prespecified outcome of this study, whether there is a difference in bone response to high-dose vitamin D supplementation between males and females is not known.

Our previous work in a population-based cohort has shown age-related changes in BMD, as measured by HR-pQCT, are different for males and females,[6] particularly for the age-range corresponding to our clinical trial. The objective of this exploratory analysis of the Calgary Vitamin D Study data was to compare the sex- and age-related changes in BMD and bone strength over 3 years of vitamin D supplementation in vitamin D–sufficient individuals.

Subjects and Methods

Study design

This 3-year randomized, double-blind clinical trial was designed to investigate the effects of high-dose daily vitamin D supplementation on bone density and strength[10] (Clinical Trial Registration NCT01900860). The trial was conducted between August 2013 and December 2017.

Participants

A total of 542 individuals were screened; 373 met inclusion criteria, of which the first 62 participants were enrolled in a pilot cohort and 311 were enrolled in the main cohort.[9] The present study focuses on the main cohort, consistent with our publication of the primary outcome data.[9] Recruitment and randomization occurred between August 2013 and November 2014. Participants were males and females, age 55 to 70 years (females at least 5 years postmenopause), with DXA (lumbar spine and total hip) areal BMD *T*-scores above −2.5 SD, serum 25-hydroxyvitamin D [25(OH)D] between 30 and 125 nmol/L and normal serum calcium (2.10–2.55 mmol/L). Participants were excluded if their screening serum 25(OH)D was <30 nmol/L or >125 nmol/L; serum calcium was >2.55 mmol/L or <2.10 mmol/L; or vitamin D supplement use >2000 IU/day for the past 6 months. Additional exclusions have been published previously.[9] The trial was approved by the Conjoint Health Research Ethics Board at the University of Calgary, and Health Canada. Each participant provided written informed consent before randomization.

Randomization and intervention

Participants were randomized, within sex, in a 1:1:1 ratio to receive either 400 IU, 4000 IU, or 10,000 IU vitamin D_3 cholecalciferol, taken orally once per day in the form of liquid drops (Ddrops®; Woodbridge, ON, Canada): five drops/day (400: 80 IU/drop; 4000: 800 IU/drop; or 10,000: 2000 IU/drop). A statistician unrelated to the trial generated the randomization table, which was stratified by sex using simple randomization blocks of 60 or 30. Adherence to treatment was calculated from daily diaries as number days of supplement administration versus total number of days of follow-up, expressed as a percentage.

During the trial, participants were permitted to take up to 200 IU/day of additional vitamin D. In addition to vitamin D_3 supplementation, each participant's dietary calcium intake was assessed by a food frequency questionnaire.[11] Participants not consuming the recommended daily allowance (1200 mg/day) received additional calcium supplement tablets (300 mg elemental calcium as citrate) as needed, up to a maximum of 600 mg supplemental calcium/day.

Outcomes and procedures

As reported previously,[9] the four co-primary outcome variables for the trial were total volumetric BMD (TtBMD by HR-pQCT) and bone strength (estimated by finite element analysis) at the distal radius and tibia. The secondary bone outcomes for the trial included total hip areal BMD by DXA; HR-pQCT measures of cortical and trabecular volumetric BMD (CtBMD, TbBMD), cortical porosity (CtPo), and trabecular number (TbN); and assessment of balance (postural sway), physical function (timed up-and-go and grip strength) and quality of life (Short Form 36 Health Survey [SF-36] questionnaire).

Volumetric BMD and bone microarchitecture were assessed with HR-pQCT (nominal isotropic voxel of 60.7 μm; XtremeCT II; SCANCO Medical AG, Brüttisellen, Switzerland), following three-dimensional image registration[12] at baseline, 6, 12, 24, and 36 months. Bone strength was assessed as failure load, estimated by finite element analysis (FAIM, v8.0; Numerics88 Solutions, Calgary, AB, Canada). Scans were assessed for motion[13] and percent overlap between registered images. Scans with motions scores of four or higher and scans where percent overlap was <75% were removed from the analysis. Areal BMD was measured annually by DXA (GE Lunar iDXA; GE Healthcare, Piscataway, NJ, USA; enCORE v16). HR-pQCT precision scores in our laboratory are <2.4% for density and <3.3% for microarchitecture parameters, with the exception of cortical porosity (<13.7%),[14] and DXA precision is 0.55% coefficient of variation (CV) for the total hip.

Balance was assessed as postural sway using the modified Clinical Test of Sensory Interaction and Balance (m-CTSIB; Biosway machine; Biodex, Shirley, NY, USA; 950–460).[15] The most difficult testing condition involving standing on a foam surface with eyes closed is presented. Physical function was evaluated with grip strength (Jamar 200-lb hand dynamometer; Austin, TX, USA; 12–0600),[16] and timed up-and-go.[17] Participants completed a self-administered SF-36 v2 to assess quality of life.[18] Assessments of balance, physical function, and quality of life were completed during annual visits.

In addition, morning fasting blood samples were assessed in real time for serum 25(OH)D, intact parathyroid hormone (PTH), and C-telopeptide of type 1 collagen (CTx). Serum 25(OH)D was measured by DiaSorin Liaison XL system (DiaSorin, Stillwater, MN, USA). PTH was measured using the DiaSorin Liaison 1–84 PTH assay and CTx was measured by Cobas e immunoassay analyzer (Roche Diagnostics, Mannheim, Germany) electrochemiluminescence immunoassay (ECLIA). Since the primary manuscript was published, additional analysis on stored frozen serum was performed to measure intact amino-terminal propeptide of type I collagen (P1NP). P1NP was assessed using the UniQ® P1NP radioimmunoassay (Orion Diagnostica, Espoo, Finland), in the laboratory of Dr. Richard Kremer (McGill University, Montreal, QC, Canada). Measurements of 25(OH)D and PTH were captured at baseline and at months 3, 6, 12, 18, 24, 30, and 36. CTx was assessed at baseline and at months 6, 12, 24, and 36;

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and P1NP was assessed at baseline and at months 3, 6, 12, 24, and 36.

Statistical analysis

The primary aim of our original 3-year trial was to investigate the effect of the three doses of vitamin D supplementation on bone density and strength.[10] The detailed description of the sample size calculation for the primary outcome variables has been previously published.[9] For this post hoc analysis we are unlikely to have enough power to detect all three-way interactions in these variables, should they exist. In addition, because this was an exploratory analysis after publication of the primary analysis, we did not apply formal corrections for multiple comparisons. The large number of variables examined increases the probability of type II error.

Participant characteristics were described using means and standard deviations (SDs) or total number and percentage. Any individuals who received at least one dose of study drug and at least one follow-up visit were included in this analysis. Previously, a constrained linear mixed effects model was used to analyze each outcome variable.[9] Fixed effects included treatment group, and a quadratic effect of time if warranted. Random effects included time in which both intercept and slope could vary. In the previous model, a treatment effect was identified by a significant treatment-by-time interaction. The correlation within participants was modeled as an autoregressive error of order 1. Missing data were accounted for by using the mixed effects model.[19] To look for a subgroup effect of sex, the previous model was extended to include a time-by-treatment group-by-sex interaction, which if statistically significant indicated a significant sex effect. To compare age-related change in TtBMD the linear relationship between age and the 3-year change from baseline was examined. The distribution of serum PTH, CTx, and P1NP, stratified by sex were examined using boxplots and sex differences. Due to inadequate model fit, serum 25(OH)D differences between males and females were examined at baseline, at months 18 and 36, using a *t* test. Analyses were conducted using the R project for Statistical Computing (R, version 3.4; R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org/). An independent Data Safety and Monitoring Board oversaw the study.

Results

A total of 311 participants (53% male) were randomized and enrolled in the study. A flow diagram of participants throughout the study is presented in Fig. 1. Number of participants who discontinued study intervention were similar between supplementation groups and sex, with 287 (92%) participants completing the trial and >99% adherence to treatment protocol in all groups. A total of 303 participants were included in analyses, having received at least one dose of the study intervention and at least one follow-up HR-pQCT scan. A summary of participant characteristics at baseline are shown in Table 1.

Some participants have missing HR-pQCT data due to motion artifact (12 participants had at least one radius scan removed) or inadequate (<75%) overlap in scan region across all study visits (13 participants had radius scans at one time point removed). Two participants had all radius scans removed due to inadequate scan region overlap and the presence of a cyst in the scan region.

The effect of time-by-treatment group-by-sex was significant for TtBMD at the radius (p = .002) and tibia (p = .005) (Fig. 2*A–B*). Both the overall and the dose-dependent effect in TtBMD was greater for females than males at both the sites. At the radius in females, the 10,000 IU group lost the largest amount of bone over the 3 years, followed by the 4000 IU group whereas the 400 IU group lost the least amount of TtBMD. Although the same trend was observed in males, the results were not statistically significant. At trial end, for females the difference between the 4000 and 400 IU group was −6.3 mg hydroxyapatite (HA)/cm^3 (95% CI, −9.7 to −2.8) and between the 10,000 and 400 IU group was −11.6 mg HA/cm^3 (95% CI, −15.1 to −8.2) for TtBMD at the radius, based on mixed model analysis. This is equivalent to changes from the mean baseline values of −1.8% (400 IU), −3.8% (4000 IU) and −5.5% (10,000 IU). For males, the difference between the 4000 and 400 IU group was −1.1 mg HA/cm^3 (95% CI, −4.2 to 2.0) and between the 10,000 and 400 IU group was −3.4 mg HA/cm^3 (95% CI, −6.4 to 0.3) for TtBMD at the radius. This is equivalent to changes from the mean baseline values of −0.9% (400 IU), −1.3% (4000 IU), and −1.9% (10,000 IU) at the radius.

At the tibia, a similar effect was seen, with a dose-related response for females, but almost no effect (<0.6%) over time or between groups for males. At trial end, for females, the difference between the 4000 and 400 IU group was −3.0 mg HA/cm^3 (95% CI, −5.6 to −0.4) and between the 10,000 and 400 IU group was −6.2 mg HA/cm^3 (95% CI, −8.7 to −3.6) for TtBMD at the tibia, based on mixed model analysis. This is equivalent to changes from the mean baseline values of −1.0% (400 IU), −2.0% (4000 IU), and −3.2% (10,000 IU). For males, the difference between the 4000 and 400 IU group was +0.5 mg HA/cm^3 (95% CI, −1.8 to 2.9) and between the 10,000 and 400 IU group was −1.5 mg HA/cm^3 (95% CI, −3.9 to 0.8) for TtBMD at the tibia. This is equivalent to changes from the mean baseline values of −0.1% (400 IU), 0.0% (4000 IU) and − 0.6% (10,000 IU) at the tibia.

There was an effect of time-by-treatment group-by-sex for CtBMD at the tibia (p = .036) and a similar trend was seen at the radius (p = .072) (Fig. 2*C–D*). The 10,000 IU group lost the largest amount of CtBMD over the 3 years, followed by the 4000 IU group and 400 IU group. At trial end, for females the difference between the 4000 and 400 IU group was −7.9 mg HA/cm^3 (95% CI, −15.3 to −0.5) and between the 10,000 and 400 IU group was −15.4 mg HA/cm^3 (95% CI, −22.7 to −8.0) for CtBMD at the tibia, based on mixed model analysis. This is equivalent to changes from the mean baseline values of −1.6% (400 IU), −2.5% (4000 IU) and −3.4% (10,000 IU). Whereas for males, the difference between the 4000 and 400 IU group was +0.6 mg HA/cm^3 (95% CI, −6.1 to 7.4) and between the 10,000 and 400 IU group was −2.5 mg HA/cm^3 (95% CI, −9.1 to 4.1) for CtBMD at the tibia. This is equivalent to changes from the mean baseline values of −0.4% (400 IU), −0.3% (4000 IU), and −0.7% (10,000 IU) at the tibia.

At the radius at trial end, the difference for CtBMD in females between the 4000 and 400 IU group was −10.8 mg HA/cm^3 (95% CI, −17.2 to −4.2) and between the 10,000 and 400 IU group was −13.0 mg HA/cm^3 (95% CI, −19.5 to −6.5), based on mixed model analysis. This is equivalent to changes from the mean baseline values of −1.3% (400 IU), −2.5% (4000 IU), and −2.8% (10,000 IU). In males, the change in CtBMD at the radius between the 4000 and 400 IU group was −1.9 mg HA/cm^3 (95% CI, −7.8 to 3.9) and between the 10,000 and 400 IU group was −4.1 mg HA/cm^3 (95% CI, −9.9 to 1.8). This is equivalent to changes from the mean baseline values of −0.5% (400 IU), −0.8% (4000 IU), and −1.0% (10,000 IU) at the radius.

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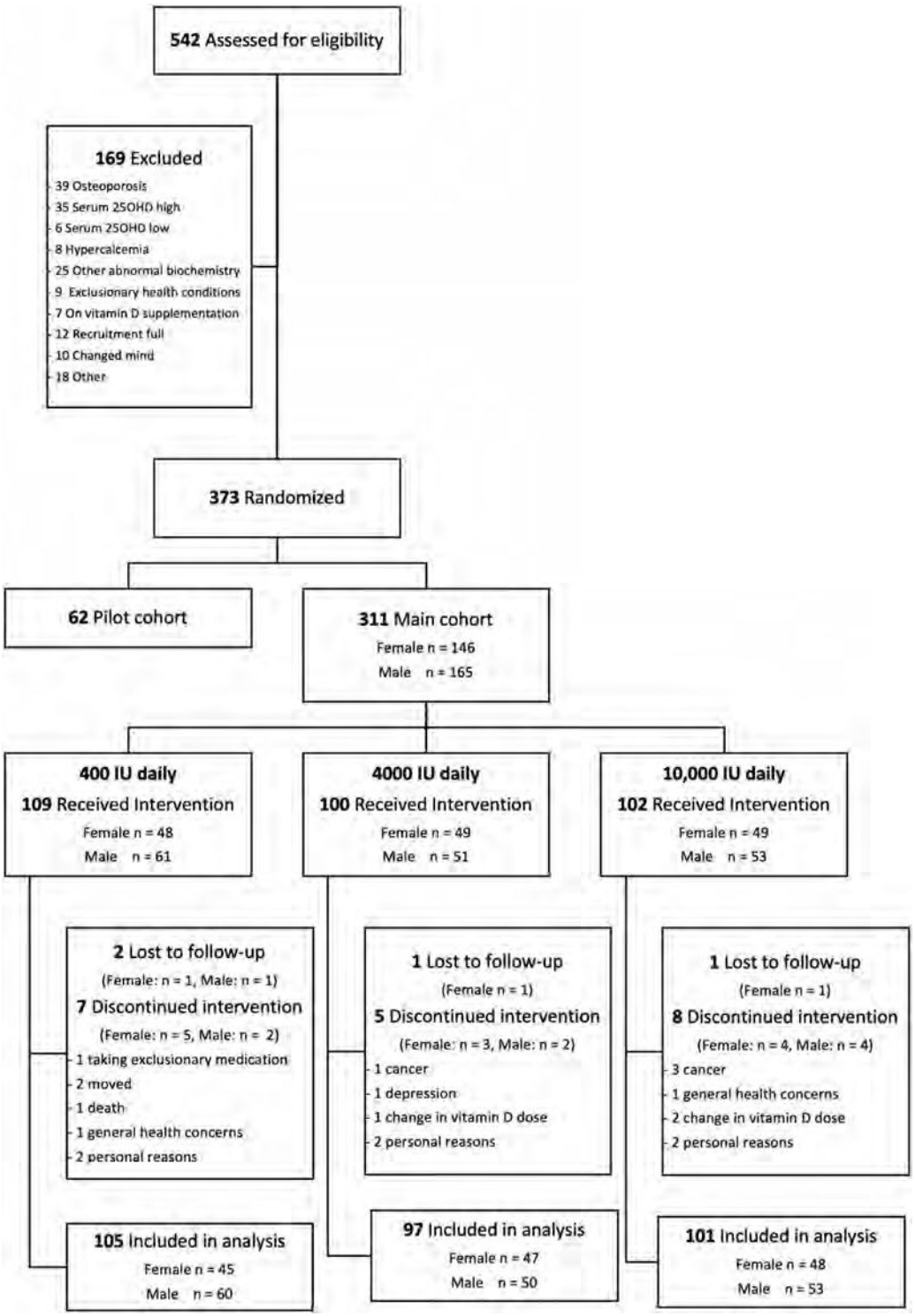


Fig 1. Flow of participants through the study.

