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Review

Does Vitamin D Make the World Go 'Round'?

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Abstract

Vitamin D has emerged from obscurity, and its effects on various organ systems throughout the body down to the cellular level are being discovered. What was once thought to be a simple hormone affecting only bone and calcium metabolism has shifted. We no longer see vitamin D as a "vitamin" important only in childhood, but as a complex hormone that is involved not only in calcium homeostasis but also in the integrity of the innate immune system. Vitamin D deficiency is linked to inflammatory and long-latency diseases such as multiple sclerosis, rheumatoid arthritis, tuberculosis, diabetes, and various cancers, to name a few. In this review, we trace how we came to view vitamin D and how that view led to one of the largest epidemics of nutrient deficiency beginning in the late 20th century. We then discuss the needs of vitamin D in the context of the breastfeeding mother and her infant and child, why breastfed infants are particularly at risk, and what to do about it.

Introduction

EVIDENCE OF THE RESURGENCE of interest in vitamin D abounds. One only has to pick up a health magazine or a local newspaper, turn on the television, or do a search on the internet to find a plethora of information. The renewed interest reflects the health attributes of vitamin D beyond bone metabolism and the widespread deficiency that affects all groups but particularly those of darker pigmentation.[1–31] Long-standing vitamin D deficiency is linked to a myriad of disease states through its putative effect on the innate immune system.[32] It is only with large numbers of individuals who suffer from vitamin D deficiency that such connections between deficiency and disease could be discerned. How did we get to this place—this place of widespread vitamin D deficiency? What is the evidence that we, in fact, have vitamin D deficiency at epidemic proportions in the United States?

A Historical Perspective

As early as the mid-1600s, rickets was identified as a major health problem for young children as people began the exodus from rural farming communities to urban areas, which in turn brought about lifestyle and environmental changes that limited sunlight exposure. Those with the disease of rickets were identified by deformities of the skeleton, including enlargement of the head, joints of the long bones, and rib cage and curvature of the spine and thighs, coupled with generalized muscle weakness. The incidence of rickets escalated during the industrial revolution: By the early 19th century, rickets was epidemic in northern Europe and in industrialized northern regions of the United States. In 1822, Sniadecki, as noted by Mozolowski,[33] published the first observation that lack of sun exposure could be the cause of rickets: He found that children who lived in Poland had a higher incidence of rickets compared with children from the countryside who were disease-free. By the mid-1800s, fish liver oils were discovered to heal rickets.[34] These clinical observations led many to believe that some type of nutritional deficiency caused rickets. It was not until the 1920s that vitamin D was identified and the link was made to rickets.[35,36]

General Metabolism of Vitamin D

It would take another 50 years before vitamin D and its metabolites could be measured consistently and with accuracy.[37] We were to learn that vitamin D occurs as vitamin D_3 (cholecalciferol), a 27-carbon derivative of cholesterol, and vitamin D_2 (ergocalciferol), a 28-carbon molecule derived

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from the plant sterol ergosterol. Compared to vitamin D_3, vitamin D_2 has an extra methyl group and a double bond between carbons 22 and 23. Vitamin D has a unique *cis*-triene structure that makes the vitamin and its related metabolites susceptible to oxidation, ultraviolet (UV) light-induced conformational changes, and attack by free radicals. It is the second process—that of a light-induced conformational change—that allows the body to make endogenous vitamin D_3 following sunlight exposure. Specifically, as shown in Figure 1, vitamin D_3 is produced in the skin from the provitamin D_3, 7-dehydrocholesterol.[38,39] It is the exposure of skin to sunlight in the UVB range of the spectrum (290–315 nm) that results in the photolytic conversion of 7-dehydrocholesterol to previtamin D_3. Through the action of thermal energy, previtamin D_3 is isomerized to vitamin D_3.[39]

Processing of Vitamin D Within the Body

Once vitamin D enters the circulation (see Fig. 1), through either epidermal transfer or intestinal absorption, it associates with vitamin D-binding protein (DBP), a 58-kDa globular protein that binds vitamin D and its metabolites.[40] The initial step in the metabolic activation of vitamin D is the enzyme-catalyzed insertion of an OH group at carbon 25 to produce 25-hydroxyvitamin D [25(OH)D], the most abundant circulating form of vitamin D.[37,41] Following formation in the liver, 25(OH)D enters the circulation where it is bound to DBP with high affinity.[42] Only small amounts of 25(OH)D are free—an important point because only the "free" concentration of the vitamin has transmembrane diffusion capabilities, thus exerting its biologic function. The conversion of 25(OH)D to 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] occurs predominantly in the kidneys; however, extrarenal conversion has been reported in cell types throughout the body, including the brain.[32,43–48] The half-life ($t_{1/2}$) of 25(OH)D in the circulation is about 2–3 weeks in normal individuals.[49] Because of its relatively long $t_{1/2}$ as compared with vitamin D (1–2 days) and $1,25(OH)_2D$ (12–24 hours), circulating 25(OH)D is the best indicator of nutritional vitamin D status.[37]

