

# Symposium: Vitamin D Insufficiency: A Significant Risk Factor in Chronic Diseases and Potential Disease-Specific Biomarkers of Vitamin D Sufficiency

## Circulating 25-Hydroxyvitamin D Levels Indicative of Vitamin D Sufficiency: Implications for Establishing a New Effective Dietary Intake Recommendation for Vitamin D<sup>1</sup>

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**ABSTRACT** It has been more than 3 decades since the first assay assessing circulating 25-hydroxyvitamin D [25(OH)D] in human subjects was performed and led to the definition of “normal” nutritional vitamin D status, i.e., vitamin D sufficiency. Sampling human subjects, who appear to be free from disease, and assessing “normal” circulating 25(OH)D levels based on a Gaussian distribution of these values is now considered to be a grossly inaccurate method of identifying the normal range. Several factors contribute to the inaccuracy of this approach, including race, lifestyle habits, sunscreen usage, age, latitude, and inappropriately low dietary intake recommendations for vitamin D. The current adult recommendations for vitamin D, 200–600 IU/d, are very inadequate when one considers that a 10–15 min whole-body exposure to peak summer sun will generate and release up to 20,000 IU vitamin D-3 into the circulation. We are now able to better identify sufficient circulating 25(OH)D levels through the use of specific biomarkers that appropriately increase or decrease with changes in 25(OH)D levels; these include intact parathyroid hormone, calcium absorption, and bone mineral density. Using these functional indicators, several studies have more accurately defined vitamin D deficiency as circulating levels of 25(OH)D  $\leq$  80 nmol or 32  $\mu$ g/L. Recent studies reveal that current dietary recommendations for adults are not sufficient to maintain circulating 25(OH)D levels at or above this level, especially in pregnancy and lactation. *J. Nutr.* 135: 317–322, 2005.

**KEY WORDS:** • vitamin D • 25-hydroxyvitamin D • parathyroid hormone • vitamin D requirement • bone mineral density • calcium absorption

What is a normal circulating level of 25-hydroxyvitamin D [25(OH)D]<sup>3</sup> indicative of sufficient levels of 25(OH)D to meet all physiological needs, not simply skeletal in humans? In the past, this was addressed by simply gathering a diverse popula-

tion of subjects who were asymptomatic for disease, measuring circulating 25(OH)D, and plotting the data using a Gaussian distribution. This approach yields normative data that are used to assess circulating 25(OH)D in that population. This is how Haddad and Chyu (1) performed their assessment of 25(OH)D status more than 30 years ago. The data from their initial study is summarized in **Table 1**. They referred to their normal volunteers as the normal population for circulating 25(OH)D levels. Their study also presented a group of lifeguards that had circulating 25(OH)D levels 2.5 times that of the “normals.” Countless similar studies have been performed in the ensuing decades, reiterating the same conclusion. I, however, interpret the original Haddad data differently; I suggest that the 25(OH)D levels in the lifeguards are normal and the “normals” are actually vitamin D deficient. The justification for my conclusion will be forthcoming in this text.

### Cutaneous generation of vitamin D-3

For all practical purposes, vitamin D does not naturally occur in foodstuffs that humans eat. There are exceptions,

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<sup>3</sup> Abbreviations used: 7-DHC, 7-dehydrocholesterol; 25(OH)D, 25-hydroxyvitamin D; BMD, bone mineral density; DRI, dietary intake recommendation; MED, minimal erythemic dose; PTH, parathyroid hormone; UVB, UV blue.