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Table 1. Participant Characteristics for Females and Males at Baseline

Variable	400 IU		4000 IU		10,000 IU	
	Female	Male	Female	Male	Female	Male
Participants, *n*	45	60	47	50	48	53
Age (years), mean ± SD	61.8 ± 3.8	62.5 ± 4.4	61.8 ± 4.4	63.3 ± 4.2	61.7 ± 4.1	62.1 ± 4.1
Years since menopause, mean ± SD	12.6 ± 5.9	–	11.7 ± 7.3	–	12.5 ± 5.6	–
BMI (kg/m^2), mean ± SD	27.8 ± 5.3	27.5 ± 3.4	28.6 ± 6.2	27.7 ± 3.6	26.7 ± 5.0	27.4 ± 3.2
Body fat (%), mean ± SD	39.8 ± 7.5	28.5 ± 6.7	39.5 ± 8.6	28.8 ± 5.5	37.0 ± 7.7	27.8 ± 6.6
Ethnicity (% white), *n* (%)	44 (97.8)	54 (90.0)	45 (95.7)	49 (98.0)	46 (95.8)	52 (98.1)
Systolic blood pressure (mmHg), mean ± SD	130.9 ± 17.8	126.7 ± 13.3	126.6 ± 13.1	136.3 ± 18.4	124.0 ± 18.3	127.1 ± 13.6
Diastolic blood pressure (mmHg), mean ± SD	81.5 ± 8.2	80.9 ± 7.4	78.0 ± 6.7	83.4 ± 8.9	78.9 ± 10.2	81.8 ± 10.7
History of skin cancer, *n* (%)[a]	6 (13.3)	6 (10.0)	1 (2.1)	6 (12.0)	6 (12.5)	9 (17.0)
History of non-skin cancer, *n* (%)	1 (2.2)	4 (6.7)	4 (8.5)	3 (6.0)	4 (8.3)	6 (11.3)
History of CV problems (%)	46.7	58.3	51.1	70.0	56.2	56.6
Type 2 diabetes (%)	0.0	3.3	4.3	4.0	0.0	5.7
Rheumatoid arthritis (%)	2.2	0.0	4.3	0.0	2.1	0.0
Asthma (%)	6.7	3.3	8.5	4.0	12.5	5.7
Smoker, *n* (%)	2 (4.4)	0 (0.0)	0.0	0.00	2 (4.2)	2 (3.8)
Falls, *n* (%)[b]	9 (20.0)	13 (21.7)	11 (23.4)	9 (18.0)	11 (22.9)	7 (13.2)
Fracture since 50 years, *n* (%)	11 (24.4)	6 (10.0)	7 (14.9)	6 (12.0)	11 (22.9)	6 (11.3)
Lumbar spine *T*-score, mean ± SD[c]	−0.6 ± 1.1	0.5 ± 1.5	−0.2 ± 1.2	0.6 ± 1.3	−0.8 ± 1.0	0.9 ± 1.4
Total hip *T*-score, mean ± SD[d]	−0.5 ± 0.8	0.6 ± 1.0	−0.3 ± 1.0	0.7 ± 1.0	−0.6 ± 0.8	0.5 ± 1.1
Serum 25(OH)D (nmol/L), mean ± SD	78.5 ± 20.5	75.3 ± 21.5	79.9 ± 21.2	82.7 ± 19.0	81.7 ± 19.6	75.5 ± 16.9
Deficient/insufficient/sufficient, *n* (%)[e]	0/4/41 (0/9/91)	0/6/54 (0/10/90)	0/3/44 (0/6/94)	0/2/48 (0/4/96)	0/4/44 (0/8/92)	0/4/49 (0/8/92)
Serum PTH (ng/L), mean ± SD[f]	23.1 ± 7.4	21.6 ± 7.9	21.9 ± 6.9	21.0 ± 6.3	21.5 ± 5.5	22.7 ± 8.8
Serum calcium (mmol/L), mean ± SD[g]	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
Serum phosphate (mmol/L), mean ± SD[h]	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	1.1 ± 0.2	1.0 ± 0.1
Serum creatinine (μmol/L), mean ± SD	70.9 ± 10.5	88.0 ± 11.5	69.2 ± 10.6	89.6 ± 11.7	70.6 ± 8.8	88.7 ± 13.0
Estimated GFR (mL/min/1.73m^2), mean ± SD	80.3 ± 12.4	81.1 ± 10.4	81.8 ± 12.9	79.0 ± 11.1	80.3 ± 11.2	80.2 ± 11.8
24-Hour urine calcium (mmol/day), mean ± SD[i]	4.2 ± 1.8	4.2 ± 2.2	4.3 ± 2.0	5.4 ± 2.2	3.9 ± 1.8	4.5 ± 2.2
Serum alkaline phosphatase (U/L), mean ± SD[j]	74.8 ± 21.3	64.9 ± 14.0	66.9 ± 13.8	64.7 ± 16.6	70.3 ± 18.6	69.8 ± 15.8

(Continues)

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Table 1. Continued

Variable	400 IU		4000 IU		10,000 IU	
	Female	Male	Female	Male	Female	Male
Plasma CTx (beta-crosslaps) (ng/L), mean ± SD[k]	371.2 ± 125.5	308.7 ± 106.9	372.8 ± 130.6	307.9 ± 124.0	390.1 ± 117.2	303.8 ± 121.6
Hemoglobin A1C (%), mean ± SD	5.7 ± 0.3	5.6 ± 0.4	5.7 ± 0.3	5.6 ± 0.3	5.6 ± 0.3	5.7 ± 0.9
Fasting blood glucose (mmol/L), mean ± SD	5.0 ± 0.7	5.2 ± 0.9	5.0 ± 0.7	5.1 ± 0.6	4.8 ± 0.7	5.5 ± 2.0
P1NP (µg/L), mean ± SD[l]	49.7 ± 19.3	39.7 ± 13.8	50.0± 18.1	38.5 ± 12.7	52.4 ± 16.6	40.8 ± 17.9

25OHD = 25-hydroxyvitamin D; BMI = body mass index; CTx = C-telopeptide of type 1 collagen (beta-crosslaps), a marker of bone resorption; GFR = glomerular filtration rate; P1NP = N-terminal propeptide of type I collagen; PTH = parathyroid hormone.

[a]Includes melanoma.

[b]Number of falls in the last year.

[c]Lumbar spine *T*-scores range from +4.7 to −2.4 meaning participants were not osteoporotic (osteoporosis is defined as a *T*-score below −2.5).

[d]Total hip *T*-scores range from +3.8 to −2.3 meaning participants were not osteoporotic (osteoporosis is defined as a *T*-score below −2.5).

[e]Deficient 25OHD = <30 nmol/L, insufficient 25OHD = 30–50 nmol/L, sufficient 25OHD = >50 nmol/L.

[f]Expected normal range for PTH 7–37 ng/L.

[g]Expected normal range for serum calcium 2.10–2.55 mmol/L.

[h]Expected normal range for serum phosphate 0.80–1.50 mmol/L.

[i]Expected normal range for 24-hour urine calcium 2.5–7.5 mmol/day.

[j]Expected normal range for serum alkaline phosphatase 30–145 U/L.

[k]Premenopausal reference range for plasma CTx 0–400 ng/L.

[l]Expected normal range for P1NP: postmenopausal female, 19–83 µg/L; male, 22–87 µg/L.

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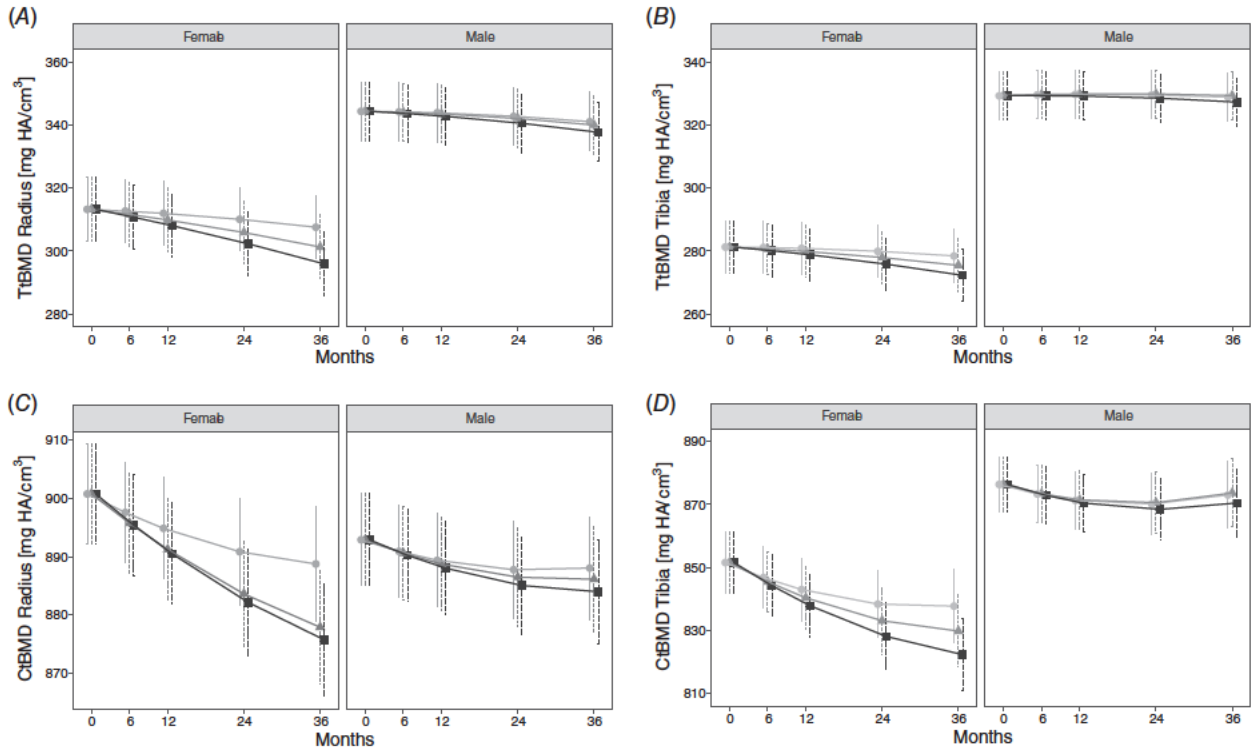


Fig 2. Sex differences in changes in TtBMD (*A*,*B*) and CtBMD (*C*,*D*) over 3 years of vitamin D supplementation, by treatment group. The modeled data of both the radius (*A*,*C*) and tibia (*B*,*D*) show the mean 95% CIs for the predicted values. Females are on the left-hand side and males on the right. Circle, light gray = 400 IU group; triangle, medium gray = 4000 IU group; square, black = 10,000 IU group. TtBMD = total bone mineral density; CtBMD = cortical bone mineral density.

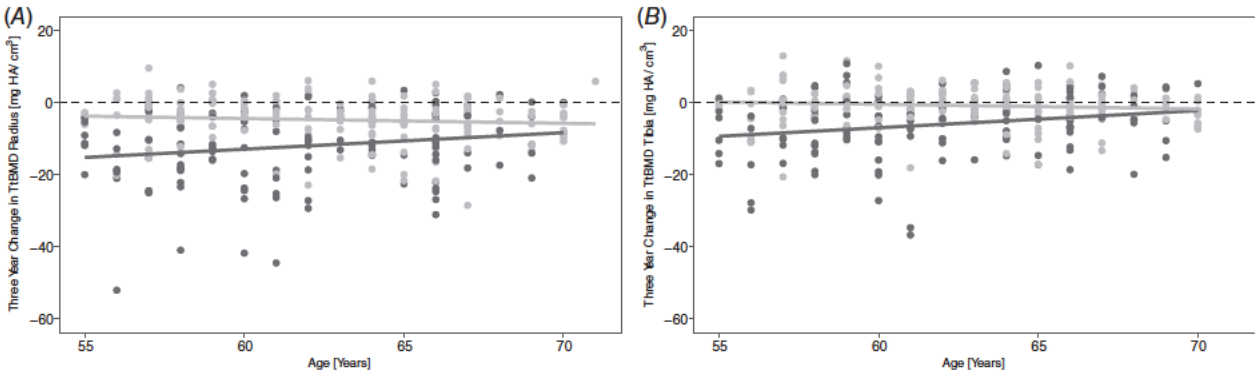


Fig 3. Sex differences in the relationship between age and TtBMD during 3 years of vitamin D supplementation. The modeled data show the mean (solid line) for the predicted values, for the radius (*A*) and the tibia (*B*). The dashed line represents zero change. Values above the dashed line show gains in TtBMD and values under represent loss in TtBMD. Light gray, males; dark gray, females. TtBMD = total bone mineral density.

Figure 3 shows age-related differences in TtBMD change for males and females. Over the 3-year study, younger females (closer to age 55 years) lost more bone than older females (closer to age 70 years) at both the radius (p = .013, Fig. 3*A*) and tibia (p = .001, Fig. 3*B*). There were no significant age-related differences for males at the radius (p = .275) or tibia (p = .197).

There was no evidence of significant time-by-treatment group-by-sex interaction effects for balance (p = .852), grip

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strength (p = .981), timed up-and-go (p = .474), or quality of life (p = .507) over the duration of the study. Although the time-by-treatment group-by-sex interaction was significant for DXA-based total hip areal BMD (p = .041), the interaction was not significant because there was no dose-response relationship in females (p = .732).

The distribution of serum 25(OH)D, PTH, CTx, and P1NP, stratified by sex, are displayed in Fig. 4*A–H*. There were no significant differences between males and females in serum 25(OH)D at baseline (p = .295), month 18 (p = .258) or month 36 (p = .717). Highest serum 25(OH)D values were reported in month 18 in the 10,000 IU group. There was no evidence for a treatment group-by-sex effect for serum PTH (p = .097); however, there was a nonlinear treatment group effect over time that was the same for both sexes (p = .002). Values were stable for the 400 IU group and declined in a dose-response manner, with the lowest values recorded by the 10,000 IU group at month 12 to 18. There was no evidence for a treatment group-by-sex effect for plasma CTx (p = .299); however, females in the 10,000 IU group had higher CTx values than the 400 IU group, but this was not significant (p = .507). When treatment group was not considered, there was a trend over time for plasma CTx to be higher in females than males (p = .097). For P1NP there was a treatment group-by-sex trend with higher values in females than males (p = .096).

Discussion

This analysis examined the sex- and age-related changes in bone density and strength following 3 years of vitamin D supplementation. The results show significant differences in dose-related change of TtBMD between males and females at both the radius and tibia. In males, there was no detected dose-response effect of vitamin D supplementation on bone density. However, in females the effect was significant, with those taking 4000 or 10,000 IU of daily vitamin D losing more bone than those taking 400 IU. In addition to TtBMD, a similar dose-response relationship was found for CtBMD. Furthermore, younger females, closer to menopause, lost larger amounts of TtBMD than older females at both the radius and tibia. These findings are clinically relevant, because vitamin D supplementation is widely administered to postmenopausal females for osteoporosis prevention. Although differences in sex and supplementation group were observed over the 3 years for volumetric BMD, similar interactions for bone strength were not observed.

Unlike the HR-pQCT volumetric BMD results, the DXA-based total hip areal BMD results show no interaction effect of sex and vitamin D supplementation dose over the duration of the study. Preceding vitamin D trials reported similar results, failing to observe differences in treatment and sex effects using DXA,[20,21] although there was a trend for smaller decreases in areal BMD at the spine for females.[20] Similarly, no vitamin D supplementation and sex effects were observed using peripheral quantitative computed tomography (pQCT) in the VITAL Trial.[20]

In normal aging populations, age-by-sex-by-time interactions have been reported for TtBMD using pQCT at the tibia, where females had a steeper rate of decline than males.[22] That finding is supported by HR-pQCT studies where age-by-sex interactions were observed at the radius and tibia for total and cortical volumetric BMD but not trabecular BMD,[7] consistent with our findings. We speculate increased endocortical remodeling due to estrogen deficiency is likely responsible for the cortical bone loss observed in this study.

The increase in bone resorption with higher doses of vitamin D, indicated by higher CTx in females, is not unexpected. High-dose vitamin D_3 supplementation results in increased conversion into the active vitamin D metabolite, calcitriol,[23] and may also stimulate local (autocrine) synthesis of calcitriol within the bone cell environment.[24,25] Calcitriol has been demonstrated to act directly on bone cells through the vitamin D receptor.[26,27] Although the vitamin D receptor is expressed in osteoblasts and osteoclasts, it appears calcitriol's main effect on bone resorption is primarily through stimulation of osteoblasts to facilitate recruitment of osteoclast precursors and activation of osteoclasts.[28 31]

The bone formation marker, P1NP, did not change over the duration of the trial, although there was a trend for P1NP to be higher in females than males. This may reflect a physiological response to increase bone formation to match bone resorption; although, the overall decrease in BMD observed in females taking high does vitamin D supplementation was likely driven by increased resorption (CTx). Within this study, any increase in bone formation is likely to be independent of PTH, as the well-described suppressive effect of vitamin D on PTH secretion was observed in both sexes, and was dose-dependent (Fig. 4).