While 25(OH)D is the best indicator of an individual's vitamin D status, $1,25(OH)_2D$ or calcitriol is the most active of the vitamin D moieties and thus considered the "true" hormonal form of vitamin D. Calcitriol works together with parathyroid hormone to maintain proper levels of calcium in the blood through enhanced intestinal absorption of calcium, decreased urinary calcium loss, or release of calcium from the bones. The body works to maintain a normal serum calcium level at the expense of bone loss if calcium is not readily available in the diet. The stages of vitamin D deficiency and their effect on calcium and phosphorus metabolism are reviewed in two recent reviews.[50,51]

Sunlight Synthesis

Several studies have shown that a single minimal erythemic dose of exposure to sunlight or UV light is equivalent to an oral vitamin D intake of 250–625 μg (10,000–25,000 IU) of vitamin D.[52–55] Importantly, a single minimal erythemic dose in dark-skinned individuals may require up to 10 times the UV exposure when compared with fair-skinned subjects.[56,57] Despite differences in skin pigmentation, humans share the same capacity to synthesize vitamin D but have different sunlight exposure requirements to trigger the endogenous process of vitamin D synthesis in the skin. These points become critical when one realizes that the photoconversion of vitamin D_3, even in fair-skinned individuals, does not occur in northern latitudes (or southern latitudes in the southern hemisphere) for several months during the winter,[58,59] a problem exacerbated in darkly pigmented individuals.

In Western cultures, how much sunlight does the average individual receive? We derive an indirect answer from

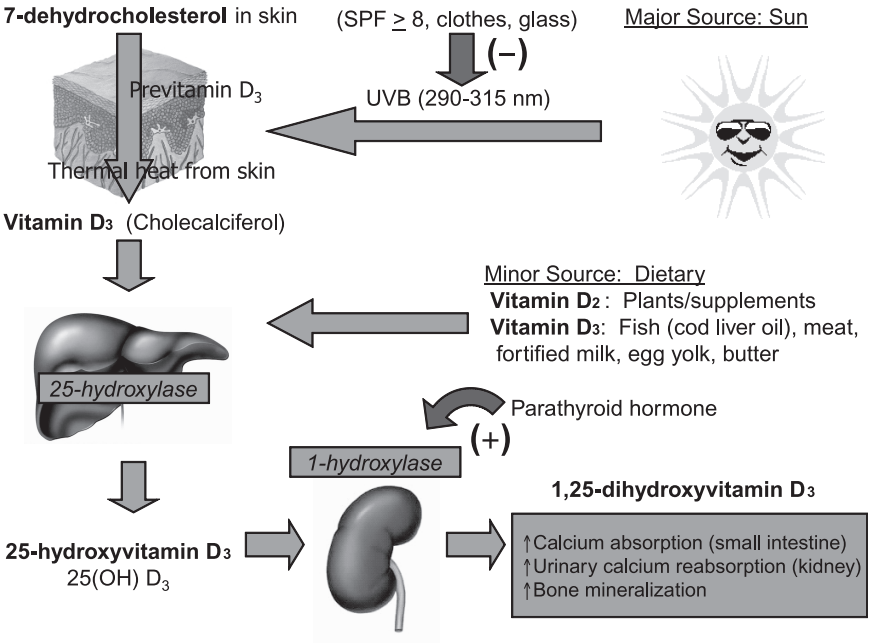

FIG. 1. The synthesis and fate of vitamin D.

studying the Indoor Air Quality Act that was passed by Congress.[60] In this national U.S. study, it was found that the average American spent 93% of their 24-hour day indoors. Since that time, air conditioning, computers, video games, and extensive television programming have become more readily available, increasing time spent indoors. Because of such changes in current lifestyles, humans are now more dependent on oral vitamin D supplementation than in our distant past.

Dietary Contribution to Our Body's Vitamin D Stores

Both vitamin D_2 and vitamin D_3 can be obtained from the diet; however, vitamin D is distributed *very* poorly in natural foodstuffs. Vitamin D is found primarily in oily fish such as salmon and swordfish, egg yolk, butter, and liver, and the average Western diet provides less than 10% of the total concentration of vitamin D in the body. Because of its extremely low abundance in foods, vitamin D commonly is fortified in food products, the most common of which is milk, at low concentrations. The vitamin D found in foods or in a supplement is easily absorbed, but the body's requirements are higher than what is provided in a traditional Western diet. Only those peoples who ingest large quantities of fish, seal, or whale—including the fat, such as traditional Eskimos—will have adequate intake (AI) of vitamin D in their food.

What Determines Your Vitamin D Status?

As shown in Table 1, there are a number of factors that affect your vitamin D status, not the least of which is your sunlight exposure and, for a given exposure, the degree of skin pigmentation, your fat mass, and use of protective clothing and/or sunscreen.[54,61–63] Seasonality is an important issue as well. There is the claim that is purported by many in medicine that if one puts one's face and hands out the window for 15 minutes three times a week, such sunlight exposure will generate enough vitamin D to keep you in the sufficient range.[54,57,59,62–65] Unless you live at the equator, this is fallacious thinking. Sunlight exposure's effect on the body's synthesis of vitamin D depends on the surface area of the body that is exposed.[59] It also depends on the season: During winter months for many parts of the world, the angle of the sun is altered such that the UVB that reaches the skin is below the necessary range for vitamin D synthesis.[55] In this way, latitude and season play significant roles. One does not need to worry about seasonality per se in areas of the world nearest the equator—of course, assuming that an individual's skin is exposed to sunlight. These factors influence your overall vitamin D status and thus your vitamin D requirements.