TABLE 1

Original assessment of nutritional vitamin D status circa 1971<sup>1</sup>

Group	n	Age	Weekly consumption of vitamin D	Weekly exposure to sunlight	Plasma 25(OH)D
		y	IU	h	nmol
Normal volunteers	40	30.2 ± 12.9	2230 ± 1041	8.8 ± 6.1	68.3 ± 29.5
Biliary cirrhosis	4	1.5–55	2500 (est.)	—	16 ± 6.5*
Lifeguards	8	18.5 ± 2.0	2895 ± 677	53.0 ± 10.3	161 ± 21.8*

<sup>1</sup> From reference (1). Values are means ± SD. \* *P* < 0.001.

such as oily fish and fish liver oil; however, for the most part, vitamin D does not naturally exist in significant amounts in the human food chain. The fact is, from an evolutionary standpoint, humans did not require vitamin D in their food supply, because, over millions of years humans, along with many animal species, evolved a photosynthetic mechanism in their skin to produce large amounts of vitamin D-3. Thus, our skin is part of the vitamin D endocrine system, and vitamin D-3 is really a prohormone.

Approximately 50,000 y ago, small bands of people, almost certainly darkly pigmented, migrated gradually from sub-Saharan Africa to eventually populate more northern latitudes. This migration resulted in a profound evolutionary adaptation, a gradient in skin pigmentation loss to the point of almost total depigmentation as evidenced by northern European populations. Why would this dramatic change have occurred so rapidly? The most obvious answer is to maximize the limited sunlight exposure as occurs when moving north from the equator. Darkly pigmented individuals in an equatorial environment literally would be bathed in intense sunlight year round, and thus, vitamin D-3 production would not be a problem. However, as these darkly pigmented individuals migrated to a northern sun-restricted environment, they would rapidly become vitamin D depleted, with the resulting mobility and reproductive problems associated with deficiency. For humans to survive in this new northern environment, skin depigmentation had to occur. Eskimos are an exception to this, because they retain significant pigmentation; however, their migration from Asia was relatively recent, and the Eskimos' diet is unique in that it contains significant levels of vitamin D-3 due to the fat and oily fish content.

The cutaneous generation of vitamin D-3 in humans has been well characterized both in vitro and in vivo (2,3). Vitamin D-3 is produced in the skin from 7-dehydrocholesterol (7-DHC) (2). This 7-DHC is distributed throughout the epidermis and dermis, with highest concentrations in the stratum spinosum and stratum basale (4). Exposure of skin to sunlight, specifically to the UV blue (UVB) range of the spectrum (290–315 nm), results in the photolytic conversion of 7-DHC to previtamin D-3. Previtamin D-3 is transformed to vitamin D-3 by a thermally induced isomerization (2). The production of vitamin D-3 is thought to be regulated by the amount of UVB reaching the 7-DHC and not by hormonal feedback (5). It is interesting that vitamin D intoxication has never been reported because of excessive exposure to sunlight. How the production of vitamin D-3 is limited in face of excessive UV irradiation and a continuous supply of the precursor 7-DHC can be explained (2). On exposure to excessive sunlight, previtamin D-3 is transformed not only into vitamin D-3 but also into lumisterol or tachysterols, which are biologically inactive and thus, reduce the amount of previtamin D-3. It is also known that excess sunlight can degrade vitamin D-3 into

inert photoproducts, including suprasterols I and II (2). A number of other natural factors can limit or regulate the cutaneous production of vitamin D-3, including aging (6), increased melanin pigmentation (4,7), and season and latitude (8). Clothing and sunscreen also will eliminate the cutaneous generation of vitamin D-3 (9,10). Thus, there are a number of factors that cause a greater dependency on dietary sources of vitamin D, the most important of which are the quality of the sunlight and the degree of skin exposure.

#### All sunlight is not equal

Growing up in northern Ohio, I can remember being told to go outside on a sunny, midwinter day and that the sunshine on my face would provide me with the vitamin D that I required. This statement is a gross misconception on 2 counts. Webb et al. (8) first demonstrated in vitro that a UVB irradiation threshold of 20 mJ/cm<sup>2</sup> is required to induce the transformation of 7-DHC to previtamin D-3. This in vitro study soon was confirmed by Matsuoka et al. (3) in vivo using human subjects. Matsuoka et al. demonstrated that a UVB irradiation threshold of 18 mJ/cm<sup>2</sup> was required to induce vitamin D-3 production and its subsequent release into the circulation (Fig. 1). These data also demonstrate that further increases in UVB energy delivered caused an exponential increase in the rate of production and release into the circu-