Over the duration of the study, females lost TtBMD between −1.8% and −5.5% at the radius and between −1.0% and −3.2% at the tibia, depending on vitamin D dose. Previously published 3-year changes in TtBMD for normal aging postmenopausal females are −2.1% and −1.3% at the radius and tibia, respectively.[7] Similar 3-year results have been estimated based on 5-year longitudinal change data, in a normative population-based cohort, where TtBMD decreased −2.9% at the radius and −1.4% at the tibia in females.[6] For males, the results presented in the current study with vitamin D supplementation were between −0.9% and −1.9% at the radius and less than −1% irrespective of vitamin D dose at the tibia. Our findings align with others that show males lose less than −1% of TtBMD over 3 years at both the radius and tibia.[6,7] It appears that high-dose vitamin D supplementation does not have the same negative impact on bone adaptation in males that we showed for females. However, in females a dose-response acceleration of bone loss with vitamin D supplementation is clear. Rates of bone loss for females in this study who were taking 400 IU daily were comparable with previously published rates; however, females taking 4000 and 10,000 IU daily vitamin D lost TtBMD at a higher rate.[6,7]

Over the 3-year study, younger females receiving vitamin D supplementation lost more TtBMD than older females. This is consistent with previous HR-pQCT studies where younger females (50–59 years) had a larger annual loss than older females (60–69 years),[6,7] but differs from DXA studies that report larger areal BMD losses at the hip in older males and females.[1 3] Furthermore, a pQCT study reported accelerated bone loss after the age of 65 years in females.[22] It is concerning that bone loss is greater in younger females (closer to 55 years of age), but perhaps this emphasizes that the increased bone turnover around menopause is compounding the effects of high-dose vitamin D supplementation.

To our knowledge, this study is the first to look at sex-specific bone changes following high doses of vitamin D supplementation (4000 and 10,000 IU daily) for 3 years. However, our findings should be interpreted in the context of some limitations. First, an important limitation is that sex differences were an exploratory

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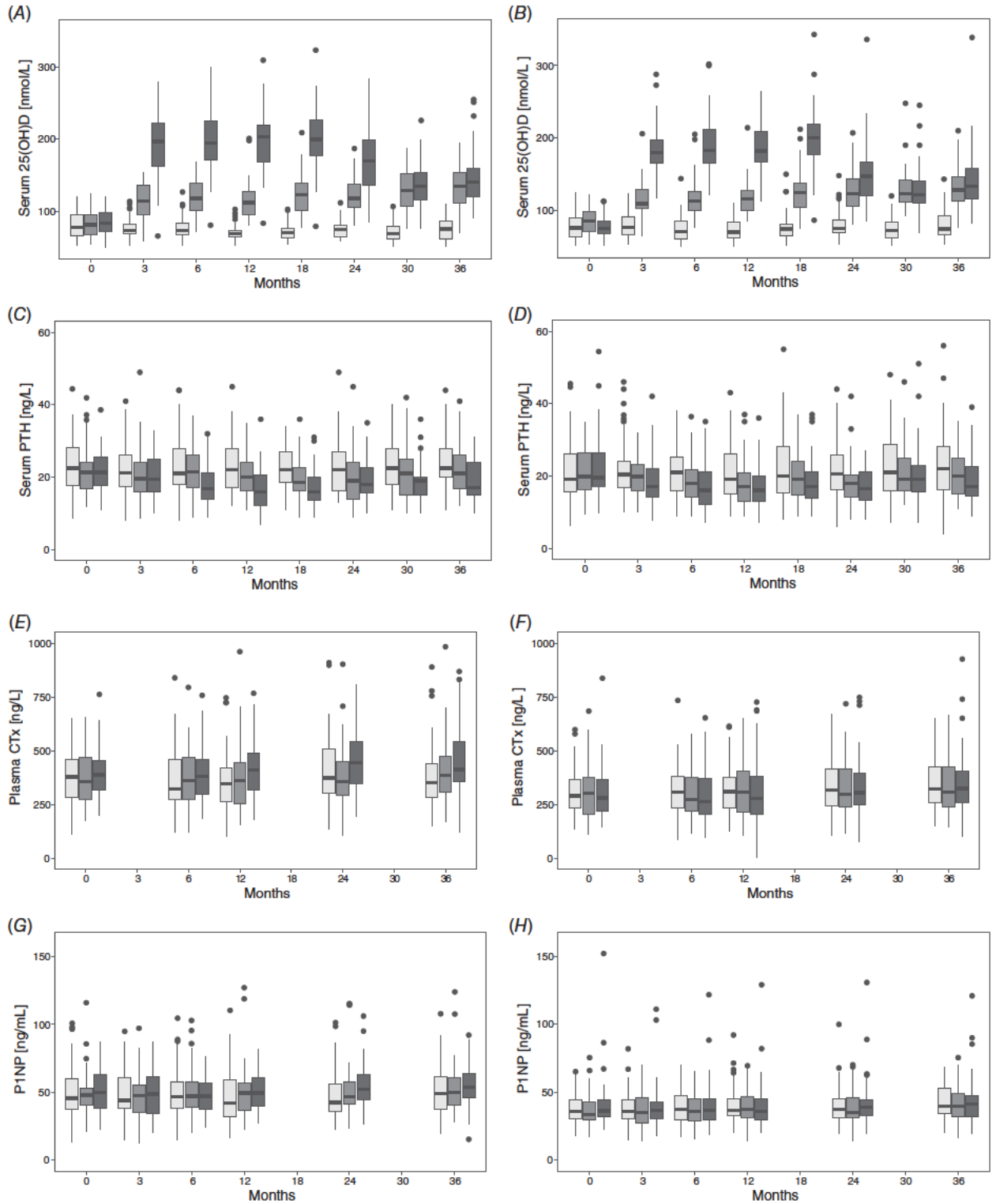


Fig 4. Distribution of 25(OH)D (*A,B*), PTH (*C,D*), CTx (*E,F*), and P1NP (*G,H*), by treatment group over the study duration, stratified by sex. The horizontal line in the middle of each box indicates the median, the top and bottom borders of the box mark the 75th and 25th percentiles, the whiskers above and below the box indicate the 90th and 10th percentiles, and the points beyond the whiskers are outliers beyond the 90th or 10th percentiles. (*A,C,E,G*) Females and (*B,D,F,H*) males. Light gray, 400 IU group; dark gray, 4000 IU group; black, 10,000 IU group. 25OHD = 25-hydroxyvitamin D; PTH = parathyroid hormone; CTx = C-telopeptide of type 1 collagen (beta-crosslaps); P1NP = N-terminal propeptide of type I collagen.

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outcome of the trial; thus, we may be underpowered to detect sex and treatment group effects over the 3 years, particularly in males where no statistical effects were found. Second, as we have reported previously,[9] two lots of the vitamin D supplementation administered to the 10,000 IU daily group between months 18 and 36 suffered varying degrees of degradation, observed in serum 25(OH)D levels (Fig. 4*A*–*B*). The estimated delivered dose to these participants ranged between 2000 to 10,000 IU daily. Nevertheless, it is important to note that despite this unfortunate problem, the 10,000 IU group maintained the highest serum 25(OH)D levels across the trial except for month 30 where the 10,000 and 4000 IU groups were similar. Third, participants with osteoporosis or with 25(OH)D levels <30 nmol/L were excluded. These factors may have altered the sex-related changes observed over study duration. Fourth, although we observed sex differences for the change in TtBMD, we did not find a concurrent effect on bone strength. The sensitivity of finite element analysis to detect changes in bone strength in a longitudinal study design is hampered by the inability to leverage image registration effectively.

Conclusion

Our findings suggest that the dose-dependent effect of high-dose vitamin D supplementation reported previously was primarily driven by females.[9] Among healthy vitamin D–sufficient females, supplementation with vitamin D at a dose of 4000 IU or 10 000 IU per day, compared with 400 IU per day, resulted in greater losses of TtBMD and CtBMD over 3 years. In males, there was no dose-response effect of vitamin D supplementation on volumetric BMD. These findings do not support a benefit of high-dose vitamin D supplementation for bone health, and raise the possibility of harm for females. Future studies of vitamin D supplementation should be designed to evaluate differences in response between males and females.

Disclosures

LAB, MSR, and DAH have nothing to declare. EOB has previously received honoraria from Amgen and Eli Lilly, and a research grant from Amgen. RK has previously received honoraria from Amgen, Eli Lilly, Novartis, Roche, and Servier, as well as a research grant from Merck, and is President and CEO of Biochrom Pharma Inc. SKB has received honorariums from Amgen and Servier, and is co-owner of Numerics88 Solutions, Inc.

Acknowledgments

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Authors' roles: LAB cleaned the data in preparation for statistical analysis, drafted the manuscript and Fig. 1. EOB and DAH were the study MDs and reviewed participant health and adverse events throughout the trial. MSR completed the statistical analysis, provided insights regarding data interpretation, and created Figs. 2, 3, and 4. RK was responsible for the P1NP data. DAH and SKB devised the study, and provided insights throughout the study and data analysis. SKB registered the HR-pQCT scans and performed the finite element analysis. All authors revised and finalized the manuscript.

Author contributions: LAB: Data curation; investigation; writing-original draft; writing-review and editing. EOB: Investigation; writing-review and editing. MSR: Formal analysis; visualization; writing-review and editing. RK: Resources; writing-review and editing. DAH: Conceptualization; funding acquisition; project administration; supervision; writing-review and editing. SKB: Conceptualization; funding acquisition; project administration; software; supervision; writing-review and editing.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1002/jbmr.4152.

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Exhibit L

Mom's Choice Award GOLD Seal

- The Mom's Choice Awards® (MCA) evaluates products and services created for children, families and educators. The program is globally recognized for establishing the benchmark of excellence in family-friendly media, products and services. The organization is based in the United States and has reviewed thousands of items from more than 55 countries.
- Around the world, parents, educators, retailers and members of the media look for the MCA mother-and-child Honoring Excellence seal of approval when selecting quality products and services for children and families.
- An esteemed panel of evaluators includes education, media and other experts as well as parents, children, librarians, performing artists, producers, medical and business professionals, authors, scientists and others.
- MCA evaluators volunteer their time and are bound by a strict code of ethics which ensures expert and objective analysis free from any manufacturer association.
- The evaluation process uses a proprietary methodology in which items are scored on a number of elements including production quality, design, educational value, entertainment value, originality, appeal and cost. Each item is judged on its own merit.
- MCA evaluators are especially interested in items that help families grow emotionally, physically and spiritually; are morally sound and promote good will; and are inspirational and uplifting.

Mom's Choice Awards®
Honoring Excellence

Honoring excellence in family-friendly media, products and services.

Presented to

Baby Ddrops®

Gold Award Recipient

September 27, 2012

Dawn Matheson | CEO

Roxanne D. Rask | COO

Exhibit M

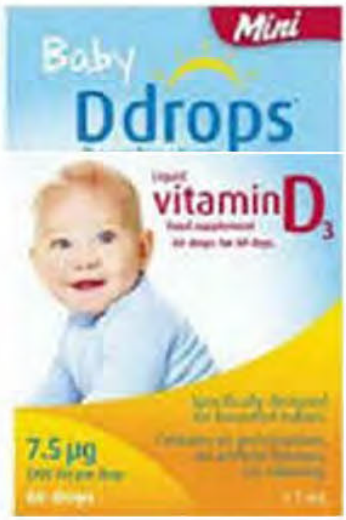


2015 Cribsie Awards New Arrival "Academy Star"

- Founded in 2010 and hosted by StrollerTraffic.com, the Cribsie Awards recognize the best products, services, and websites for babies and tots. The Cribsies offer a secure voting platform that limits users to one unique vote per email address, meaning the Cribsies are a true, untainted measure of the public's opinion on the top parenting brands.
- With hundreds of thousands of votes cast and a reach in the millions, the Cribsies have quickly become the definitive awards for parents.
- You nominate. We select. Parents vote.


ACADEMY STAR
2015
NEW ARRIVAL
THE
CRIBSIE AWARDS
CRIBSIEAWARDS.COM

Exhibit N



2014 Bounty Mums Love "Commended"

- Our mums have voted in their thousands for their fave brands, from the first set of wheels to the products that keep those cute chubby baby bottoms clean and dry.
- As a highly-commended product you will automatically benefit from the following activity that will drive to the BML page on Bounty.com:
    - Ongoing social media (FB & Twitter) shout outs
    - Inclusion in weekly newsletters (pre & post-natal) driving to the relevant Bounty MUMS LOVE sections
    - All winners / commended hosted on Bounty.com by category

bounty®

Contract No: CT9959
Opportunity No: 179135
Date Sent: 28 January 2015
AM: Trish Dominy

FOR AND ON BEHALF OF THE LICENSOR		FOR AND ON BEHALF OF THE LICENSEE	
SIGNATURE:	Susan Jenkins	SIGNATURE:	
NAME:	Susan Jenkins	NAME :	
TITLE:	Director Sales & Marketing	TITLE:	
DATE:	Jan. 30 2015	DATE:	

Exhibit O



2015 Well.ca Green Choice Award Winner

- In recognition of Earth Month, we launched our **First Annual *Green Choice Awards***, allowing our customers (that's you!) to vote on what their most loved and trusted green and natural brands were.
- There were 88 products in contention for the Green Choice Awards in 14 categories plus a Well.ca Staff Pick, allowing staff to choose their favourite from all of our Green and Natural brands.
- Earth Month is an important time for everyone to take stock and see what each one of us can do to help improve our planet. If we each made one small change, the earth would reap the rewards. This Earth Month, we encourage all Canadians to try a new, environmentally friendly product. Then come back and vote in the annual Green Choice Awards next year!

[redacted]

From: Susan Jankus <susan.jankus@ddrops.ca>
Subject: **FW: Congratulations from Well.ca - Green Choice Awards**
Date: February 23, 2015 at 2:16:55 PM MST
To: Angie Duz <angie.duz@ddrops.ca>, Sydney Poulos <sydney.poulos@ddrops.ca>, Anthony Talotta <anthony.talotta@ddrops.ca>

Hi Everyone,

This just came through from Well.ca

Be Well & Smile!

Susan

Ddrops The sunshine vitamin in just one drop

Susan Jankus
Director Sales & Marketing

126 Trowers Road, Woodbridge, Ontario L4L 5Z4 Canada
T. +1-866-761-9333 ext.240 F. +1 866 654 5212 E. susan.jankus@ddrops.ca

DdropsCompany.com

Baby Ddrops® awarded GOLD by Mom`s Choice Awards

Parent Tested Parent Approved

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From: Kathy Song <kathleen.song@well.ca>
Date: Monday, February 23, 2015 at 4:14 PM
To: Susan Jankus <susan.jankus@ddrops.ca>
Subject: Congratulations from Well.ca - Green Choice Awards

Hi there,

On behalf of the entire team at Well.ca, it is my pleasure to inform you that Ddrops Liquid Vitamins (https://well.ca/products/ddrops-liquid-vitamin-d3_7335.html) has been nominated as a potential recipient of the Healthy Baby 2015 Green Choice Awards!

This is the first annual Well.ca Awards and we couldn't be more excited. We are selecting 15 companies for their excellence as green and natural brands to be voted on. With over 3,000 brands, obviously the decision process was not easy. We hand selected our nominees in order to properly represent our growing Well.ca community.

How it works:

There will be 15 awards to be voted on, as well as 1 staff chosen award.

On March 9th, 2015 , voting will be opened up to the public.

It will run for 2 weeks, closing at 11:59 p.m. EST on March 20th,2015.

The winning brands will be selected and announced on April 2nd; time TBD.

Spread the Word:

The 2015 Well.ca Awards will be promoted via all of our social media channels and we hope that you will join us in their promotion! The first week of March we will launch all promotional activities for the awards, so feel free to cross-promote on your own websites and on Facebook, Twitter, Instagram and Pinterest. We invite you to share in this voting opportunity with your own community of customers and we have attached the official Well.ca awards stamp for you to use. Once the voting is live, we will provide you a link to the appropriate site.

If this has reached a Broker, Distributor or anyone who is not affiliated directly with the brand, please pass along updated contact details to Gillian Geremia (gillian.geremia@well.ca) or Kathy Song (kathy.song@well.ca).

Once again, congratulations! Please contact us with any questions, we'd be happy to hear from you.

Thank you,

--

Kathy Song | Marketing Coordinator
365 Bloor Street East, Suite 1910
Toronto, Ontario. M4W 3L4
W: 416.479.0313ext: 319





Exhibit P



2016 National Parenting Product Awards Winner

- The National Parenting Product Awards (NAPPA) is one of the longest running and most respected awards programs in the country. With 25 years of experience in the industry, we have been continuously celebrated as the "go-to" source for parents and professionals seeking the best products for their children and families.
- NAPPA enlists a team of independent expert judges and family testers to review each submission thoroughly. Together, they select the most entertaining, appealing, safe, educational, age-appropriate and enduring products as NAPPA winners. A NAPPA recognition is more than just a seal of approval. It signifies that a product has been rigorously evaluated against stringent criteria and that it stands out among hundreds of similar products from around the world. Products that receive the prestigious NAPPA award are quality products that parents and professionals can trust.
- All Ddrops products have been awarded Gold: Baby, Booster, Adult (1000 IU and 2000 IU) and Vegan Ddrops by Ddrops Company.