Table 1. Main Factors Affecting an Individual's Vitamin D Status

Sunlight exposure
Degree of skin pigmentation
Use of sunscreen (SPF ≥8)
Latitude
Season
Time spent outdoors
Protective clothing: type of clothing and degree of body covered
Body mass and percentage body fat
Diet—intake of fish oil, oily fish, foods with vitamin D fortification
Vitamin D supplementation

Vitamin D Requirements

Adult requirements for vitamin D have been a moving target. Four decades ago, the American Academy of Pediatrics' Committee on Nutrition noted that there were few data concerning the vitamin D requirements of older children and adults. Based on the data available at the time and the premise that vitamin D's action were limited to bone and calcium metabolism, the American Academy of Pediatrics recommended one-half the infant dose for adults or 200 IU/day and 400 IU/day for pregnant and lactating women, respectively.[66] Similar recommendations for adults also were made in England.[67] The adult AI of 200 IU was described as "generous" in the 1989 version of recommended intakes by the Institute of Medicine/National Academy of Sciences.[68] Yet, at this dose (10 μg/day) in an adult, circulating 25(OH)D levels usually remain unchanged or decline, especially during winter months.[69–73] This was first demonstrated in both adolescent girls and young women.[17,71] In another study involving adult submariners with no sunlight exposure for up to 6 months, even 600 IU/day vitamin D *did not* maintain circulating 25(OH)D.[74] Evidence is mounting that the recommendations for older children and adults perhaps valid a few decades ago are no longer valid today.[75–77] Yet, why have medical experts been reluctant to increase vitamin D requirements? The answer is in the history books.

The Dangers of Vitamin D

As early as the 1920s, reports of vitamin D toxicity surfaced.[78–80] Individuals were prescribed or given hundreds of thousands of international units of vitamin D that resulted in the classic symptomatology of toxicity within weeks: anorexia, nausea/vomiting, weakness, fatigue, lassitude, polyuria/polydipsia, and nocturia. Laboratory parameters showed hypercalcemia, acute and/or chronic renal failure, and variable degrees of hyperphosphatemia.[81,82] Excessive calcification of the epiphysis and metaphysis and extramedullary calcifications were found on radiographs.[81,83] Harris and Innes,[79] Harris and Moore,[84] Ham and Lewis,[85] and others reported that hypervitaminosis D was a real and reproducible entity that could be replicated in the laboratory using rat and rabbit models. Investigators gave pharmacological doses to rats, which were similar to the amounts prescribed or given to some children and adults.[79] Since that time, reports of vitamin D toxicity have occurred—in each case involving ingestion of hundreds of thousands of international units of vitamin D taken for weeks to months.[83,86] An excerpt from Debré[86a] gives us a glimpse of the dosage that causes such toxicity: "What are signs by which one can make a prognosis? The total dosage of the drug is not the main factor. However, it is true that the children who died (at age of 20 and 16 months) had received respectively 11,200,000 and 18,200,000 units. The mild cases occurred when only 3,000,000 to 6,000,000 units had been given." Clearly, these children were given pharmacological doses of vitamin D and not doses within the physiological range.[69,70,72,86b]

What made it more difficult to discern vitamin D's safety was that for a subset of infants and children, vitamin D's toxicity appeared even at much smaller doses of vitamin D.[87–89] In addition, when vitamin D was given to pregnant women, there were reports of affected offspring with a specific constellation of findings.[88,90] First described by Lightwood[91] in 1932 and again as a case series in 1952,[92] by Anderson and Schlesinger[93] in 1940 and as a case series by Schlesinger et al.[88] in 1956, by Baggenstoss and Keith[94] in 1941, by Fanconi et al.[95] in 1952, and by Creery[96] in 1953, the entity of idiopathic hypercalcemia of childhood was discovered and redefined. Russell and Young[97] described to the Royal Society of Medicine two cases of idiopathic hypercalcemia of infancy with the following conclusion: "It may be concluded that the pathological process underlying the severe and chronic form of hypercalcaemia in infancy is intoxication with vitamin D or with some factor resembling its effects, probably initiated prenatally . . . are likely due to the same causative factor operating later or with less intensity than in the cases with manifest skeletal changes and gross mental and physical retardation."