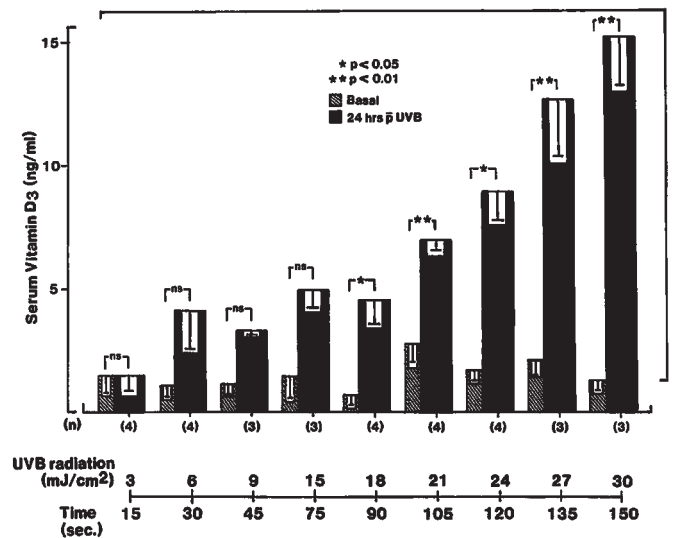
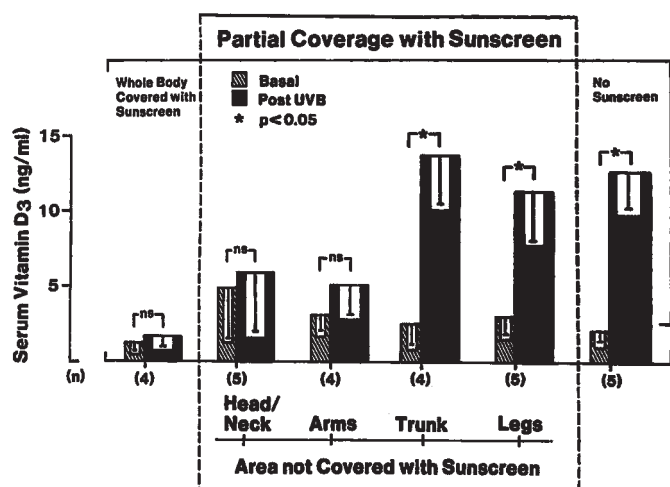


FIGURE 1 Effect of graded doses of whole-body UVB irradiance in untanned, healthy white subjects with skin type III. Results are expressed as means ± SEM. Time represents exposure period in phototherapy unit. From reference (3).

lation. Further work by Matsuoka et al. (9) demonstrated what portions of the human body should be exposed to UV light to maximize *in vivo* vitamin D-3 production (Fig. 2). First, these data demonstrate just how effective sunscreen is at blocking cutaneous vitamin D-3 production. Secondly, the data clearly demonstrate that minimal skin exposure of arms and face will only result in a nominal production of vitamin D-3. This limited cutaneous production of vitamin D-3 is further exacerbated by increasing cutaneous melanin content, the extent of which is dependent on race (7). The relevance of these findings to public health is clear. The exposure level of 18–20 mJ/cm<sup>2</sup> is not generally reached during the winter in northern United States above latitude 40°. For example, in Boston (42° N latitude), the accumulated daily UVB solar irradiance (from 1130 to 1430 h EST) remains below 20 mJ/cm<sup>2</sup> from November through February. Thus, a Caucasian individual in a bathing suit outside on a sunny January day in Boston would not produce endogenous vitamin D-3. Further, even in the summer with only one's arms and face exposed, minimal endogenous vitamin D-3 production would be achieved in that individual. In the African-American population, the situation is far worse, with heavy melanin effectively blocking UVB absorption.