National Parenting Publications Awards

presents

Ddrops Company

With a 2015 Parenting Resources Gold Award for

Ddrops: Baby, Booster, Adult (1000 IU and 2000 IU) and Vegan



Exhibit Q


BAG
$1.29
$11.99
$6.99
$2.49
CBS
Baby
Ddrops
The sunshine vitamin in just one drop
Liquid
vitamin D3
Dietary supplement
60 drops for 60 days
Babies need vitamin D for healthy growth and development.
Contains no preservatives, no artificial flavors, no coloring.
400 IU
per drop
60 drops


BA
$11.99
$6.99
MARTHA
$1,000
$2,000
Ddrops
Ddrops
$6.9


The Price Is Right
3/31/2016
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Full Episodes: Season 44
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GRANOLA
CREAM CHEESE
COATING MIX
SUPPLEMENT
CANDY

Exhibit R

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Crocker et al. BMC Public Health 2011, 11:905
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BMC Public Health

RESEARCH ARTICLE Open Access

Very high vitamin D supplementation rates among infants aged 2 months in Vancouver and Richmond, British Columbia, Canada

Barbara Crocker[1], Tim J Green[2*], Susan I Barr[2], Bridgid Beckingham[1], Radhika Bhagat[1], Beata Dabrowska[1], Rachel Douthwaite[1], Carmen Evanson[1], Russell Friesen[2], Kathy Hydamaka[3], Wangyang Li[2], Kelly Simmons[1] and Lillian Tse[1]

Abstract

Background: Vitamin D deficiency during infancy may lead to rickets and possibly other poor health outcomes. The World Health Organization recommends exclusive breastfeeding for the first 6 months. Breast milk is the best food for infants but does not contain adequate vitamin D. Health Canada recommends all breastfed infants receive a daily vitamin D supplement of 400 IU; however, there appears to be limited current Canadian data as to whether parents or caregivers are following this advice. The aim of this study was to determine the rates of vitamin D supplementation among 2-month old infants in Vancouver and Richmond, British Columbia, Canada.

Methods: Mothers of all healthy infants born between April and May 2010 were approached to participate. Telephone surveys were conducted with 577 mothers (response rate 56%) when their infants turned 2 months.

Results: Over half of the infants received only breast milk in the week prior to the survey. One third received a mixture of breast milk and infant formula and 10% received only formula. About 80% of the infants were supplemented with vitamin D at 2 months. Infants who received only breast milk were most likely to be supplemented with vitamin D (91%). Over 60% of the infants had a total vitamin D intake of 300- < 500 IU/d from supplements and formula and only 5% did not receive any vitamin D. Most parents were advised to give vitamin D supplement by health professionals, such as public health nurses, midwives, and doctors.

Conclusions: About 90% of the infants received breast milk at 2 months of age. The vitamin D supplementation rate was 80%. Future studies are needed to monitor breastfeeding duration and vitamin D supplementation rates as infants get older.

Background

Worldwide public health authorities recommend exclusive breastfeeding for the first 6 months of life for healthy term infants [1-3]. Breast milk is the best food for optimal growth of the infant and breastfeeding has been associated with improved health outcomes for mother and infant [1]. While breast milk is the ideal food for infant, it does not generally supply adequate amounts of vitamin D [4]. As such, breastfed infants are at risk of vitamin D deficiency [5]. In its most serious form vitamin D deficiency in infancy leads to rickets, a condition characterized by weakened bones, resulting from poor mineralization of newly formed bone tissue [6]. Additionally, there is emerging evidence that lack of vitamin D during infancy, is associated with altered calcium metabolism [7], early childhood tooth decay [8], and increased risk of Type 1 Diabetes [9], and asthma later on [10].

While infant formula is fortified with vitamin D, breastfed infants are reliant on skin synthesis of vitamin D through the action of sunlight or supplemental vitamin D. Due to the risk of skin cancer it is generally recommended that infants under 1 year be kept out of direct sunlight [11]. Owing to concerns about vitamin D

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deficiency, Health Canada [4], the American Academy of Pediatrics [3], as well as several European countries [12-14] recommend that breastfed infants receive a daily vitamin D supplement, usually 400 IU. In Canada this recommendation has been in place since 1967 [4], yet, other than in the province of Quebec [15,16], there are few data as to whether parents or caregivers are following this advice. As part of the Canadian Community Health Survey 2007-2008 [17], Statistics Canada reported that among women who had given birth in the past 5 years and exclusively breastfed their infant, 67% provided a vitamin D supplement to the infant. However, this survey did not consider the frequency, dose, or form of supplement, nor were supplementation practices assessed among women who fed their infants a combination of breast milk and formula. Further, this survey included women who gave birth as early as 2002 and there may now be greater awareness about the importance of vitamin D. Indeed, Gallo et al. [18] reported that in one Montreal hospital 98% of exclusively breastfeeding mothers who gave birth in 2007-2008 supplemented their infants at some point prior to 6 months of age.

As part of British Columbia's (BC) publicly funded medical system, public health nurses provide breastfeeding support including advice on vitamin D supplementation within the first few days postpartum. To assess the effectiveness of this health promotion strategy, we conducted a survey in 2010 to determine the rates of breastfeeding and vitamin D supplementation among 2-month old infants in Vancouver and Richmond, BC. We also wanted to determine the type and dose of vitamin D supplementation given, examine the association of socio-demographic factors and vitamin D supplementation and determine barriers to infant vitamin D supplementation.

Methods

Sampling frame

Ethical approval to conduct the study was obtained from the University of British Columbia Behavioural Research Ethics Board. The survey was conducted in Richmond and five of six Community Health Areas in Vancouver. Vancouver (population 578,041; 2006) and Richmond (population 174,461; 2006) are the largest and fourth largest cities, respectively, in BC, Canada. All parents or caregivers whose infant was born between April and May of 2010 were invited to participate in the survey. Parents or caregivers were ineligible: if they moved out of the catchment area or were involved in a program that provided care to high-risk mothers (i.e. substance-abuse); or if their infant was adopted, in foster-care, or under the care of a neonatal intensive care unit. An infant age of 2 months was chosen because we wanted to survey women who had established breastfeeding and before the breastfeeding rates decline. Initial recruitment took place through public health nurses who asked the parent at an early postpartum contact whether they would be willing to participate in a short telephone interview. At an infant age of 2 months (range = 7-10 weeks) a research assistant phoned the parent to complete the survey after again obtaining verbal consent to participate.

Survey

The survey questionnaire was designed to obtain information on infant feeding practices; vitamin D supplementation including the form, frequency, and dose; who recommended that the infant be supplemented and (if appropriate) the reasons for not supplementing; and basic socio-demographic questions. The questionnaire was initially developed by the study investigators through face-to face meetings and through consultation and focus testing with relevant stakeholders. We piloted the questionnaire with ten new parents and revised it accordingly. The questionnaire was administered in English or when required in Mandarin, Cantonese, Punjabi, Vietnamese, or Spanish.

Data analysis

In our study, we defined "All breast milk" as infants who had received only breast milk (vitamins, minerals, and medicines permitted) in the week prior to the survey [2]. "Mixed breast milk and formula" was used to define infants who had received a mixture of breast milk and infant formula in the week prior to the survey. "Infant formula" was used to define infants who had received only formula in the week prior to the survey. Based on data indicating that 2-month old infants are fed an average of 8 times per day [3], we further divided the "mixed breast milk and formula" group into: "≥75% of breast milk" defined as ≤2 feedings of other liquids/food per day; "50- < 75% breast milk" as 3-4 feedings of other liquids/food per day; and " < 50% breast milk" as > 4 feedings of other liquids/food per day. Average daily formula intake was calculated by multiplying frequency of consumption in 24 hours by the amount of formula received in each bottle feed. The amount of vitamin D provided by formula was calculated assuming infant formula contains 40 IU vitamin D/100 ml, and the average intake of vitamin D provided by supplements was calculated based on the amount per dose and the frequency of administration. These two sources were summed to determine daily vitamin D intake. Descriptive statistics were used to describe breastfeeding and vitamin D supplementation rates (proportion with 95% confidence interval). Chi Square tests were used to determine differences in vitamin D supplementation by feeding practice.

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Rates were also stratified by socio-demographic variables such as ethnicity. The proportion of infants receiving a total vitamin D intake of < 300 IU from supplements and/or formula was also examined by feeding practice using Chi-square. A cut-point of 300 IU was selected because in order to achieve 400 IU per day a caregiver of breastfed infant would need to be 100% compliant with daily supplementation during the week surveyed. Further, the evidence base for infant requirements is poor [19] and recent trials suggest that 400 IU vitamin D exceeds requirements [20]. Multiple logistic regression was used to determine significant predictors (from among age, ethnicity, parity, income, and education) of receiving vitamin D supplements in two separate models; one in women whose infants had only received breast milk in the week prior to the survey and the other in those providing at least 50% of feeds from breast milk.

Results

Of the 1028 women eligible to participate, 577 completed the survey giving a response rate of 56% (Figure 1). Participant characteristics are given in Table 1. The majority of the mothers were over 31 y. One third of the infants were of European ethnicity and another one third was Chinese. Among those who responded to the annual family income question (n = 454), almost half had an income greater than $80,000. Participants were generally well educated with 87% having completed some post-secondary education.

The breastfeeding initiation rate was 99% (n = 570) and by 2 months of age over 40% of women were exclusively breastfeeding, using the WHO definition (2). Table 2 displays feeding practices and vitamin D supplementation practices at 2 months of age. Nearly 90% of infants were still receiving some breast milk. Over half received only breast milk in the past week; about one third received a mixture of breast milk and formula ranging from ≤2 to > 4 feedings of other liquids/food per day; and about 10% received only infant formula. Of the 577 infants, about 80% were supplemented with vitamin D at the time mothers were surveyed. Rates of vitamin D supplementation were significantly higher in those receiving only breast milk in the past week than those who received both breast milk and formula, which in turn were higher than exclusively formula fed babies. Within the mixed breast milk and formula group, infants receiving < 50% breast milk were less likely to be supplemented with vitamin D than those receiving 50% or more breast milk (P < 0.001). The proportion of

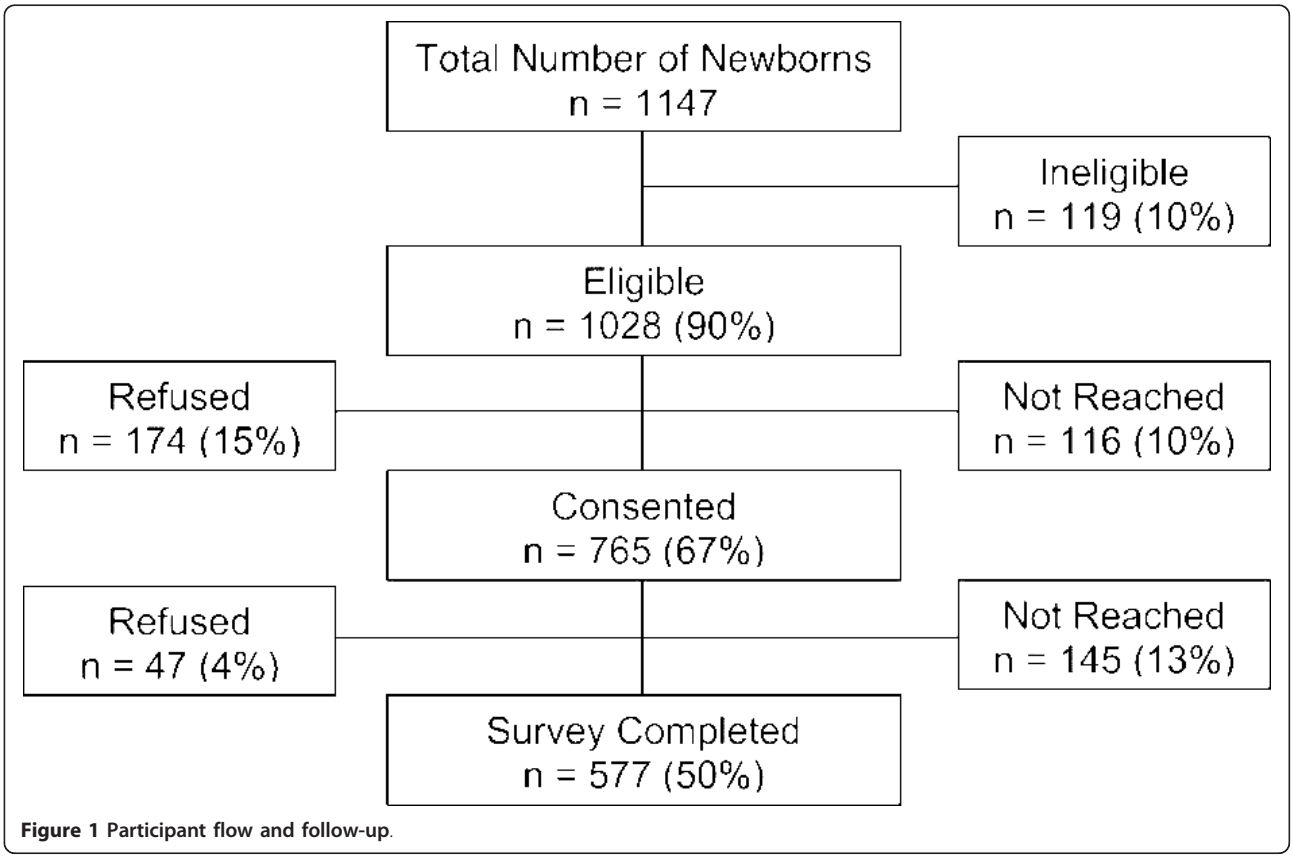


Figure 1 Participant flow and follow-up.

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Table 1 Participant characteristics

Characteristic	% (*n*)
Maternal Age	
< 31	33.3 (192)
≥31	66.7 (385)
Baby's ethnicity	
European	36.7 (212)
Chinese	31.2 (180)
Other[1]	32.1 (185)
Annual family income	
<$40,000	19.6 (113)
$40,000-59,000	11.3 (65)
$60,000-80,000	10.4 (60)
>$80,000	37.4 (216)
Unknown[2]	21.3 (123)
Education	
< High school	0.3 (2)
Some and completed high school	12.5 (72)
Some trade/vocational training and college/university	8.8 (51)
Completed trade/vocational training and college/ university	78.3 (452)
Parity	
Primipara	52.0 (300)
Multipara	48.0 (277)
Baby's gender	
Male	50.6 (292)
Female	49.4 (285)
Marital status	
Single	4.9 (28)
Married	85.6 (494)
Common-law	8.5 (49)
Other	1.0 (6)

[1]South Asian 42.0% (63), South East Asian 42.7% (64), Korean 6.0% (9), Japanese 9.3% (14), Aboriginal 25.7% (9), Black 22.9% (8), Middle Eastern 42.9% (15), Iranian 2.9% (1), Afghan 2.9% (1), Turkish 2.9% (1)

[2]Do not know and do not want to say

infants receiving less than 300 IU vitamin D per day (supplement and/or formula) was higher among infants receiving only breast milk than among those fed mixed breast milk and formula. However, within this group there was no difference in the percentage of infants receiving less than 300 IU per day.

About 5% of the infants did not receive any vitamin D from supplements and/or infant formula and 61% had a total vitamin D intake of 300- < 500 IU/d (Figure 2). Approximately 10% received 500 IU or more per day with the highest intake being 1130 IU/d (1000 IU from the supplement). Of those infants who were given vitamin D supplements, 80% received D-Drops® (a concentrated formula that provides 400 IU in a single drop) and 16% received D-Vi-Sol® (a formula that provides 400 IU per 1 mL). As shown in Table 3, over 90% of participants recalled receiving one or more recommendations to give their infant a vitamin D supplement. Public health nurses and physicians were the most frequent sources of this recommendation. Women who did use a supplement were asked to respond to an open question on why they chose to do so. The most common reasons were related to the inadequate amounts of vitamin D in breast milk, that a supplement had been recommended, that vitamin D had health benefits for the infant, and that lack of sunlight exposure meant supplementation was needed. Women who did not provide a supplement were asked to choose from a list on why they did not. Among those providing a response, the most common reasons were that the infant was being given formula, or that they did not think supplementation was necessary.

In multiple regression analysis of infants receiving only breast milk in the week prior to the survey (n = 331), only parity was associated with vitamin D supplementation. Primiparas were more likely to supplement their infants than multiparas (95% versus 84%; P = 0.03). Table 4 shows the results of the logistic regression of selected variables associated with vitamin D supplementation in infants who received greater than 50% of their feeds from breast milk (n = 517). None of the variables studied was significantly associated with supplementation, although there was a tendency for a higher rate of vitamin D supplementation in infants from families in the two higher income categories than those in the lowest income category.