Definitive "proof" of vitamin D's toxicity and teratogenicity surfaced in the early 1960s. In 1963, Black and Bonham-Carter[98] recognized that elfin facies observed in patients with severe idiopathic infantile hypercalcemia resembled peculiar facies observed in patients with supravalvular aortic stenosis (SAS) syndrome. Shortly thereafter, Garcia et al.[99] documented the occurrence of idiopathic hypercalcemia in an infant with SAS. The infant also had peripheral pulmonary stenosis, mental retardation, elfin facies, and an elevated blood concentration of vitamin D. Additional support came from the work of Friedman and Roberts.[100]

What is interesting is that in 1964, no quantitative means of assessing circulating concentrations of vitamin D existed.[101] In fact, at that time, it was unproven that vitamin D was further metabolized within the body. Despite these limitations, by 1967, vitamin D was viewed by many in the medical community as the cause of SAS syndrome[102–105]; specifically, it was thought that maternal vitamin D supplementation during pregnancy and its associated toxicity caused SAS syndrome in a subgroup of susceptible fetuses and infants resulting in the constellation of findings that included the elfin facies and other described findings. Animal models were developed to show that toxic excesses of vitamin D during pregnancy would result in SAS.[106,107] In those studies, pharmacologic doses—not physiologic doses—of vitamin D were given to animals, creating hypervitaminosis D with hypercalcemia.

What we were to find out was that SAS was not caused by too much vitamin D per se, but in fact is a genetic disorder called Williams' syndrome.[108] Williams' syndrome is a severe genetic affliction related to elastin gene disruption caused by deletion of elastin and contiguous genes on chromosome 7g11.23. The syndrome is characterized by multiorgan involvement (including SAS), dysmorphic facial features, and a distinctive cognitive profile.[109] Williams' syndrome patients often exhibit abnormal vitamin D metabolism with an exaggerated increase in circulating 25(OH)D to orally administered vitamin D, and therefore such patients are susceptible to bouts of idiopathic hypercalcemia. This relationship was suspected as early as 1976[110] but was not made definitively until 1991.[109]

As mentioned earlier, those cases of vitamin D toxicity that have occurred in infants, children, and adults without Williams' syndrome occurred when excessive doses (well in excess of 10,000 IU/day) were given. Despite the enhanced understanding about the cause of SAS in patients with Williams' syndrome, it was not known until recently what doses of vitamin D were physiologic and what were pharmacologic. Because of this lack of understanding, fear of causing hypervitaminosis D in individuals, particularly pregnant women, has continued to present.[111,112]

What Constitutes Sufficiency?

What then should the AI for vitamin D be in the neonate, the infant, the young child, the 10-year-old, the adolescent, the adult, and the adult who is pregnant or lactating in order to achieve optimal circulating concentrations of 25(OH)D? Before that question can be answered, the optimal concentration of circulating 25(OH)D needs to be determined across the lifespan and based on body mass. Most studies have concentrated on how much vitamin D is required to avoid deficiency as manifested by bony changes such as rickets and osteopenia.[113] Available evidence in which circulating intact parathyroid hormone and 25(OH)D were measured in adult patients indicates that secondary hyperparathyroidism occurs when serum 25(OH)D values fall below the range of 15–20 ng/mL.[114–116] A recent report by Vieth et al.[117] demonstrates that maximal suppression of parathyroid hormone by circulating 25(OH)D occurs at >80 nmol (32 ng/mL) of 25(OH)D. Heaney et al.[118] have demonstrated in normal adults that intestinal calcium absorption is reduced in individuals who exhibit circulating 25(OH)D levels of 20 ng/mL compared to subjects with circulating levels >32 ng/mL. The authors concluded that individuals with circulating 25(OH)D levels at the low end of the current reference range may not be getting the full benefit from their calcium intake. Recent, additional retrospective and interventional studies suggest that circulating 25(OH)D needs to exceed 80 nmol to maximize skeletal integrity.[119,120]

As was mentioned earlier, health professionals need to "broaden their horizon" and think of vitamin D in more global health terms that incorporate vitamin D's true role as a hormone. The vitamin D endocrine system is the *only* steroid endocrine system in the body that is almost always limited by substrate availability because of latitude, lifestyle, race/skin pigmentation, sunlight exposure, and other factors. This limitation of substrate affects both the conversion of vitamin D to 25(OH)D and the conversion of 25(OH)D to $1,25(OH)_2D$ in renal and extrarenal sites.

Health Implications of Vitamin D

Increased circulating 25(OH)D has been linked with improved glucose handling and beta-cell function[22] and a growing list of long-latency diseases that include cardiovascular disease,[28,29,121,122] multiple sclerosis,[123–125] rheumatoid arthritis,[126] type 1 and 2 diabetes,[126] and at least 15 types of cancers.[26,127–134] While these studies describe strong correlation with vitamin D deficiency, they do not provide proof of causality or a mechanism of action. Two studies have begun to decipher the riddle of vitamin D's role in maintaining the innate immune system with profound implications.[32,135] Some of these data, as well as additional studies, have been

summarized in a recent review regarding the optimization of circulating 25(OH)D levels to reduce the risk of long-latency disease states.[136]

Ideally, the total circulating 25(OH)D should mirror what is attained by those who live and work in a sun-rich environment who have levels of 54–90 ng/mL[65,137,138] and not by those who are sunlight-deprived or covered from sunlight.[139] The debate about what constitutes frank deficiency, insufficiency, and sufficiency continues. Depending on what biomarker one chooses, there could be a different cutoff point for each category. Most, however, would agree that levels below 50 nmol/L (or 20 ng/mL) represent deficiency; whether that label extends to 70 or even 80 nmol/L is less clear.