The question then is, how much vitamin D-3 do humans generate *in vivo* through solar exposure? Several investigators have addressed this question, and the answer has been fairly consistent (3,5,11,12). Using synthetic UV sunlamps, a total-body minimal erythemic dose (MED) can be determined for a given individual by graded exposure of small areas of the back to increasing doses of UVB. The individual returns 24 h later, and the least amount of exposure that causes an erythemic response (pinkening of the skin) is the MED for that individual. What does an MED mean from a practical sense? A Caucasian individual in a bathing suit who is not tanned would receive a total body MED from ~10–12 min of peak July summer sun (1130 to 1430 h EST) in Boston. For an Asian Indian, that same MED could take perhaps 30 min of exposure, and, for a very darkly pigmented African-American, it could require 120 min of exposure to synthesize the same amount of vitamin D-3 as a Caucasian exposed for 10–12 min.

To determine the amount of vitamin D synthesized in a



**FIGURE 2** Regional synthesis and release of vitamin D-3 in untanned young white subjects with skin type III. Sunscreening agent (SPF 15) was applied 1 h before exposure to 27 mJ/cm<sup>2</sup> of UVB irradiance. Vitamin D-3 levels were monitored at baseline and 24 h after exposure. From reference (9).

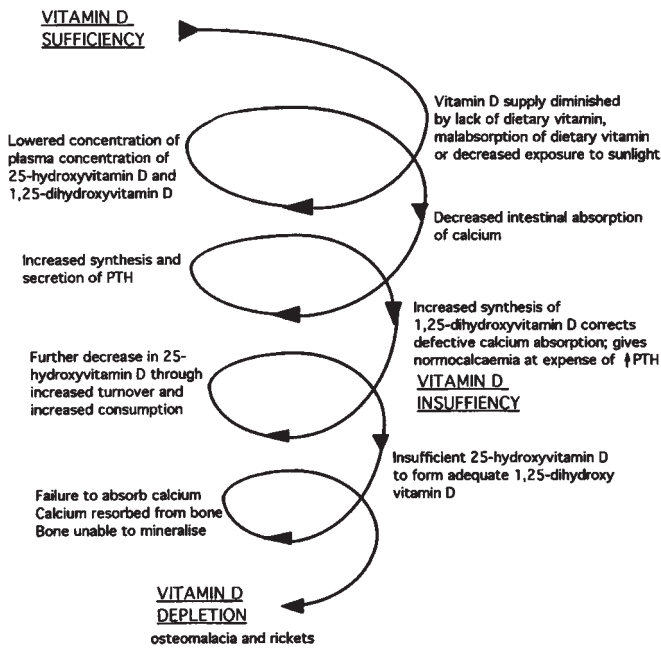
single MED, one simply compares circulating levels of vitamin D-3 at various time points from individuals provided a known oral supplement of vitamin D-3 with those receiving an MED of UVB (3,5,11–13). When this comparison is made, it is clear that a total-body MED will release ~10,000–20,000 IU vitamin D-3 into the circulation within 24 h of exposure. Remember that the exposure time required to achieve that MED is highly dependent on skin pigmentation. Thus, at least in Caucasians, an intense but very brief sun exposure causes the release of a “large amount” of vitamin D-3 into the circulation. The “large amount” is defined relative to the current dietary intake recommendations (DRI) of 400 IU/d for vitamin D.

### What is the normal level of circulating 25(OH)D in humans?

Past attempts to define “normal” circulating 25(OH)D were seriously flawed. To properly define “normal” 25(OH)D status in humans, it makes more sense to measure 25(OH)D in “healthy subjects” who are sunbathers, fieldworkers, construction workers, or other individuals who work outside, who are not overly clothed, and who are without sunblock. Humans did not evolve in today's sun-shy culture, so “normal” with respect to circulating 25(OH)D levels should not be defined by the current average or median population level. In sun-rich environments where clothing or cultural practices do not prevent sun exposure, circulating 25(OH)D ranges from 135 to 225 nmol/L (54–90 μg/L) (1,14,15). Thus, we must be very careful how we define “normal” or adequate or sufficient with respect to circulating 25(OH)D.