Discussion

In this study we build on previous Canadian observations indicating high rates of breastfeeding and vitamin D supplementation, and provide new information regarding the dose of vitamin D provided, and mothers' reasons for choosing to supplement or not supplement. Almost 60% of infants had received only breast milk in the week prior to the survey and of these greater than 90% had received vitamin D supplements. This is much higher than the 67% vitamin D supplementation rate reported in a 2007-2008 survey for Canadian women

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Table 2 Feeding practices and vitamin D supplementation of infants aged two months

Feeding practice	Feeding Practice	Vitamin D Supplement	< 300 IU/d Vitamin D[5]
	% (*n*)	% yes (*n*)	% (*n*)
Total	100.0 (577)	79.9 (461)	28.9 (167)
All breast milk[1]	57.4 (331)	91.2 (302)[a]*	33.5 (111)[a]
Mixed breast milk and formula	32.2 (186)	79.0 (147)[b]	22.0 (41)[b]
≥75% breast milk[2]	53.8 (100)	86.0 (86)	21.0 (21)
50- < 75% breast milk[3]	20.4 (38)	84.2 (32)	26.3 (10)
< 50% breast milk[4]	25.8 (48)	60.4 (29)	20.8 (10)
Infant formula	10.4 (60)	20.0 (12)[c]	25.9 (15)[ab]

[1]Only breast milk in the past week
[2] ≤2 feedings of other liquids/food per day
[3]3-4 feedings of other liquids/food per day
[4] > 4 feedings of other liquids/food per day
[5]Total daily vitamin D intake from supplement and formula
*Rows showing different superscripts are significantly different from each other; χ^2 $P < 0.001$

who had exclusively breastfed an infant in the past 5 years [17]. In that study, the supplementation rate in BC was marginally higher than the national average (70%), but still well below the rate in our study. Our rates of vitamin D supplementation are comparable to those reported more recently in one Montreal hospital where 98% of exclusively breastfed (WHO definition) infants had been supplemented with vitamin D at some point during the first 6 months [18]. Data from the Infant Feeding Practices Study II (2005-2007) suggest that US vitamin D supplementation rates are markedly lower than in Canada; 43% of infants were breastfed at 2 months and of these only 10% were receiving vitamin D [21]. However, the American Academy of Pediatrics only began recommending infant supplementation in November 2008, whereas the Canadian recommendation has been present in some form since 1967. Further, breastfeeding rates have been historically higher in Canada than in the US and Canada's higher latitude may have created a greater impetus for infant supplementation in this country. As expected infant supplementation at 2 months was lower amongst those receiving mixed feeds (79%) and lower still in infants receiving only infant formula (20%). In Montreal, an apparently higher 88% of mixed feeders had received supplemental vitamin D; however, this was anytime during the first 6 months [18]. In the US study, amongst mixed feeders, only 5% were receiving vitamin D supplements at 2 months of age [21]. Despite a high proportion of breastfed infants receiving vitamin D supplements, one third were not receiving at least 300 IU/d; mainly because of less than daily supplement administration. Although we report less frequent vitamin D supplementation rates among infants who were receiving mixed breast milk and formula in the week before the survey than among those fed only breast milk

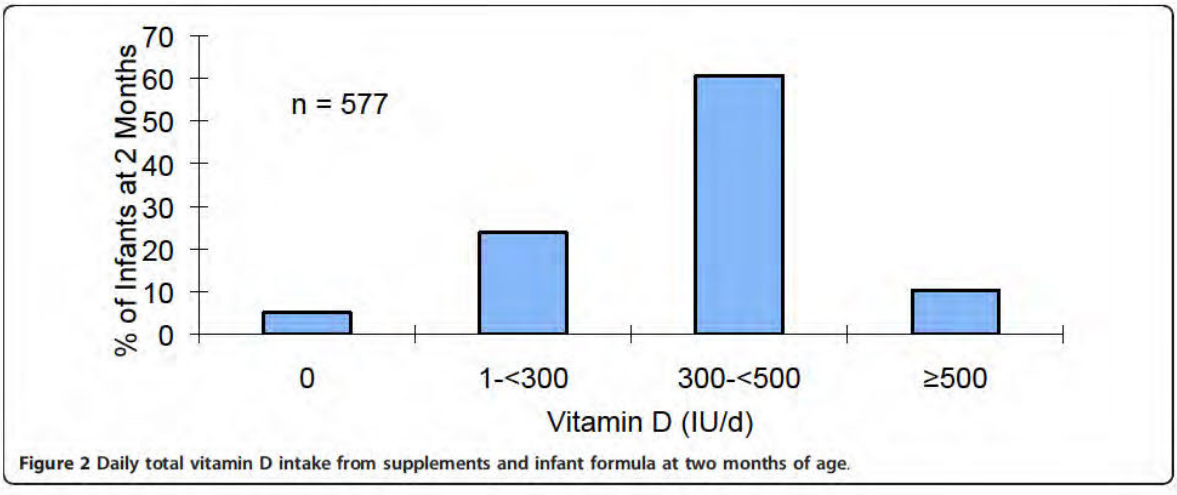


Figure 2 Daily total vitamin D intake from supplements and infant formula at two months of age.

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Table 3 Advice and decisions on vitamin D supplementation

	% (n)
Did anyone ever recommend a vitamin D supplement?	
Yes	92.2 (532)
No	7.8 (45)
Who recommended the supplement? (n = 532)[a]	
Public health nurse	80.2 (426)
Doctor	69.7 (370)
Midwife	9.6 (51)
Dietitian/pharmacist	5.1 (27)
Family member or friend	19.4 (103)
Other[1]	7.7 (46)
Reasons for providing a vitamin D supplement (n = 452)[a]	
Not in breast milk/I am breastfeeding	43.6 (197)
It was recommended	30.3 (137)
Health benefits for infant	28.3 (128)
Lack of sunlight/northern climate	21.0 (95)
Other[2]	13.1 (59)
Reasons for not supplementing vitamin D (n = 116)[a]	
Baby is being fed formula	35.3 (41)
I didn't know to give	10.3 (12)
I don't think the baby needs it	19.0 (22)
Forgot to give	3.4 (4)
Baby did not tolerate (vomit/spit up)	0.9 (1)
Other[3]	5.2 (6)
No response	28.4 (33)

[a]Multiple responses possible

[1]Reading, television, internet, South Community Birth Program, Baby's Best Chance, prenatal class, lactation clinic, natural path, doula, specialist, from first child

[2]Knew from first baby, read about it, did not know why, it is important

[3]Confused with the recommended dose, will purchase later, will ask doctor, first baby did not tolerate, had not started yet

(79% versus 91%), fewer of the infants receiving mixed feeding had vitamin D intakes below 300 IU/d vitamin D (22%, versus 33.5% in the fully breastfed group). Further, within the group receiving mixed feeds, as the amount of formula increased infant supplementation dropped; however, the percentage of those receiving less than 300 IU/d remained constant at around 20%. This was not unexpected as formula is fortified with vitamin D and it would take about 700 ml of formula to achieve an intake of 300 IU/d. Consuming less than 700 ml/d also explains why 25% of formula fed infants failed to achieve this intake. Using a stricter cut-point of 400 IU/d, the Montreal researchers reported that 74% of exclusively breastfed infants and 51% receiving mixed feeds achieved an intake of 400 IU/d at 6 months [18].

Up to a third of infants not achieving 300 IU/d vitamin D and even less achieving the recommendation of 400 IU/d may appear high. However, only 5% of infants were receiving no vitamin D. Further, it is acknowledged that the evidence base used to derive the infant recommendation is limited. Serum 25-hydroxyvitamin D (25OHD) concentration is the best indicator of vitamin D status. Although controversial the US Institute of Medicine recently affirmed a 25OHD of 50 nmol/L as desirable in all age groups including infants [19]. Greer et al. [22] showed that breastfed infants (n = 9) receiving 400 IU had mean 25-hydroxyvitamin D concentrations of 95 nmol/L after 12 weeks. More recently, infants randomized to 250 or 500 IU per day (n = 20 per group) at birth achieved mean (95% CI) 25OHD concentrations of 139 (114-164) and 151 (126-176) nmol/L, respectively after 6 weeks [20]. Thus, it appears that the recommended intake of 400 IU exceeds the requirements of almost all infants, perhaps by a considerable margin. There have been reports of infant overdosing with vitamin D in the US resulting in the Food and Drug Administration issuing a warning of the potential risk of overdosing infants with liquid vitamin D [23]. In our study only one infant was receiving greater than the upper limit for vitamin D of 1000 IU suggesting this was not a problem.

Among caregivers there was generally good awareness of the need to supplement and why it was important. For example, caregivers indicated that they used a supplement because vitamin D was not present in adequate amounts in breast milk and/or that sunlight exposure was limited or not recommended; and many women who used formula appeared to be aware that supplementation wasn't required. Over 90% of caregivers recalled receiving advice primarily from public health nurses and doctors to supplement with vitamin D, which may explain the high rates of supplementation. In a Seattle study [24], parents who reported that their child's pediatrician recommended vitamin D were 8 times more likely to provide the supplementation than parents whose child's pediatrician did not. However, only a third of parents recalled receiving any recommendation and of these under half supplemented with vitamin D. In contrast to our study, where < 5% of caregivers thought supplementation was unnecessary, 67% of parents in the Seattle study believed that supplementation was unnecessary because breast milk has all needed nutrition.

Multivariate regression revealed little in the way of predictors of supplement use. There was a non-significant tendency for family incomes less than $40,000 to be associated with lower rates of supplementation. However, vitamin D supplements cost less than $40 for 6 months and cost of the supplements was not given as a reason for not supplementing. Interestingly 80% of caregivers reported giving their infants D-Drops® versus

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Table 4 Percentages and multivariate adjusted odds ratio (and 95% CIs) for infant vitamin D supplementation in infants receiving greater than 50% of feeds from breast milk by select characteristics (*n* = 420)

Characteristic	% (*n*)	OR (95% CI)	*p*-value
Maternal Age			
< 31 y	87.6 (134)	1.00	
≥31 y	89.9 (286)	1.28 (0.65, 2.50)	0.479
Baby's ethnicity			
European	88.9 (160)	1.00	
Chinese	89.6 (129)	1.34 (0.62, 2.86)	0.456
Other	89.1 (131)	1.32 (0.62, 2.80)	0.469
Annual family income			
<$40,000	83.1 (74)	1.00	
$40,000-80,000	92.2 (95)	2.51 (0.98, 6.41)	0.054
>$80,000	91.4 (170)	2.24 (0.94, 5.30)	0.068
Non-responder[1]	87.1 (81)	1.35 (0.58, 3.14)	0.488
Education			
Less than completed trade/vocational training and college/university	87.0 (87)	1.00	
Completed trade/vocational training and college/university	89.8 (333)	0.99 (0.48, 2.04)	0.984
Parity			
Primipara	90.5 (219)	1.00	
Multipara	87.8 (201)	0.69 (0.37, 1.29)	0.244

Note: *CI* confidence interval, *OR* multivariate adjusted odds ratio

[1]Do not know and do not want to say

only 16% who supplemented with D-Vi-Sol® . The reason for the popularity of D-Drops® may be their ease of administration requiring only a single drop that can be placed on the mother's breast prior to nursing, versus the need to use a dropper to administer D-Vi-Sol® [25].

A strength of our study was that we had access to a database that contained the names of nearly all infants born in Vancouver and Richmond over the study period. Also, we sampled an ethnically diverse population where breastfeeding rates are high relative to the rest of North America. Finally, data were collected prospectively at 2 months rather than relying on recall of up to 5 years in one study. In studies of this type, selection bias is always an important consideration. For example, people who choose to participate versus those who do not, may be more educated and of higher socioeconomic status and thus more likely to breastfeed and supplement with vitamin D. Thus a limitation of our study is that we had only a moderate response rate of 56%. Unfortunately, we do not have any data on our non-responders and there are no representative data on pregnant women in Vancouver and Richmond to compare our results with. However, with respect to ethnicity [26], education [27] and family income [28] our sample compares well with 2006 census data for women from Vancouver and Richmond. Further, only half of the non-responders refused participation, while the other half could not be contacted initially or at follow-up. Because the survey was conducted in the summer months it appeared that many women were away on vacations or staying with family outside the area. We acknowledge that our findings cannot be extrapolated to the rest of Canada or even BC. Breastfeeding rates are higher in BC than elsewhere and Vancouver and Richmond have a unique ethnic mix not present in the rest of the province or Canada. Second, we only sampled at 2 months; this was an intentional decision but more data are needed on older infants. In the US, supplementation rates remained relatively constant out to 12 months; however, in this study both breastfeeding rates and infant supplementation were much lower than ours [21]. In Montreal, of all supplemented breastfed infants around a third had stopped taking the supplement by 6 months [18]. More data are needed on older infants especially around the time of introduction of solids and as breastfeeding rates drop with age.

Conclusions

Breastfeeding rate was high among this group of mothers. About 90% of the infants received breast milk and 57% were exclusively breastfed at 2 months. Over 60% of the infants had a total vitamin D intake of 300- < 500 IU/d from supplements and formula. The vitamin D supplementation rate was 80%. There was good awareness of the need to supplement with vitamin D and the reasons why. Most parents were advised to give their infants vitamin D

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supplement by public health nurses and doctors. Overall it appears that the level of knowledge translation about the importance of vitamin D is high. This points to the success of Vancouver Coastal Heath's system of universal contact by public health nurses, especially for providing breast feeding support and also for providing key public health messages such as the use of vitamin D. Future studies are needed to monitor vitamin D supplement rates of older infants and toddlers.

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Authors' contributions
BC and TG were responsible for the conception of the study and obtaining funds. All authors had a role in the design, acquisition of data, analysis and interpretation of data. BC, TG, SB, and WL drafted the initial manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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doi:10.1186/1471-2458-11-905
Cite this article as: Crocker *et al.*: **Very high vitamin D supplementation rates among infants aged 2 months in Vancouver and Richmond, British Columbia, Canada.** *BMC Public Health* 2011 **11**:905.

REDACTED

Vieth Ex 2071 IPR2023-00726 200

Exhibit S

United States

U.S. Ddrops Sales and % Market Change 2015-2018:

	2015	2016	2017	2018
Baby Ddrops US sales	Redacted			
% market change				

U.S. Ddrops Market Share Nielson Report Data 2018-2021:

	2018	2019	2020	2021
Nielson Ddrops US Marke share	Redacted			

CONFIDENTIAL OUTSIDE ATTORNEYS' EYES ONLY HIGHLY SENSITIVE FINANCIAL INFORMATION

Canada

Canada Ddrops Sales and % Market Change 2009-2016:

	2009	2010	2011	2012	2013	2014	2015	2016
CA Ddrops Sales	Redacted							
CA % market change								

CONFIDENTIAL OUTSIDE ATTORNEYS' EYES ONLY HIGHLY SENSITIVE FINANCIAL INFORMATION

Exhibit T


mama_and_baby_jax
Hitchin
Follow
Ddrops
161 likes
mama_and_baby_jax M Y S U N S H I N E
AD
Jaxon loves those sunny days but wrapping up warm and crunching around in the leaves can be fun too. However it's vital to get our daily dose of


161 likes
mama_and_baby_jax M Y S U N S H I N E
AD
Jaxon loves those sunny days but wrapping up warm and crunching around in the leaves can be fun too. However it's vital to get our daily dose of vitamin D, especially to support Jaxon's bones & teeth!
I just love how easy the drops are to add to food/ drink making for easy consumption. It's also natural with no preservatives or artificial colours/ flavouring - just coconut oil & pure vitamin D3!
You can get yours at Boots or Via Amazon
#BabyDrops#DDrops#ParentSquad#Gifted#Baby #VitaminD#MumLife#MumBlogger#JourneyThrou ghMotherhood
October 18, 2022

grace_and_co_x Follow ···



22 likes

grace_and_co_x Vitamin D...
We were kindly gifted VitaminD drops from



22 likes

grace_and_co_x Vitamin D...
We were kindly gifted VitaminD drops from @ddrops_uk.

Did you know the NHS recommend that breastfed babies from birth to 1 year should be given one drop a day, and with their Ddropper technology it's never been easier.

Adults are also encouraged to take one drop daily.
VitaminD contributes to the maintenance of normal bone and teeth, it also contributes to the normal function of the immune system and normal cell division.

#ddrops #babyddrops #parentsquad #breastfeeding #vitamins #discoverunder5k #gifted #ad

November 11, 2022

glasgowmammy Follow ···



Liked by **chaudharyhaiderali2023** and **others**

glasgowmammy ☀️ D-Drops ☀️

AD / we were kindly gifted these vitamin D drops to try from @ddrops_uk ☀️

Perfect for fussy babies as they are basically flavourless due to only containing 2 ingredients 👌

And can you believe just one drop contains 10ug, which is the daily recommended amount 🥰

Simply put 1 drop straight onto your nip if breastfeeding or onto a (clean) finger

Ideal to use from birth

@officialparentsquad

#mummyblogger #mumfluencers #homeaccount #under10k #glasgowmums #glasgow #hinch #hincher #hinching #mrshinchhome #mrshinch #hincharmy #staceysolomon #mama_support_group #mumssupportingmums #mumsofinstagram #newmum #girlmum #mixingmamas #instamumsquad #parentsquad #ddrops #vitamind #gifted

November 17, 2023



612 likes

military_mummyof4 Sunshine 🌞

Sunshine in a bottle, these vitamins drops are amazing so easy to use and are scientifically proven as a safe way for infants to get more vitamin D.

I have used these with all my babies to give them



612 likes

military_mummyof4 Sunshine 🌞

Sunshine in a bottle, these vitamins drops are amazing so easy to use and are scientifically proven as a safe way for infants to get more vitamin D.