Effect of Oral Supplementation on Circulating 25(OH)D

Several studies suggest that intakes of 1,000 IU/day in adults raise serum 25(OH)D values only to slightly above 24 ng/mL.[115,140–144] In a recent landmark study, Vieth et al.[70] examined the efficacy and safety of relatively high intakes of vitamin D by assessing the effects of vitamin D of 1,000 and 4,000 IU/day in 61 adults for up to 5 months. They found that vitamin D at a dose of 4,000 IU/day was effective in elevating the serum 25(OH)D concentration to normal values (40 ng/mL). It is important to note that in this study a steady-state value of circulating 25(OH)D was achieved approximately 90 days following initiation of supplementation at the 4,000 IU/day level. In another study, Heaney et al.[72] supplemented male Caucasian subjects during winter months with 1,000, 5,000, or 10,000 IU of vitamin D/day for a period of 4 months. As in the previous study,[70] these investigators observed a steady-state value of circulating 25(OH)D levels following approximately 90 days of supplementation. At the end of the study period, the average increase from baseline of circulating 25(OH)D was 4.8, 36.7, and 63.8 ng/mL for the 1,000, 5,000, and 10,000 IU daily dose groups, respectively. The final circulating levels of 25(OH)D in these treatment groups were 33.6, 64.5, and 90.0 ng/mL, respectively. In this entire study, not a single episode of hypercalcemia or hypercalciuria was observed.

Vitamin D Content of Human Milk and Factors Affecting Content

Human milk had long been thought to be an adequate source of antirachitic activity for the neonate and growing infant. Even before the discovery of vitamin D, McCollum et al.[35] and Park[36] stated that rickets was due to the deprivation of sunlight and a dietary factor X. They observed that factor X was found in "good breastmilk" and cod liver oil and that although rickets did develop in breastfed children it was rarely as severe as in artificially fed infants. Early attempts to quantify the antirachitic potential of human milk were crude and yielded little information.[145–147] For a time, it was believed that vitamin D sulfate was responsible for the antirachitic activity in human milk[148,149]; however, this was shown not to be the case.[150]

In the 1980s, antirachitic activity of human milk from mothers receiving 400 IU of vitamin D/day was defined with sensitive assay technology to be 20–70 IU/L.[101,151,152] Further, almost all of the activity was attributable to vitamin D and 25(OH)D. These studies also demonstrated that dietary maternal vitamin D supplementation and UV light exposure increased the vitamin D content of human milk.[101,153,154] Specker et al.[155] determined that the antirachitic content of human milk was lower in African American than in Caucasian mothers. This difference was attributed to variation in dietary intake of vitamin D and UV exposure.

An interesting study involved a woman with hypoparathyroidism who was treated with 100,000 IU/day vitamin D_2 for the maintenance of her plasma calcium throughout pregnancy, delivered a normal child at term, and then breastfed her infant.[156] Analysis of breastmilk from this mother showed it to contain over 7,000 IU/L antirachitic activity. While a study by our group involving lactating mothers receiving up to 4,000 IU of vitamin D_2/day did raise the antirachitic activity of their milk, it did not rise above 200 IU/L.[23] In a subsequent study of maternal supplementation with 6,400 IU of vitamin D_3/day, however, milk antirachitic activity was observed to increase to nearly 800 IU/L, which resulted in substantial increases in neonatal 25(OH)D levels. This was achieved without toxicity to the mother. Thus, it is clear that the vitamin D content of human milk can be influenced by maternal diet and/or UV exposure. *If a lactating mother has limited exposure, has darker pigmentation, and/or limited vitamin D intake (such as occurs with the current 400 IU/day AI), the vitamin D content of her milk will be low.*

Vitamin D Supplementation During Lactation

Scientific data pertaining to vitamin D supplementation during lactation in the human subject are extremely scarce. An arbitrary AI has been set at 400 IU/day for the lactating mother.[157] Three studies prospectively examined vitamin D supplementation during lactation.[23,158,159] The first study involved supplementation of lactating mothers with either 1,000 or 2,000 IU of vitamin D/day for a period of 15 weeks. The rise in circulating 25(OH)D levels during this period of supplementation was 16 and 23 ng/mL for the 1,000 and 2,000 IU dose groups, respectively. A recent study performed in our laboratory involved supplementing lactating mothers with 2,000 and 4,000 IU of vitamin D/day for a period of 3 months.[23] As was mentioned earlier, we found an increase in maternal circulating 25(OH)D, antirachitic content of milk, and circulating 25(OH)D in the recipient infant using maternal vitamin D_2 supplementation with 2,000/IU day, but more significantly with 4,000 IU/day.[23] The levels were less than predicted by pharmacokinetics, which may be explained by the observation that in some circumstances vitamin D_2 appears inferior to vitamin D_3 at maintaining circulating 25(OH)D levels in humans.[160] In a subsequent supplementation study with vitamin D_3, we found an improved vitamin D status in nursing infants whose mothers were randomized to the 6,400 IU/day group compared to the 400 IU/day group.[73] It is clear that larger, more detailed studies are required to determine the vitamin D requirement of the lactating mother. We have reviewed this subject previously in detail.[136]