A more serious problem is how we define nutritional vitamin D deficiency in the human population. In recent years, investigators have turned to the use of biomarkers or functional end points to more clearly define adequacy of circulating 25(OH)D levels with respect to the calcium homeostatic function of vitamin D. These biomarkers include the functional indicators parathyroid hormone (PTH), calcium absorption, and bone mineral density (BMD) (16–20). What do these biomarkers of 25(OH)D function tell us about a set point for the onset of nutritional vitamin D deficiency? Let us begin with PTH, because a significant amount of work has been done with this biomarker. Many studies have shown the significant, inverse relationship between circulating 25(OH)D and PTH (16–18). The biological consequences, with respect to calcium homeostasis, due to the decline in nutritional vitamin D status are illustrated in Figure 3 (21). Secondary hyperparathyroidism is the key hallmark of poor nutritional vitamin D status in the elderly, because this inverse relationship is more pronounced with increasing age (18). Secondary hyperparathyroidism may subside in the elderly when circulating 25(OH)D levels reach ~80 nmol (32 μg/L) (Fig. 4). However, a more sophisticated mathematical model suggests that circulating 25(OH)D has the ability to suppress PTH at concentrations well above 80 nmol (Fig. 4).

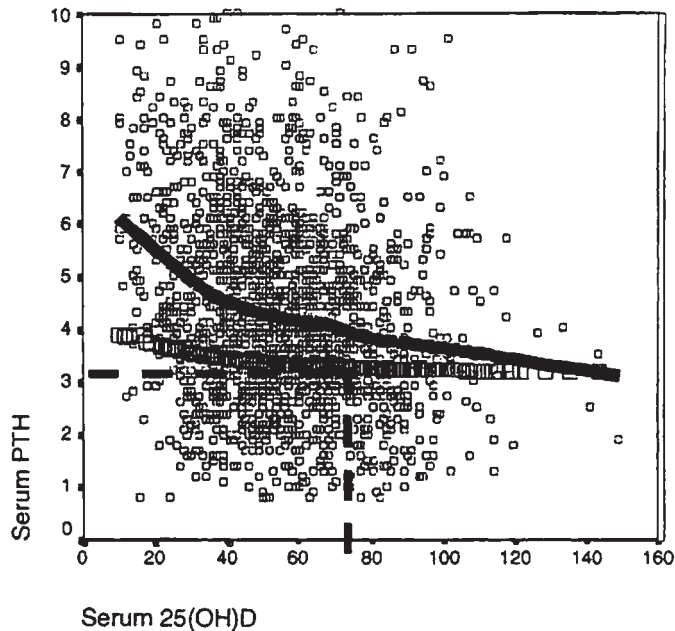
Similar results have been observed using calcium absorption as the functional endpoint (19). When circulating 25(OH)D drops below 80 nmol, calcium absorption is impaired (Fig. 5). It is logical that impaired calcium absorption and secondary hyperparathyroidism due to nutritional vitamin D deficiency would have an adverse impact on skeletal integrity. Ironically, a recent publication details such a relationship (20). This remarkable relationship between circulating 25(OH)D and BMD in several thousand subjects who participated in the NHANES III survey is presented (Fig. 6). Here, again, it is evident that circulating levels of 25(OH)D of at least 80 nmol (32 μg/L) are required to optimize this func-



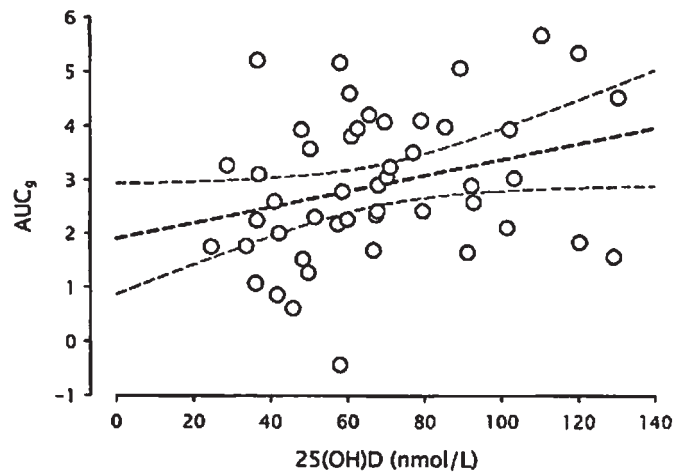
**FIGURE 3** Spiral of physiological developments as vitamin D deficiency progresses. From reference (21).