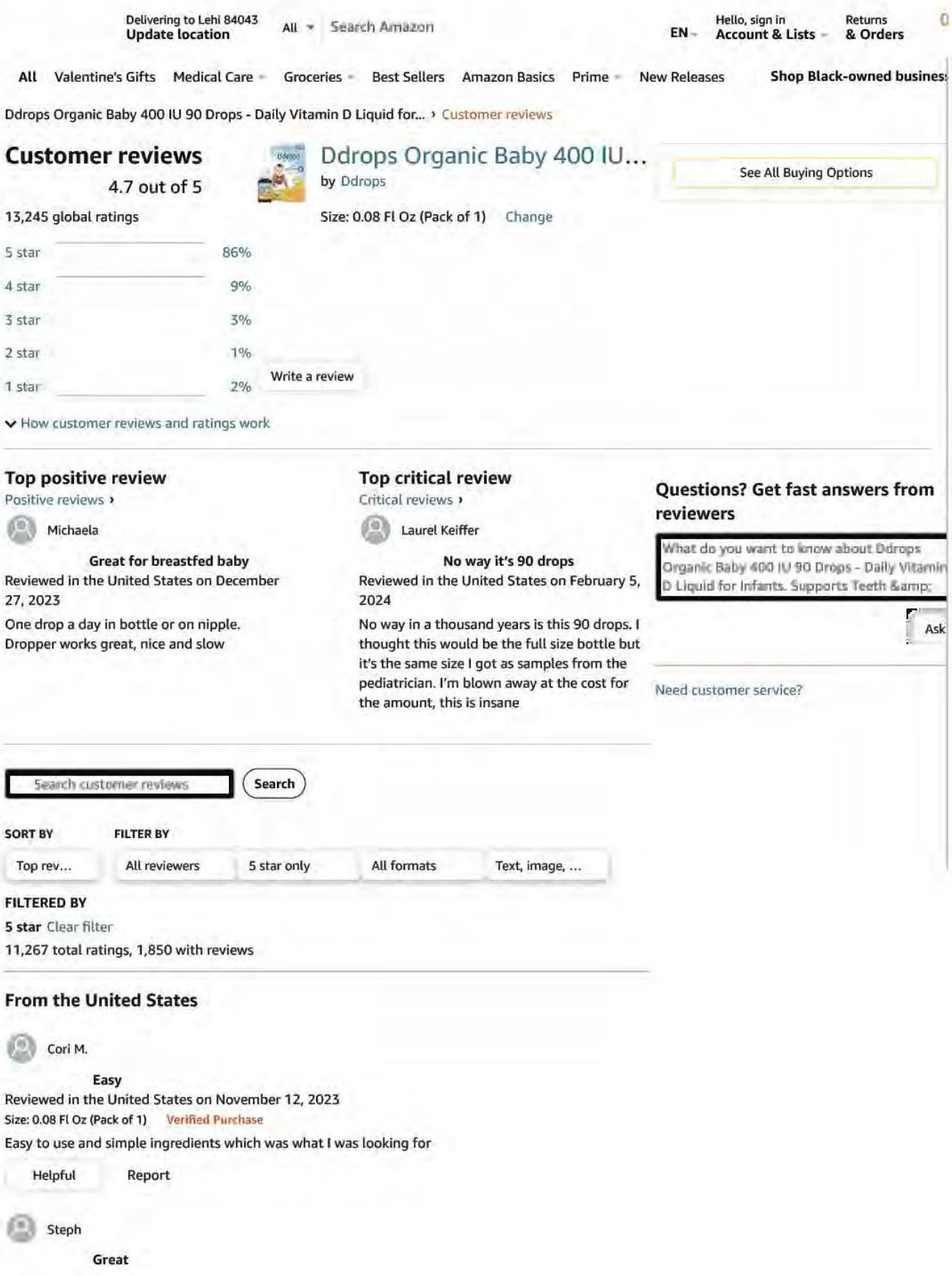
I have used these with all my babies to give them that little bit extra especially with the UK's unpredictable weather 🙈

Breastfed babies are at more risk of being vitamin D deficient as this is poorly transferred through breatmilk. Vitamin D is crucial for healthy mineralization of bones, teeth and helps support immune health. To use all you do I put on a washed finger, onto the nipple it can also be added into food and drink once weaning too.

Ad/Gifted @ddrops_uk @officialparentsquad

Swipe ➡️ to see Harlows current pose and how she thinks we wear sunglasses 😅

May 9, 2021


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Update location
All
Search Amazon
EN
Hello, sign in
Account & Lists
Returns
& Orders
All
Valentine's Gifts
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Ddrops Organic Baby 400 IU 90 Drops - Daily Vitamin D Liquid for... › Customer reviews
Customer reviews
4.7 out of 5
13,245 global ratings
5 star 86%
4 star 9%
3 star 3%
2 star 1%
1 star 2%
Write a review
How customer reviews and ratings work
Ddrops Organic Baby 400 IU...
by Ddrops
Size: 0.08 Fl Oz (Pack of 1) Change
See All Buying Options
Top positive review
Positive reviews ›
Michaela
Great for breastfed baby
Reviewed in the United States on December 27, 2023
One drop a day in bottle or on nipple. Dropper works great, nice and slow
Top critical review
Critical reviews ›
Laurel Keiffer
No way it's 90 drops
Reviewed in the United States on February 5, 2024
No way in a thousand years is this 90 drops. I thought this would be the full size bottle but it's the same size I got as samples from the pediatrician. I'm blown away at the cost for the amount, this is insane
Questions? Get fast answers from reviewers
What do you want to know about Ddrops Organic Baby 400 IU 90 Drops - Daily Vitamin D Liquid for Infants. Supports Teeth &
Ask
Need customer service?
Search customer reviews
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SORT BY
FILTER BY
Top rev...
All reviewers
5 star only
All formats
Text, image, ...
FILTERED BY
5 star Clear filter
11,267 total ratings, 1,850 with reviews
From the United States
Cori M.
Easy
Reviewed in the United States on November 12, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase
Easy to use and simple ingredients which was what I was looking for
Helpful
Report
Steph
Great

Reviewed in the United States on June 8, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase

Way better than the prescription vitamin d that is overly sweet and is "butterscotch" flavor that the pediatrician prescribed. My newborn hated it, I tried it and it the amount of sweetness made my mouth feel disgusting. Found these drops and they have a butterty unflavored sweetness to them. The drop coats the mouth and cant be spit out, baby didn't hate it and didn't gag or spit it out due to the taste unlike the prescription. Super easy to administer, not sure why people are complaining, definitely a "user error" situation. I sometimes miss because my baby moves but it's rare to miss since the drop is slow and can calculate when it will drop and that's when I open my baby's mouth.

3 people found this helpful

Helpful Report

CW.

Pediatrician recommended

Reviewed in the United States on November 1, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase

Easy to use and recommended by the pediatrician.

Helpful Report

Moonprincesslizzy

Love it

Reviewed in the United States on October 12, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase

Love the ingredients and the ease of use on this product. It has a lot in a tiny bottle and great value.

Helpful Report

Jason Osterman

Fine product

Reviewed in the United States on October 25, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase

Just vitamin drops, but no issues.

Helpful Report

Frankie07

The best d drops

Reviewed in the United States on August 11, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase

We have used these with all 3 of our kids and they are great. Always a quality product, easy to either drop a drop in babies mouth directly or place on the mom breast before breastfeeding. The bottle is easy to use and none of our babies ever had a negative reaction Would definitely recommend

3 people found this helpful

Helpful Report

Kelli

Life saver

Reviewed in the United States on August 30, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase

These drops are a life saver, my little baby would get a good amount of gas's when she was 3-5 weeks old. These drops work so quickly for her. This is definitely something you need to have when you have a baby.

3 people found this helpful

Helpful Report

Melissa

great

Reviewed in the United States on October 16, 2023

Size: 0.08 Fl Oz (Pack of 1) Verified Purchase

great product for the littles

Helpful Report

Priscila

Very good

Reviewed in the United States on October 11, 2023

Size: 0.08 Fl Oz (Pack of 1) Verified Purchase

Very easy to use.

Helpful Report

SheShe

They are what they are.

Reviewed in the United States on April 15, 2023

Size: 0.08 Fl Oz (Pack of 1) Verified Purchase

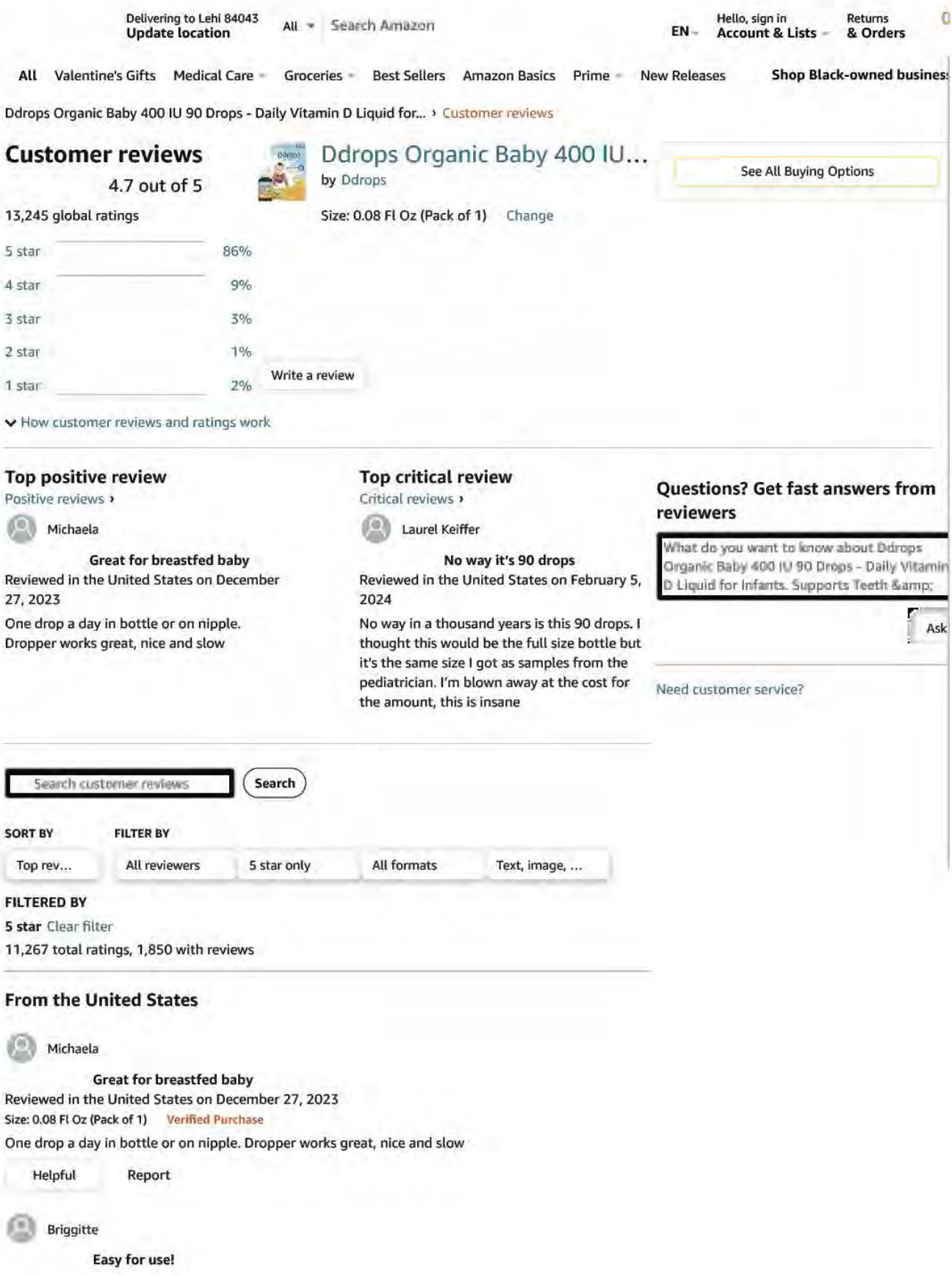
They are vitamin D drops. I know there are alot of brands out there. I was given a tester of these at my ob office while i was pregnant and since my baby is breastfeeding his pediatrician said he needed D drops. I said ohh i got these as a tester and she said yes those are perfect compared to others on the market. Since the tester only came with 15 drops. I needed to buy more. So here we are. I put a drop on his binki and pop it in hos mouth and its super easy. Super concentrated too so you just need a drop.

3 people found this helpful

Helpful Report

‹ Previous page Next page ›

‹ See all details for Ddrops Organic Baby 400 IU 90 Drops - Daily Vitamin D Liquid for...


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4 star 9%
3 star 3%
2 star 1%
1 star 2%
Write a review
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Ddrops Organic Baby 400 IU...
by Ddrops
Size: 0.08 Fl Oz (Pack of 1) Change
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Positive reviews ›
Michaela
Great for breastfed baby
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Ask
Need customer service?
Search customer reviews
Search
SORT BY
FILTER BY
Top rev...
All reviewers
5 star only
All formats
Text, image, ...
FILTERED BY
5 star Clear filter
11,267 total ratings, 1,850 with reviews
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Michaela
Great for breastfed baby
Reviewed in the United States on December 27, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase
One drop a day in bottle or on nipple. Dropper works great, nice and slow
Helpful
Report
Briggitte
Easy for use!

Reviewed in the United States on December 30, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase
You can apply it to the pacifier, clean finger or nipple. very good

Helpful Report

Tia

Easiest Vitamin D!

Reviewed in the United States on November 11, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase
I am glad I found these vitamins. After researching the better brands for infant vitamin D, I saw this one recommended over and over. I love that it is just one drop vs the vitamin D we were prescribed (where you have to administer an entire syringe). I find the vitamin comes out at a good rate and I haven't had any issues with it. It's free from any of the gross and unnecessary stuff (dyes, etc). Easy for breastfeeding or just put a drop on a pacifer. I'll continue to use this brand.

One person found this helpful

Helpful Report

Mary

A must have for breastfeeding moms

Reviewed in the United States on December 18, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase
Easy to use for your kiddos.

Helpful Report

Mad Gyal J

For strong baby results...

Reviewed in the United States on January 12, 2024
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase
My newborn's pediatrician recommended this particular vitamin supplement at her 5th day check up. I ordered it that same day and have been using it 'til present day. My baby is now 4 months and 2 milestones ahead of her time. It's only one tiny clear and tasteless drop I put in the milk. Moms, this is a winner vitamin!!!

Helpful Report

claire

Better than the prescription drops!

Reviewed in the United States on February 1, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase
Our doctor sent in a prescription to our pharmacy for some infant oral vitamin d that was total garbage (one dose was a huge amount for her at 1ml, full of preservatives, vitamin d was 8th on the list of ingredients, and it contained butterscotch flavoring... yuck). I felt terrible at the idea of giving something with artificial flavoring in it to my exclusively breastfed 3w old infant, and shocked those would be prescribed by our dr. After hearing other parents' recommendations, and what their pediatricians recommended, I decided to order these. These drops pretty much taste and smell like nothing, only require one tiny drop, and have just 2 straightforward ingredients! Only downside is that the dropper can be kind of tricky for my partner and he sometimes accidentally does more than one drop and has to start a new bottle (I find it easy to get one drop because the dropper is similar to essential oil bottles I've used). I'll save the other drops with flavoring in them for when my baby is over 6mos, and I'll definitely be reordering these.

10 people found this helpful

Helpful Report

Jun Skeenes

Easy to use
Reviewed in the United States on November 24, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase
Only one drop and it comes out pretty quickly

Helpful Report

NARESH

Baby Loves It
Reviewed in the United States on January 20, 2024
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase
My baby's pediatrician recommended this brand and a few others to try. This was the first I ordered and it was a success. I think my baby actually enjoys the taste.

Helpful Report

Emily Y

Does the job
Reviewed in the United States on November 13, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase
I love that only 1 drop is required to get babies dose of vitamin D so you're not fighting to get a whole mL down. My infant does not seem to notice it at all when placed in her bottle or directly on the breast so I don't imagine there's any concerning flavor. There is really no smell. I do think the drops can be a bit difficult to get out and sometimes I find myself vigorously shaking the little vial to get one. But on the flip side I would hate it if it spilled so I suppose this is the lesser of two evils. Overall a great product and I plan to purchase again when this bottle is empty.

Helpful Report

hiolee

Good
Reviewed in the United States on December 19, 2023
Size: 0.08 Fl Oz (Pack of 1) Verified Purchase
Good



Helpful Report

< Previous page Next page >

‹ See all details for Ddrops Organic Baby 400 IU 90 Drops - Daily Vitamin D Liquid for...

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Your Account
Your Orders
Shipping Rates & Policies

4.6
494 star ratings

86
86% would recommend
120 recommendations

5 stars	82%
4 stars	9%
3 stars	3%
2 stars	2%
1 star	5%



Shop all Ddrops
Ddrops Baby Vitamin D 400 IU Organic Liquid Drops - 0.06 fl oz
494 | 4 Questions

~~$11.99~~
at American Fork

Qty 1 | Add to cart

Review images





Write a review

Reviews related to

Ingredient | Design | Application

sort by **highest rated** | filter by **all ratings**

With photos
Verified purchases

We found 142 matching reviews

easy to use
Would recommend
libramom - 2 days ago, Verified purchaser

easy to use when on the go nice size bottle

5.0 **Quality** out of 5
5.0 **Value** out of 5

Did you find this review helpful?
Helpful | Not helpful

Report review

Our favorite vitamin D drops!
Would recommend
bhanke - 12 days ago, Verified purchaser

I am currently breast feeding my 4th baby and these are my favorite vitamin D drops! I always forget to grab the drops before I start nursing, so I love that these can be used by putting a drop on a clean finger and just having baby suck on it for a bit.