Breastfeeding's Effect on Infant Vitamin D Status and Its Relationship to Nutritional Rickets

Thirty-five years ago the incidence of nutritional rickets was thought to be disappearing.[159] Many reports since then, however, indicate that this is not the case.[162–168] The major-

ity of the cases reported in the last decade involved darkly pigmented infants who were exclusively breastfed. The marginal vitamin D status of mothers and breastfeeding infants even in sunny climates such as Charleston, SC is underscored by our own recent data.[169] Hypovitaminosis D in the breastfed infant also is a severe problem in sun-rich environments such as the Middle East.[20] This hypovitaminosis D results because sun exposure to both mother and infant is extremely limited. Further, dietary supplementation in this population is not a common practice.

Antirachitic Activity of Human Milk

From the prior discussion in this report, it is clear that the antirachitic content of human milk is quite variable and is affected by season, maternal vitamin D intake, form of vitamin D taken (D_2 or D_3), and race. Cancela et al.[170] have reported that circulating 25(OH)D levels in breastfed infants are directly related to the vitamin D content of mother's milk. This was also shown by Hollis and colleagues.[23,73,171]

Greer and Marshall[172] reported that exclusively breastfed Caucasian infants nursed during the winter in a northern climate maintained a "minimally normal" vitamin D status for a period of 6 months. During the study, circulating 25(OH)D levels in the breastfeeding infants from the this study actually declined as winter progressed. This decline occurred in spite of a maternal vitamin D intake of approximately 700 IU/day.[172] Similarly, a Finnish study showed that maternal supplementation with 1,000 IU/day vitamin D had little effect on either *maternal* or *nursing infants*' circulating 25(OH)D values. Interestingly, these same investigators repeated a similar study with 2,000 IU/day and found nursing infants' vitamin D status to improve significantly.[159] In this latter study, the authors added a disclaimer, "A sufficient supply of vitamin D to the breastfed infant is achieved only by increasing the maternal supplementation up to 2,000 IU/day. As such, [this] dose is far higher than the daily dietary allowance recommended for lactating mothers [and therefore] its safety over prolonged periods is not known and should be examined." Hollis and Pittard[41] previously showed that vitamin D status at birth is closely related to that of the mother and is related to race. These data from more than 2 decades ago clearly demonstrated that urban African American women and their infants have circulating 25(OH)D levels well below those that constitute vitamin D deficiency as it is defined today.[136]

The relationship between circulating vitamin D_2, D_3, 25-hydroxyvitamin D_2 [25(OH)D_2], and 25-hydroxyvitamin D_3 [25(OH)D_3] and corresponding milk levels in 51 lactating mothers was described in 1986 by Hollis et al.[171] There was a significant correlation seen in regression analyses between vitamin D_2 in maternal serum and human milk. Similar significant relationships were found between plasma and milk concentrations of vitamin D_3, 25(OH)D_2, and 25(OH)D_3. In contrast, the plasma DBP levels were not related in these fluids. The parent vitamins gain access into the milk much more readily than do their 25-hydroxylated metabolites: vitamin D in milk was 20% of the plasma concentration, whereas 25(OH)D in milk was approximately 0.5–1.0% of that in plasma. Prior studies suggest that 25(OH)D is the most stable antirachitic compound, whereas vitamin D is the compound that provides the greatest potential for "adjustment" of antirachitic activity in milk.[101,154,156,171]

The transfer of the antirachitic sterols from the circulation to milk is most likely a function of their ability to associate with the plasma DBP. The DBP functions as a "sink" for vitamin D and its metabolites, and the vast majority of these antirachitic sterols are bound by this globulin in the circulation.[37] The antirachitic sterols can only enter a cell by diffusion once they are dissociated from their carrier protein (DBP). This is referred to as the "free concentration" of the sterol and follows the law of mass action.[42] The free concentration of the sterol is determined by two factors: (1) the sterol's affinity to bind to the DBP and (2) the concentration of the DBP in the circulation. The higher the binding affinity of the sterol towards the DBP, the lower the free concentration of the sterol and the less sterol available for cell membrane translocation, or, in other words, less transfer into the milk. This translocation of vitamin D from blood to milk probably occurs through a lipoprotein-containing particle, much like that of cholesterol.[173] From previous work, we know that the association constant for the antirachitic sterols with the DBP is 25(OH)D >>> vitamin D.[40] Thus, this model predicts what has been observed: The circulating parent vitamin D gains access to milk at a much greater rate than does the 25-hydroxylated metabolite.