tional end point of BMD. It is quite apparent from Figure 6 that a “normal range” for circulating 25(OH)D set at 25 nmol/L (10 µg/L) or even 37.5 nmol/L (15 µg/L) will be associated with lower BMD and increased risk of fracture.

From these recent studies, it is obvious that we no longer have to guess what a deficient level of circulating 25(OH)D

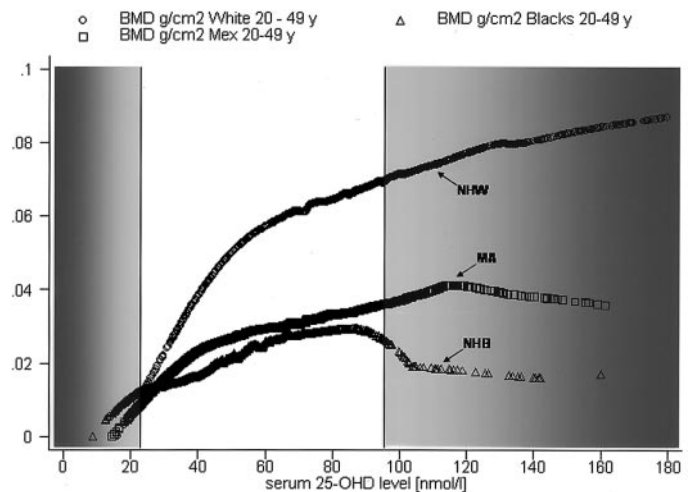


**FIGURE 4** Circulating PTH vs. circulating 25(OH)D concentrations in 1741 patients, overlaid with the locally weighted regression and scatterplot smoothing (LOWESS) technique (dash-dot line) and exponential decay function fitted to the data (dashed line). The vertical dashed line indicates the point at which PTH concentrations theoretically attain the plateau value, based on the exponential function. PTH units are pmol/L. 25(OH)D units are nmol/L. From reference (18).

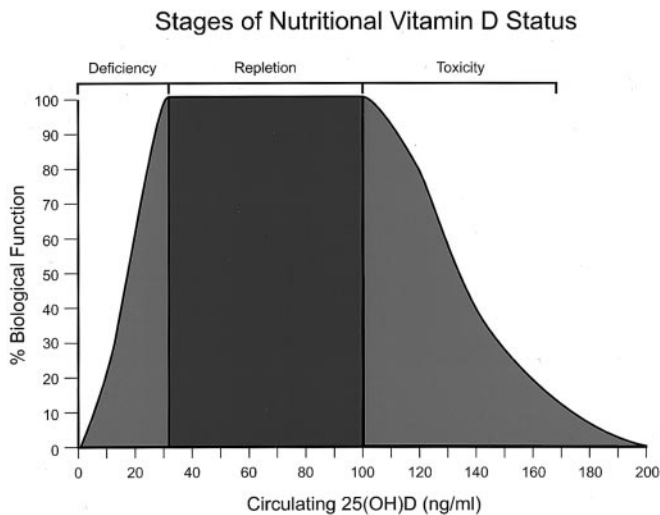


**FIGURE 5** Correlation of area under the curve with serum 25(OH)D concentrations in 48 measurements of calcium absorption in 34 postmenopausal women. The lines represent the least squares regression line through the data and its 95% confidence limits. From reference (19).