5.0 **Quality** out of 5 5.0 **Value** out of 5



Shop all Ddrops

Ddrops Baby Vitamin D 400 IU Organic Liquid Drops - 0.06 fl oz

494 4 Questions

~~$11.99~~

at American Fork

Qty 1 **Add to cart**

Vitamin D3

Would recommend

Baby - 2 months ago, Verified purchaser

I think the flavors is good, my baby use it easily.

5.0 **Value** out of 5 5.0 **Quality** out of 5

1 guest found this review helpful. Did you?

Helpful Not helpful Report review

Recommended by our pediatrician

Would recommend

SJO23 - 5 months ago, Verified purchaser

Love that it's just a single drop! Sometimes drops can come out fast tho so be careful you don't accidentally do two!

1 reply from Ddrops team - 4 months ago

Greetings from Ddrops! Thank you for your message and feedback. If the drop is coming out too fast, the bottle can be tilted slightly on an angle from vertical to slow the speed of the drop. To prevent a second drop from coming out, start tilting the bottle 45 degrees and turning it upright as soon as the first drop has been dispensed. I hope this information is helpful. May | The Ddrops Team

5.0 **Value** out of 5 5.0 **Quality** out of 5

2 guests found this review helpful. Did you?

Helpful Not helpful Report review

D Drops

Would recommend

Duni - 5 months ago, Verified purchaser

I'm thinking these have a good flavor because my baby always takes his paci when I apply these drops to it

5.0 **Quality** out of 5 5.0 **Value** out of 5

1 guest found this review helpful. Did you?

Helpful | Not helpful

Report review



Shop all Ddrops

Ddrops Baby Vitamin D 400 IU Organic Liquid Drops - 0.06 fl oz

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Qty 1 | Add to cart

5.0 **Quality** out of 5

5.0 **Value** out of 5

3 guests found this review helpful. Did you?

Helpful | Not helpful

Report review

Super easy to apply!

Anne J - 6 months ago

Purchased a few of these D drops for our baby. Super easy to administer. We would keep it on the changing table so whenever she woke up and we had to change her diaper it would easy to drop into her mouth. I did try it and did not find that it had a flavor.

1 guest found this review helpful. Did you?

Helpful | Not helpful

Report review

3 for 3 on this brand

Would recommend

AT - 10 months ago, Verified purchaser

I've bought this brand for our first born and have continued to use it. We have not had any issues and will continue to use this brand.

5.0 **Quality** out of 5

5.0 **Value** out of 5

1 guest found this review helpful. Did you?

Helpful | Not helpful

Report review

D drops

Would recommend

Neks - 1 year ago

Convenient for use, a drop does the magic

5.0 **Value** out of 5

5.0 **Quality** out of 5

1 guest found this review helpful. Did you?

Helpful | Not helpful

Report review



Shop all Ddrops

Ddrops Baby Vitamin D 400 IU Organic Liquid Drops - 0.06 fl oz

494 | 4 Questions

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at American Fork ⓘ

Qty 1 | Add to cart

5.0 **Quality** out of 5

5.0 **Value** out of 5

Did you find this review helpful?

Helpful | Not helpful

Report review

Works great!

tea145 - 1 year ago, Verified purchaser

Have used these with 2 babies and they worked great!

Did you find this review helpful?

Helpful | Not helpful

Report review

Recommend

Would recommend

Yulee - 1 year ago, Verified purchaser

Use it everyday for both my babies

5.0 **Value** out of 5

5.0 **Quality** out of 5

2 guests found this review helpful. Did you?

Helpful | Not helpful

Report review

Super easy to administer

★★★★★ | Would recommend

JReed - 1 year ago, Verified purchaser



Shop all Ddrops

Ddrops Baby Vitamin D 400 IU Organic Liquid Drops - 0.06 fl oz

★★★★★ 494 | 4 Questions

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Qty 1 | Add to cart

easy to use

★★★★★ | Would recommend

YP - 1 year ago, Verified purchaser

recommended by my doctor and easy to use

5.0 **Quality** out of 5

5.0 **Value** out of 5

Did you find this review helpful?

Helpful | Not helpful

Report review

good quality

★★★★★ | Would recommend

Ela12 - 1 year ago, Verified purchaser

I definitely recommend this to everyone

5.0 **Quality** out of 5

5.0 **Value** out of 5

1 guest found this review helpful. Did you?

Helpful | Not helpful

Report review

Great vitamin!

★★★★★ | Would recommend

happy customer - 2 years ago, Verified purchaser

Easy to use, no separate dropper to clean off.

5.0 **Quality** out of 5

5.0 **Value** out of 5

1 guest found this review helpful. Did you?

Helpful | Not helpful

Report review

Quick and easy

★★★★★ | Would recommend

Ky - 2 years ago, Verified purchaser



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Ddrops Baby Vitamin D 400 IU Organic Liquid Drops - 0.06 fl oz

★★★★★ 494 | 4 Questions

$11.99

at American Fork ⓘ

Qty 1 | Add to cart

Helpful | Not helpful | Report review

Great Buy

★★★★★ | Would recommend

kitkatana - 2 years ago, Verified purchaser

Way better ingredients and one drop back 1ml compared to other brands. Baby doesn't even notice.

5.0 **Quality** out of 5

5.0 **Value** out of 5

2 guests found this review helpful. Did you?

Helpful | Not helpful | Report review

Great drops!

★★★★★ | Would recommend

Brandi - 2 years ago, Verified purchaser

We got a sample at the hospital and decided to stick with these for my baby :) they're perfect

5.0 **Value** out of 5

5.0 **Quality** out of 5

5 guests found this review helpful. Did you?

Helpful | Not helpful | Report review

Easy to use, gets the job done

★★★★★ | Would recommend

Momma1992 - 2 years ago

My first was exclusively breastfed, I would drip this on my nipple, one drop a day, before he latched and he never knew. It got the job done and was easy. Keep it by your favorite feeding spot and it's always there when you need it. (I have also read that lack of vitamin d in babies may contribute to peanut allergies, interesting!)

5.0 **Value** out of 5

5.0 **Quality** out of 5

7 guests found this review helpful. Did you?

Helpful Not helpful Report review

Vitamin D for breastfed Baby



Shop all Ddrops

Ddrops Baby Vitamin D 400 IU Organic Liquid Drops - 0.06 fl oz

494 4 Questions

$11.99
at American Fork

Qty 1 Add to cart

out of 5 out of 5

Did you find this review helpful?

Helpful Not helpful Report review

Every drops make a difference

Esther Anayochi Obinna - 2 years ago, Verified purchaser

Soften baby's formula

1 guest found this review helpful. Did you?

Helpful Not helpful Report review

It works for us

Would recommend

Birdie - 2 years ago, Verified purchaser

Baby doesn't mind when we put these drops on her pacifier. So far so good!

5.0 **Quality** out of 5 5.0 **Value** out of 5

2 guests found this review helpful. Did you?

Helpful Not helpful Report review

Great buy

★★★★★ | Would recommend

Ravz - 2 years ago, Verified purchaser



Shop all Ddrops

Ddrops Baby Vitamin D 400 IU Organic Liquid Drops - 0.06 fl oz

★★★★★ 494 | 4 Questions

$11.99

at American Fork ⓘ

Qty 1 | Add to cart

Helpful | Not helpful

Report review

Works well

★★★★★ | Would recommend

Momof22021 - 2 years ago, Verified purchaser

My 5 month old has taken these since about 6 weeks old. No issues!

5.0 **Value** out of 5

5.0 **Quality** out of 5

1 guest found this review helpful. Did you?

Helpful | Not helpful

Report review

Easy Peasy

★★★★★ | Would recommend

DearAnja - 2 years ago, Verified purchaser

Very Easy To Give To Baby

Did you find this review helpful?

Helpful | Not helpful

Report review

Seems good

★★★★★ | Would recommend

unknown - 2 years ago, Verified purchaser

These drops seem to be working well for my little one. I like that they don't have a bunch of extra ingredients like most other brands. They're very easy to use as well.

5.0 **Value** out of 5

5.0 **Quality** out of 5

4 guests found this review helpful. Did you?

Helpful | Not helpful

Report review

So easy to use

Would recommend

Kim - 2 years ago, Verified purchaser



Shop all Ddrops

Ddrops Baby Vitamin D 400 IU Organic Liquid Drops - 0.06 fl oz

494 4 Questions

~~$11.99~~

at American Fork

Qty 1 Add to cart

Easy to use

Snags - 2 years ago, Verified purchaser

Recommended by our pediatrician and easy to use. Just apply to a clean finger and pop it in your baby's mouth. Or apply directly to nipple if breast feeding. My boy takes it with a smile.

5.0 **Quality** out of 5

5.0 **Value** out of 5

3 guests found this review helpful. Did you?

Helpful Not helpful Report review

Would recommend

Would recommend

Linz - 2 years ago, Verified purchaser

Good quality for the price. Used these on my daughter.

5.0 **Quality** out of 5

5.0 **Value** out of 5

1 guest found this review helpful. Did you?

Helpful Not helpful Report review

Easy to use

mama - 2 years ago, Verified purchaser

Easy to administer. We just put one drop on the nipple before breastfeeding!

4 guests found this review helpful. Did you?

Helpful Not helpful Report review

The only drops that didnt make my kids gassy!

★★★★★ | Would recommend

L C - 2 years ago, Verified purchaser

These are amazing. I've tried a few other brands and these are the ONLY type that didn't give my kids gas! Our pediatricians also recommended this brand and I can't say good enough things about it! My kiddos took the drops well and I am a happy mama!



Shop all Ddrops

Ddrops Baby Vitamin D 400 IU Organic Liquid Drops - 0.06 fl oz

★★★★★ 494 4 Questions

~~$11.99~~

at American Fork ⓘ

Qty 1 Add to cart

Same Drops as thr Hospital

★★★★★ | Would recommend

Jane - 2 years ago, Verified purchaser

These are the same drops that they sent us home from the hospital with. They seem to work just fine for our baby.

5.0 **Value** out of 5

5.0 **Quality** out of 5

2 guests found this review helpful. Did you?

Helpful Not helpful Report review

Better tolerance and easier to administer than other brands

★★★★★ | Would recommend

Liz - 2 years ago, Verified purchaser

So much easier to administer than other brand vitamin D drops that use a dropper. My son also tolerates taking these much better as they are not sweet and only a drop.

5.0 **Quality** out of 5

5.0 **Value** out of 5

2 guests found this review helpful. Did you?

Helpful Not helpful Report review

vitamin d

★★★★★ | Would recommend

cloud - 3 years ago, Verified purchaser

I love it is easy to apply on my nipple, no hassle, no side affect on my baby he's only 3 months so that's pretty good I had tried the enfamil one and it made his tummy hurt.

3 guests found this review helpful. Did you?

Helpful Not helpful Report review

Big fan

Would recommend

YG - 3 years ago, Verified purchaser

My baby is 16 months $ gets excited when I put it on my nipple, he just dives for it. Not sure if he's playing or just really loves it!



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Qty 1 | Add to cart

Better than Elavil

Would recommend

LC - 3 years ago, Verified purchaser

So much better than Elavil. Easy to give one drop when breastfeeding. Elavil made my baby gassy, constipated, and fussy. Having to give 1ml with all the extra additives was no fun. These are a lot more natural, just need to watch and make sure baby isn't allergic to coconut.

5.0 **Value** out of 5

5.0 **Quality** out of 5

2 guests found this review helpful. Did you?

Helpful | Not helpful

Report review

Easy to administer.

Would recommend

TargetRev - 3 years ago

It is so easy to administer, literal just a drop to the finger and then into babys mouth. My baby doesn't even notice when I give it to him. He would gag and vomit all the time with the1 mL dosages from another brand that was provided by his pediatric. I will be repurchasing these.

5.0 **Value** out of 5

5.0 **Quality** out of 5

1 guest found this review helpful. Did you?

Helpful | Not helpful

Report review

Much better than other brands

Would recommend

Kristina - 3 years ago

Daughter used to spit up/vomit milk shortly after using another brand of infant vitamin D that required a 5ml syringe full. Never had an issue once we switched to these. Used them for several months and now using for our son. It can be easy to miss a drop if you are not careful so I use my finger. Once the bottle is half gone, the drops can take a while to dispense. I found storing the bottle upside down at that point helps.

5.0 **Quality** out of 5

4.0 **Value** out of 5

4 guests found this review helpful. Did you?

Helpful Not helpful Report review



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5.0 **Value** out of 5 5.0 **Quality** out of 5

Did you find this review helpful?

Helpful Not helpful Report review

Good product

Would recommend

JulieM - 3 years ago, Verified purchaser

We have not had any issues and have been using for four months. It does take a little hit of time to come out of the bottle.

5.0 **Quality** out of 5 5.0 **Value** out of 5

2 guests found this review helpful. Did you?

Helpful Not helpful Report review

Great and safe

Would recommend

Alee2283 - 3 years ago

Great product. Easy to administer with drop on finger. Doesn't cause gas or fussiness. Seems to make baby salivate a bit more but not problematic. Prefer this over di v sol enfamil brand.

5.0 **Value** out of 5 5.0 **Quality** out of 5

1 guest found this review helpful. Did you?

Helpful Not helpful Report review

Good drops

Would recommend

Melp - 3 years ago

My baby has been on these since he was a newborn- now 7mo dr recommends these until a year. He's been doing very well on these drops it does take some time to come out of the bottle which is annoying.

5.0 **Value** out of 5 5.0 **Quality** out of 5

2 guests found this review helpful. Did you?



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Qty 1 **Add to cart**

Z - 3 years ago, Verified purchaser

Easy to get the drop out of the bottle.

Did you find this review helpful?

Helpful Not helpful Report review

Easy to use

phlalm - 3 years ago

So much easier to give to baby than other Vitamin D drops. Colorless and odorless, and baby doesn't seem to mind at all.

5.0 **Quality** out of 5 5.0 **Value** out of 5

1 guest found this review helpful. Did you?

Helpful Not helpful Report review

Would recommend

Briana - 3 years ago, Verified purchaser

Great and easy to use.

5.0 **Quality** out of 5 5.0 **Value** out of 5

Did you find this review helpful?

Helpful Not helpful Report review

Sj123 - 3 years ago

These are the drops I use for my son. He never refuses a bottle or binky that has it on/in it

Did you find this review helpful?

Helpful Not helpful Report review

No taste so baby likes it

PS - 4 years ago, Verified purchaser



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Qty 1 | Add to cart

Helpful | Not helpful | Report review

Sam - 4 years ago, Verified purchaser

Good vitamin d drops

Did you find this review helpful?

Helpful | Not helpful | Report review

Flavorless and good dispenser

Would recommend

Amanda - 4 years ago

After trying the Enfamil brand where baby requires one ml to get 400 iu and baby hated it, I asked some moms in a group what they use and this was recommended. It's odorless, tasteless and literally takes one drop. You must hold it upside down for maybe 3 seconds before the drop fully forms.

5.0 **Quality** out of 5

5.0 **Value** out of 5

5 guests found this review helpful. Did you?

Helpful | Not helpful | Report review

Doctor recommended!

Would recommend

corthilt - 4 years ago, Verified purchaser

This item was recommended by our pediatrician. So far no issues!

5.0 **Quality** out of 5

5.0 **Value** out of 5

Did you find this review helpful?

Helpful | Not helpful | Report review

★★★★★ | Would recommend

Mom 101 - 4 years ago, Verified purchaser

They worked for my baby. I put one drop in his breast milk in the morning. His doctor initially recommended De-Vi-Sol but they have too many additives. I was happy to discover these. I have not had a problem with dispenser of the drops either.



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Qty 1 | Add to cart

Report review

★★★★★ | Would recommend

hzt - 4 years ago, Verified purchaser

amazing good survice

5.0 **Quality** out of 5

2 guests found this review helpful. Did you?

Helpful | Not helpful

Report review

These drops are better

★★★★★ | Would recommend

Michelle - 4 years ago, Verified purchaser

These drops are my go-to drops for my kids. I've tried other with a syringe, but these drops are way better. And they don't spill as easily as the others.

5.0 **Quality** out of 5

5.0 **Value** out of 5

1 guest found this review helpful. Did you?

Helpful | Not helpful

Report review

Baby doesn't notice

★★★★★ | Would recommend

Sweet T - 4 years ago, Verified purchaser



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Qty 1 | Add to cart

Helpful | Not helpful | Report review

★★★★★

KristaMD - 4 years ago, Verified purchaser

Super easy to give our baby. We just put one drop on the tip of his binky and done!

5.0 **Quality** out of 5 | 5.0 **Value** out of 5

1 guest found this review helpful. Did you?

Helpful | Not helpful | Report review

So easy!

★★★★★ | Would recommend

ESavory - 5 years ago, Verified purchaser

So easy to use and didn't upset my baby's tummy. I love that it's only a drop!

5.0 **Quality** out of 5 | 5.0 **Value** out of 5

4 guests found this review helpful. Did you?

Helpful | Not helpful | Report review

★★★★★ | Would recommend

Chels - 5 years ago, Verified purchaser

One drop is all they need, much easier than a whole dropper full of the other stuff!