These data have a practical implication: The vitamin D content of human milk is directly related to the lactating mother's vitamin D status. Vitamin D status in this case refers to both circulating vitamin D and 25(OH)D. In lactating mothers taking 400 IU/day vitamin D, we found human milk to contain 33–68 IU/L antirachitic activity.[73,171] In a recent supplementation study of women at baseline taking 400 IU/day vitamin D ($n = 35$), the mean antirachitic activity of the milk was 37.9 ± 10.7 IU/L.[23] These calculations are based on various conversion factors for the biological activity of 25(OH)D. All biological assays are based on the parent vitamin containing 1 IU activity (25 ng),[174] with some disagreement, however, with regard to the biological activity of 25(OH)D, with 1 IU equaling between 5 and 18 ng depending on the biological assay.[152,174] Both the parent vitamin and the 25-hydroxylated form contribute significantly to the antirachitic properties of human milk. Given that, which form of the vitamin is most important in determining the antirachitic properties of milk?

The data suggest that the role of 25(OH)D is to supply a relatively stable amount of antirachitic activity into milk, which appear to be dictated by two factors: (1) Circulating levels of 25(OH)D are stable for relatively long periods of time ($t_{1/2}$ ~3 weeks) and therefore are not influenced greatly by day-to-day sun exposure or dietary changes. (2) The transfer of 25(OH)D from circulation to milk is greatly limited by the circulating DBP that binds 25(OH)D with high affinity and thus limits its free concentration and translocation across the mammary complex into the milk.

Effect of Sunlight Exposure on Milk's Vitamin D Content

This question was addressed by Greer et al.[154] in their study of lactating women. Following total body UVB exposure, increasing vitamin D_3 concentrations in the circulation and milk peaked within 48 hours, followed by a rapid decline in both fluids due to the relatively short $t_{1/2}$ of the parent vitamin in the circulation. In these same subjects, circu-

lating 25(OH)D_3 concentrations also increased from 13.9 to 20.5 ng/mL and remained significantly elevated for at least 14 days. There was no significant change, however, in milk 25(OH)D concentrations during this period. Conversely, because of the appearance of vitamin D_3 following simulated sunlight, antirachitic activity in mother's milk increased severalfold. What also is apparent from this study is the rapid decline in circulating and milk vitamin D_3 concentrations following a single phototherapy session due to the short parent vitamin $t_{1/2}$ in the circulation.

The question then becomes: How can the circulating level of vitamin D be kept elevated for extended periods? Very limited data exist on this point because frankly there was little attention in the past given to determining what sustained levels of vitamin D were. Rather, all of the attention was or is focused on 25(OH)D. *However, for a lactating mother, it is essential that sustained circulating vitamin D be maintained.* Again, sustained circulating vitamin D in the mother will result in a substantial increase in the vitamin D content of her milk. We estimate from our latest preliminary data that daily maternal intakes of 6,400 IU/day vitamin D will result in raising the antirachitic activity of their milk to 500–800 IU/L.[73] This level of antirachitic activity in human milk likely will be sufficient for the nursing infant to maintain adequate circulating levels of 25(OH)D.

High-Dose Supplementation During Lactation

During the past few years, we have conducted pilot studies[23,73] and now a National Institutes of Health-sponsored trial examining the effect of maternal high-dose vitamin D supplementation on the mothers and their nursing infants in a randomized, blinded fashion. The results of the two pilot studies are summarized in Table 2. The women who completed both studies were either exclusively or fully breastfeeding, with confirmation of infant dietary intake by a detailed dietary log and monthly interview. Blood, urine, and milk samples were obtained monthly from the mothers. Infant blood was collected at months 1 and 4 of Study 1 and months 1, 4, and 7 in Study 2. In Study 2, those mothers randomized to receive 6,400 IU of vitamin D_3/day were supplied with placebo drops for their infants, while mothers ingesting placebo tablets received infant drops with 300 IU of vitamin D_3 for daily dosing. In both studies, serum from the mother was monitored for total calcium, phosphorus, and vitamins D_2, D_3, 25(OH)D_2, and 25(OH)D_3. Infant serum was monitored for vitamins D_2, D_3, 25(OH)D_2, and 25(OH)D_3, calcium, and phosphorus. Mother's urine was monitored for calcium/creatinine ratio, and milk was assessed for vitamin D antirachitic activity by measuring vitamin D_2, D_3, 25(OH)D_2, and 25(OH)D_3. In Study 1, vitamin D_2 was used for maternal dosing as a specific tracking agent because the contribution of D_2 from another source would be unlikely or minimal. By using vitamin D_2 in this study, we could precisely define the rise and/or transfer of vitamin D compounds in/from mother to her infant without confounding factors such as extra dietary intake and sun exposure.

In both studies, maternal supplementation with high-dose vitamin D (2,000, 4,000, or 6,400 IU of vitamin D/day) safely resulted in increases in maternal circulating 25(OH)D concentrations and in milk antirachitic activity.[23,73] As expected, the milk antirachitic activity in the 6,400 IU/day group of lactating mothers increased the most to over 800 IU/L, which resulted in a dramatic rise in infant circulating 25(OH) levels and mirrored levels of infants in that study receiving 300 IU/day vitamin D_3 directly in drops. In contrast, the mothers receiving only 400 IU/day exhibited a substantial decline in circulating 25(OH)D over a 3-month period during the winter months that placed them in a hypovitaminotic D state.[136] As a function of seasonality, these mothers' circulating 25(OH)D levels ultimately recovered later in the study because of increased UV exposure. Finally, we made an interesting observation in one of our subjects ingesting 6,400 IU/day. Four days prior to visit 4 (3 months), this mother acquired an intestinal virus and was unable to take her supplement for 3 days prior to the scheduled visit. The rapid effect of the missed doses is apparent by the rapid decline of circulating vitamin D_3 and the resulting drop in milk antirachitic activity. This reinforces our premise that in order to maintain her milk activity at maximum levels, the lactating mother requires daily vitamin D_3 ingestion.