should be in a human subject. Clearly, there is no known harmful effect of maintaining a circulating 25(OH)D level of at least 80 nmol/L, but there are serious risks encountered at levels less than this. Thus, I strongly believe that nutritional vitamin D deficiency should be defined as <80 nmol (32 µg/L) circulating 25(OH)D as I have graphically illustrated (Fig. 7). This is a simplified version of previous figures that presented many stages of vitamin D status, including deficiency, insufficiency, hypovitaminosis, sufficiency, and toxicity (22). Complicated staging charts are difficult to apply to clinical practice, so I have simplified this by defining vitamin D deficiency, optimal vitamin D status (repletion), and toxicity. The deficiency portion of this figure is based on the calcium homeostatic functional end points discussed earlier. However, circulating levels of 25(OH)D that define optimal vitamin D status or repletion and toxicity—hypervitaminosis D—remain blurred. I have arbitrarily set the toxic level at 250 nmol (100



**FIGURE 6** Regression plot of BMD change (g/cm<sup>2</sup>) by 25(OH)D level (nmol/L) in younger adults (20 to 49 y). Circles represent Caucasians, squares represent Mexican-Americans, and triangles represent African-Americans. From reference (20).



**FIGURE 7** Stages of nutritional vitamin D status. Concentrations in ng/mL can be converted to nmol/L using a multiplication factor of 2.5.

$\mu\text{g/L}$ ), which is a conservative estimate, because true vitamin D toxicity is well beyond this concentration, as shown in a recent study that observed no harmful effects with 25(OH)D levels of 250 nmol (23). Additional studies on this topic of vitamin D toxicity are ongoing and are the subject for the 2005 Experimental Biology symposium.

This proposed vitamin D deficiency set point is much higher than the 25 nmol/L or 10  $\mu\text{g/L}$  value recognized by most physicians, assay manufacturers, and clinical chemistry laboratories. This higher deficiency set point has important implications in therapeutic treatment, particularly for the elderly, given that vitamin D deficiency is a well-established risk factor for hip fracture (24–26). Manufacturers of assay kits and clinical laboratories servicing hospitals and nursing homes should be encouraged to reevaluate their normal ranges of 25(OH)D in view of this proposed set point and should alert physicians to this change to enable early detection and correction of this risk factor for bone fracture in the elderly.

#### Modernizing the dietary recommended intake for vitamin D

Let us review how the 200 IU/d dietary recommendation for vitamin D for persons up to age 50 was established in 1997 (27). Before 1997, the Recommended Daily Allowance for vitamin D in infants and children was 400 IU (28). In essence, the scientific basis for this dose was that it approximated what was in a teaspoon of cod liver oil, and this had long been considered safe and effective in preventing rickets (29). Forty years ago, an expert committee on vitamin D provided only anecdotal support for what it referred to as “the hypothesis of a small requirement” for vitamin D in adults, and it recommended one-half the infant dose to ensure that adults obtain some from the diet (30). In England, an adult requirement of only 100 IU/d was substantiated on the basis of findings in 7 adult women with severe nutritional osteomalacia whose bones showed a response when given this amount (31). The adult DRI of 200 IU/d was described as “a generous allowance” in the 1989 version of the American recommended dietary allowances (28). What is truly disturbing is that the basis for these recommendations was made before it was possible to measure the circulating concentration of 25(OH)D, the indicator of nutritional vitamin D status (32,33). A question that

has intrigued our group for years is the following: How is it possible that the recommendation of 200 IU/d for vitamin D is the same for a 3.5-kg term infant and a 90-kg adult?