3 guests found this review helpful. Did you?

Helpful | Not helpful | Report review

★★★★★ | Would recommend

Well - 5 years ago, Verified purchaser

Vitamin D drops is working for my kid, I like it very much.

5.0 **Quality** out of 5 5.0 **Value** out of 5



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Qty 1 | Add to cart

Awesome drops for baby!

★★★★★ | Would recommend

Kehau - 5 years ago, Verified purchaser

Love this drops! Doesn't make my son have an upset stomach. Kinda expensive but worth it!

5.0 **Quality** out of 5 3.0 **Value** out of 5

Did you find this review helpful?

Helpful | Not helpful | Report review

★★★★★ | Would recommend

Cheeky kids - 5 years ago, Verified purchaser

Good for baby good products

5.0 **Quality** out of 5 5.0 **Value** out of 5

Did you find this review helpful?

Helpful | Not helpful | Report review

Love them

★★★★★ | Would recommend

Kaymae77 - 5 years ago, Verified purchaser

Love & highly recommend these drops. My baby has been exclusively breastfed & I didn't know they needed vitamin D til he was 3mo. I tried another brand with a dropper & he would choke, gag or vomit on them. These are super easy to sneak on the breast or a spoonful of food with no issues for baby.

5.0 **Quality** out of 5 5.0 **Value** out of 5

3 guests found this review helpful. Did you?

Helpful | Not helpful | Report review

Good product that has been repurchased

★★★★★ | Would recommend

Good product - 5 years ago, Verified purchaser

Very good product, the daughter used it all the time, this time is for the domestic friend's baby.

5.0 **Quality**



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Qty 1 | Add to cart

★★★★★ | Would recommend

Momof2 - 5 years ago, Verified purchaser

Easy to use, Love it!!!

1 guest found this review helpful. Did you?

Helpful | Not helpful | Report review

love these!

★★★★★ | Would recommend

Heather - 5 years ago, Verified purchaser

the best! no fillers, just one drop needed. i dropped in babies mouth when younger, now drop on small food bite (or a goldfish). no taste.

5.0 **Quality** out of 5

Did you find this review helpful?

Helpful | Not helpful | Report review

Easy to use. Dispenses one drop at a time.

★★★★★ | Would recommend

JS - 5 years ago, Verified purchaser

It is easy to use because it dispenses exactly one drop at a time. I use my finger and let baby suckle on it for about 20 seconds. Baby likes it and takes is easily.

5.0 **Quality** out of 5

Did you find this review helpful?

Helpful | Not helpful | Report review

★★★★★ | Would recommend

Amwil - 5 years ago, Verified purchaser

My pediatrician recommended this brand. I like that I only need s single drop instead on one or more droppers, and baby either doesn't notice

REDACTED Vieth Ex 2071 IPR2023-00726 232

or doesn't mind the taste. Very easy to use, and has lasted a long time.

4.0 **Quality** out of 5

Did you find this review helpful?



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Qty 1 | Add to cart

Would recommend

ShainaD603 - 5 years ago, Verified purchaser

Love these drops! I will always buy them from now on. My son got to the point he would not even take the Enfamil ones and I believe they caused him a lot of tummy upset! Also great it is only one drop and not an entire ml!

5.0 **Quality** out of 5

Did you find this review helpful?

Helpful | Not helpful | Report review

Easy and gentle on stomach

Would recommend

Lindsey24 - 5 years ago, Verified purchaser

These drops are so simple to use and did not seem to upset my baby's tummy like the enfamil vitamin d did. I love that's it's only a drop and not a syringe full. Highly recommend for breastfeeding moms. One drop on breast and it's done! Easy!

1 guest found this review helpful. Did you?

Helpful | Not helpful | Report review

Tasteless and only one drop

Would recommend

JCox - 5 years ago, Verified purchaser

We like that it's only one drop and doesn't have a bunch of extra ingredients. Also, there is no taste at all so no chance of baby not liking it.

5.0 **Quality** out of 5

Did you find this review helpful?

Helpful | Not helpful | Report review

D drops baby vitamins

Would recommend

Diane - 5 years ago, Verified purchaser

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Very easy to apply drop and taste is unnoticeable!

Did you find this review helpful?

Helpful | Not helpful

Report review



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Qty 1 | Add to cart

These drops are awesome! Just one drop and you're set..way easier than using a syringe and squirting into baby's mouth and then having to clean stuff. My baby doesn't even realize the drops are on there. Such a great concept and product!

5.0 **Quality** out of 5

Did you find this review helpful?

Helpful | Not helpful

Report review

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*See offer details. Restrictions apply. Pricing, promotions and availability may vary by location and at Target.com

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Contact Us
Feedback
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Security & Fraud
Team Member Services

Stores

Find a Store
Clinic
Pharmacy
Optical
More In-Store Services

Services

Target Circle
RedCard
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Boston, Massachusetts

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Vitamin D3- körpələr və uşaqlarda sağlam

Exhibit U

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UNITED STATES DISTRICT COURT
DISTRICT OF MINNESOTA

DDrops Company, Reinhold Vieth, And Elaine Vieth,

Plaintiffs,

v.

Walgreen Co., and drugstore.com, Inc.,

Defendants.

Case No. 15-CV-3981 (WMW/SER)

JOINT MOTION FOR DISMISSAL WITH PREJUDICE

Pursuant to Rule 41(a)(1)(B) of the Federal Rules of Civil Procedure, Plaintiffs Ddrops Company, Reinhold Vieth, and Elaine Vieth (collectively, "Ddrops") and Defendants Walgreen Co., and drugstore.com, Inc., (collectively, "Walgreens") jointly move for dismissal with prejudice as follows:

1. All claims and counterclaims in the above-captioned legal action be dismissed with prejudice.

2. Each party is to bear its own costs, expenses and attorneys' fees in this legal action.

Dated: May ___, 2016

_____
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David D. Axtell (#314596)
William D. Thomson (#396743)
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Confidential Outside Attorneys' Eyes Only DDROPS-0009226
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Dated: May __, 2016

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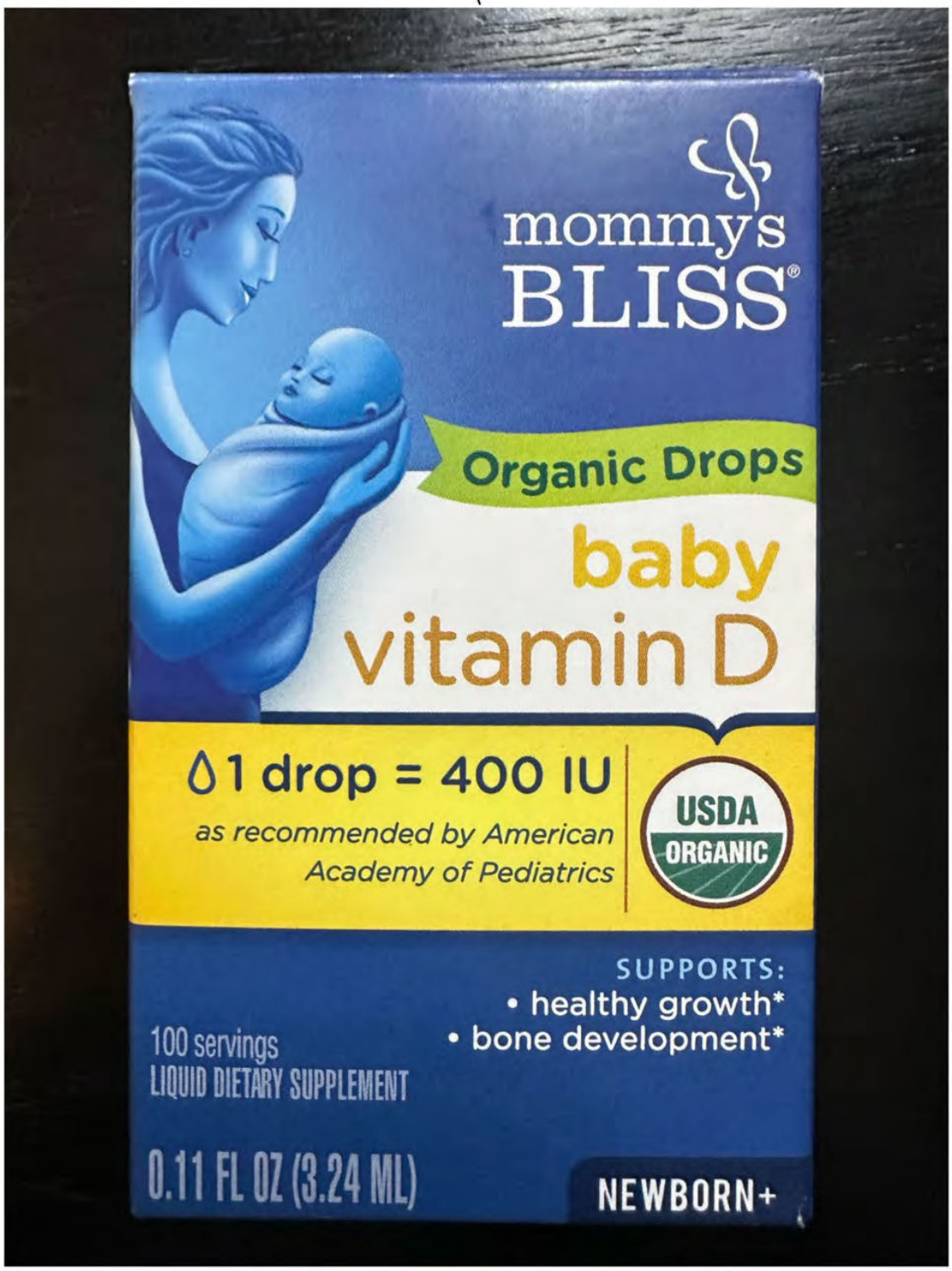
Attorneys for Plaintiffs

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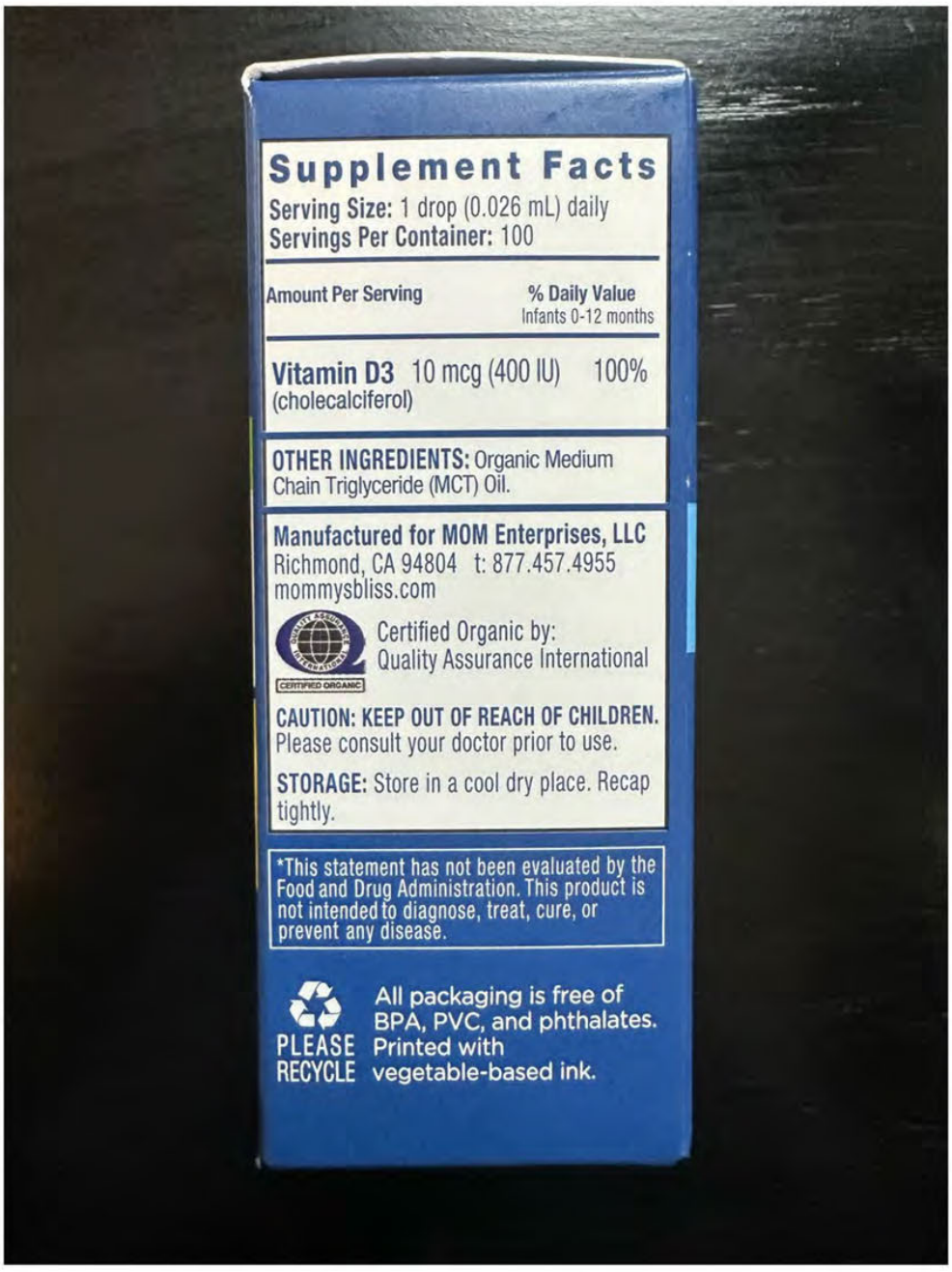
Exhibit V

Redacted

Exhibit X


mommy's BLISS®
Organic Drops
baby vitamin D
1 drop = 400 IU
as recommended by American Academy of Pediatrics
USDA ORGANIC
SUPPORTS:
• healthy growth*
• bone development*
100 servings
LIQUID DIETARY SUPPLEMENT
0.11 FL OZ (3.24 ML)
NEWBORN+


for moms, by moms
Our family-run company was founded by my mom, who raised three children while working as a nurse and certified midwife. Driven by compassion for other moms, she created Mommy's Bliss to safely and naturally bring families back to wellness. As a new mom myself, I understand how important it is to choose products that I trust to work effectively and safely and make my little ones happy!
Yasmin Kaderali
mom, daughter & CEO
Mommy's Bliss
FOUNDATION
bliss for every baby
Your purchase supports improving the health of 20,000+ children around the globe. Learn more at: www.mommysbliss.com/foundation.
MOM
Founded + Led
CONTENTS CERTIFIED
NSF
NON GMO Project
VERIFIED
nongmoproject.org


Supplement Facts
Serving Size: 1 drop (0.026 mL) daily
Servings Per Container: 100
Amount Per Serving
% Daily Value
Infants 0-12 months
Vitamin D3 10 mcg (400 IU) 100%
(cholecalciferol)
OTHER INGREDIENTS: Organic Medium Chain Triglyceride (MCT) Oil.
Manufactured for MOM Enterprises, LLC
Richmond, CA 94804 t: 877.457.4955
mommysbliss.com
Certified Organic by:
Quality Assurance International
CERTIFIED ORGANIC
CAUTION: KEEP OUT OF REACH OF CHILDREN.
Please consult your doctor prior to use.
STORAGE: Store in a cool dry place. Recap tightly.
*This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.
PLEASE RECYCLE
All packaging is free of BPA, PVC, and phthalates. Printed with vegetable-based ink.



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organic
MEDIUM CHAIN TRIGLYCERIDE (MCT) OIL
Supplement Facts
Serving Size: 1 drop (0.026 mL) daily
Servings Per Container: 100
Amount Per Serving
% Daily Value Infants 0-12 months
Vitamin D3 10 mcg (400 IU) 100%
(cholecalciferol)
OTHER INGREDIENTS: Organic Medium Chain Triglyceride (MCT) Oil.
Manufactured for MOM Enterprises, Inc.
Richmond, CA 94804 t: 877.457.4955
mommysbliss.com
Certified Organic by: Quality Assurance International
CAUTION: KEEP OUT OF REACH OF CHILDREN.
Please consult your doctor prior to use.
STORAGE: Store in a cool dry place. Recap tightly.
*This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.
PLEASE RECYCLE
All packaging is free of BPA, PVC, and phthalates. Printed with vegetable-based ink.
and
VITAMIN D3


NEW & IMPROVED SQUEEZE BOTTLE
mommy's BLISS
Organic Drops
baby vitamin D
1 drop = 400 IU
as recommended by American Academy of Pediatrics
USDA ORGANIC
SUPPORTS
• healthy growth*
• bone development*
100 servings
LIQUID DIETARY SUPPLEMENT
0.11 FL OZ (3.24 ML)
NEWBORN+


Ingredients
CONTENTS CERTIFIED NSF
CONTENTS CERTIFIED USDA ORGANIC
what's in it
Vitamin D
Organic MCT Oil
what's not in it
Artificial colors and flavors
Sucrose
Gluten
Binders or fillers
Dairy
Yeast
Dill
Petroleum based by-products
Alcohol