Table 2. High-Dose Vitamin D Supplementation During Lactation

Study	*Circulating 25(OH)D (ng/mL)*	*Vitamin D (ng/mL)*	*Milk antirachitic activity (IU/L)*
Hollis and Wagner[23] (2004)			
Group 1			
Mother 1,600 IU D_2 + 400 IU D_3	36.1	3.4	69.7
Infant 0 IU	27.9		
Group 2			
Mother 3,600 IU D_2 + 400 IU D_3	43.9	9.4	134.6
Infant 0 IU	30.8		
Wagner et al.[73] (2006)			
Group 1			
Mother 400 IU D_3	38.4	4	76.3
Infant 300 IU D_3	43		
Group 2			
Mother 6,400 IU D_3	58.6	49.7	
Infant 0 IU D_3	46		873.5

Comparison of Sun-Derived Versus Oral Vitamin D Supplementation

At a maternal intake of 6,400 IU of vitamin D_3/day, circulating vitamin D_3 and 25(OH)D increased significantly; however, these increases appeared to be limited and controlled.[73] In a comparison of individuals who reported daily sun exposure of at least >15 hours of peak sun exposure/week with the lactating maternal cohort of 400 and 6,400 IU of vitamin D/day,[139] the following differences were noted: (1) There was much variability in the 25(OH)D levels in the sun exposure group as some had limited sunlight exposure per body surface—some had only hands and head exposed (e.g., those who surfed with wetsuits). (2) The relationship between circulating vitamin D and 25(OH)D is not linear but is saturable and controlled. (3) Optimal nutritional vitamin D status may occur when equimolar levels of circulating vitamin D_3 and 25(OH)D_3 occur (>40 ng/mL); at this point the V_{max} of the enzyme appears to be achieved. Another important point about the enzyme kinetics of the vitamin D 25-hydroxylase is this: As humans live today, this enzyme operates below its V_{max} because of the chronic deficiency of substrate, vitamin D. Not a single other steroidal hormone system in the body is limited in this fashion since their starting point is cholesterol.[139] When humans are sun (or dietary) replete, the vitamin D system will function in a fashion as do these other steroid synthetic pathways, i.e., not limited by substrate availability. (4) One can be vitamin D deficient with significant sun exposure if the skin area exposed is limited. (5) Whether one receives vitamin D_3 orally or through UV exposure, the vitamin D 25-hydroxylase handles it in an equivalent fashion.[137]

Significance

Through vitamin D's effect not only on calcium and bone metabolism but also on the innate immune system, we have come to appreciate its significance in maintaining the health status of humans throughout the lifespan. In this early part of the 21st century, we have diagnosed widespread vitamin D deficiency that has occurred as a consequence of our lifestyle changes, particularly during the past 20 years, but also as a direct result of misattribution and a limited understanding of the physiologic requirements of vitamin D, the risks of toxicity, and the therapeutic range that is essential to maintain good health. Maternal vitamin D deficiency and the resulting nutritional rickets in her nursing infant are preventable disorders whose occurrence is on the rise. We understand more fully now that this deficiency is not caused by something that is inherently wrong or missing in mother's milk but rather by inadequate maternal dietary vitamin D intake and the resultant low concentrations in the mother's milk. On the surface, the problem appears easily solvable through direct supplementation of the nursing infant with oral vitamin D. Yet, this approach does not address the issue of why the antirachitic activity of human milk is low—namely, that mother's vitamin D status is poor, and thus her milk has insufficient vitamin D. While supplementation of the infant with vitamin D *may* ameliorate the problem in that age group, it does not address the needs of the mother. Only through ongoing studies to identify what dose is necessary to safely achieve normal vitamin D status in both mother and infant will we advance the practices that we recommend today. In the future, we expect that by treating the mother with a sufficient dose of vitamin D, both mother and her recipient infant will achieve normal vitamin D status. We strongly believe that the AI for vitamin D in lactating mothers, especially darkly pigmented individuals, is woefully inadequate. The effects of acute vitamin D deprivation are known to result in rickets in the rapidly growing child and osteopenia and osteoporosis in mother. As new evidence points to serious consequences of chronic vitamin D deprivation, including decreased bone mass in later life as well as increased risks of periodontal disease, infections, type 1 diabetes, neoplasia, myopathy, and depression, we must establish normative guidelines for safe and effective vitamin D supplementation during lactation in both the lactating woman *and* her infant that address modern-day lifestyles. It is clear that, at least in part, vitamin D does make the world go 'round.

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