We have ascertained the effect of a daily intake of 400 IU vitamin D/d, which is the value used for nutrient labeling in the United States, as well as the recommendation for older adults (age 50–70 y) (27) on circulating 25(OH)D levels in infants and adults. We published the results of a study in infants more than a decade ago (34). A 400 IU/d dose promoted a significant increase in circulating 25(OH)D levels that was more pronounced in the preterm infant group (34). Thus, a daily dose of 400 IU vitamin D appears to be effective in raising the 25(OH)D concentration to 80–200 nmol/L (32–80  $\mu\text{g/L}$ ) in infants. Conversely, what effect does a 400 IU/d dose of vitamin D for an extended time (months) have in adults? The answer is little or nothing. At this dose in an adult, the circulating 25(OH)D concentration usually remains unchanged or declines. This was first shown in both adolescent girls and young women (35,36). The most recent study demonstrating the ineffectuality of the current adult DRI for vitamin D was published by Datta et al. (37). These investigators studied 160 pregnant minority women in the United Kingdom. The women were provided with 800–1600 IU/d vitamin D for the duration of their pregnancies. The investigators found a mean  $\pm$  SD increase in circulating 25(OH)D concentrations (nmol/L) from  $14.5 \pm 2.25$  at the beginning of pregnancy to  $28.0 \pm 15.8$  at term after vitamin D supplementation. In other words, mothers who were vitamin D deficient at the beginning of their pregnancies were still deficient at term after receiving supplements of 800–1600 IU vitamin D/d throughout their pregnancies. This result is precisely what the regression analysis from Heaney et al. (23) predicted would happen at this level of vitamin D intake—continued vitamin D deficiency, which is a huge public health problem around the globe.

So, the question is, what vitamin D intake is required to maintain or, preferably, improve the nutritional vitamin D status in adults, including those who are pregnant or lactating? This is a complex scientific question, yet recent, well-controlled studies have provided some provisional answers (23,38,39). Remember from the previous discussion that humans evolved using sun exposure to endogenously generate tens of thousands of IU of vitamin D-3/d. So, in light of the above facts, dietary recommendations in the range of 200–600 IU/d are woefully inadequate and ineffective with respect to maintaining normal circulating concentrations of 25(OH)D in adults with minimal solar exposure.

On the basis of 25(OH)D concentrations in sun-replete adults, what vitamin D intake is required to sustain an adequate nutritional status of vitamin D? In Caucasians who experience significant solar exposure to the body routinely, this is not an important consideration. As a population, however, our unprotected exposure to the sun is declining rapidly, because of the all-pervading fear of skin cancer and premature aging resulting from public education campaigns. What do we do? We need to reevaluate the DRI for vitamin D so that the dietary intake guidelines actually have a physiological effect. Recent attempts to define effective intake guidelines show promise. Vieth et al. (38) in 2001 supplemented healthy adults with up to 4000 IU/d vitamin D-3 for a period of 5 mo. Circulating 25(OH)D levels increased substantially and not a single adverse event or episode of hypercalciuria was observed. In an even more detailed report, Heaney et al. (23) studied 67 men divided into 4 groups that received 200, 1000, 5000, or 10,000 IU/d vitamin D-3 for 5 mo. The 200 IU/d group failed to maintain circulating 25(OH)D concentrations during the

study period, whereas the remaining 3 groups responded in a dose-response fashion with respect to elevations in circulating 25(OH)D concentrations. From these data, regression analysis can be used to predict circulating 25(OH)D from a given oral intake of vitamin D. The data show that for every 40 IU of vitamin D intake, circulating 25(OH)D increases by 0.70 nmol/L (0.28  $\mu\text{g/L}$ ) over 5 mo on a given regimen. A steady state appears to be achieved after  $\sim 90$  d on each dose tested (23,38). Thus, doses of 400, 1000, 5000, and 10,000 IU/d for 5 mo will result in theoretical increases in circulating concentrations of 7, 17.5, 70, and 175 nmol 25(OH)D, respectively. Again, no adverse events were noted. Our laboratory has conducted similar studies in lactating women (39). We noted that the breastfeeding infants of the mothers receiving 4000 IU/d vitamin D<sub>2</sub> had a substantially improved nutritional vitamin D status due to the transfer of vitamin D into the mothers' milk, again in the absence of adverse events.

Given the results of these recent scientific studies that evaluated high-dose vitamin D supplementation, it appears that the current DRI for adults are woefully inadequate, misleading, and potentially harmful, placing individuals at undue risk for a number of chronic diseases. The current adult dietary recommendations of 200–600 IU/d are extraordinarily low compared with endogenous production during sun exposure. Reexamination of the requirements for vitamin D is clearly merited and may likely reveal the need for vitamin D intakes exceeding 2000 IU/d for adults.

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