Functional indices of vitamin D status and ramifications of vitamin D deficiency[1–4]

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ABSTRACT

Serum 25-hydroxyvitamin D_3 [25(OH)D_3] concentrations are currently recognized as the functional status indicator for vitamin D. Evidence is reviewed that shows that serum 25(OH)D_3 concentrations of $<$ 80 nmol/L are associated with reduced calcium absorption, osteoporosis, and increased fracture risk. For typical older individuals, supplemental oral intakes of ~1300 IU/d are required to reach the lower end of the optimal range. Evidence of substantial problems in routine clinical measurement of serum 25(OH)D_3 concentrations among patients is cited. There is great need for standardization and improved reproducibility and sensitivity of measurements of serum 25(OH)D_3 concentrations. *Am J Clin Nutr* 2004;80(suppl):1706S–9S.

KEY WORDS Rickets, osteomalacia, osteoporosis, calcium absorption, fractures, serum 25-hydroxyvitamin D_3

INTRODUCTION

In its most recent revision of recommended intakes for bone-related nutrients, the Food and Nutrition Board (FNB) identified serum 25-hydroxyvitamin D_3 [25(OH)D_3] concentrations as the appropriate functional indicator of vitamin D status (1). However, on the basis of the evidence then available, the Calcium and Related Nutrients Panel of the FNB was not able to assign specific serum concentrations of 25(OH)D_3 to various health and disease states. The report of the panel also recognized that solar vitamin D synthesis in the skin was an important source of vitamin D, but data then available did not permit estimation of either usual or optimal proportions of input from cutaneous and ingested sources. Nevertheless, the increase in intake recommendations from 200 IU/d (5 μg/d) before 50 y of age to 600 IU/d (15 μg/d) at $\geq$ 70 y of age reflected a recognition that the contribution of cutaneous sources decreases with age. Also, lacking the needed information, the FNB reverted to using the absence of rickets and osteomalacia as the de facto indicator of vitamin D sufficiency. No other health or disease outcomes were factored into the recommendations for vitamin D intake.

Although a great deal of additional work remains to be performed, sufficient information has been developed in the past 8 y to close some of the information gaps that confronted the Calcium and Related Nutrients Panel in its deliberations in the middle 1990s. This brief review highlights certain aspects of this new information.

VITAMIN D AND DISEASE

Although the index diseases for vitamin D deficiency have long been considered to be rickets (for children) and osteomalacia (for adults), there has been a growing conviction that less severe degrees of deficiency may also produce skeletal disease. The canonical function of vitamin D is facilitation of the active transport component of intestinal calcium absorption, and there has never been any evidence suggesting that absorption is optimal at vitamin D concentrations just sufficient to prevent rickets or osteomalacia.

On the basis of his extensive experience with histomorphometric analysis of adult bone samples, in 1990 Parfitt (2) introduced an heuristically important reconceptualization of the bone disease attributable to vitamin D deficiency, for which he coined the term hypovitaminosis D osteopathy. He identified 3 stages of disease, related to increasing degrees of vitamin D depletion. In stage 1, the only detectable pathophysiologic change was reduced intestinal absorption of calcium, with consequent diminution of skeletal calcium reserves and accompanying osteoporosis. In biopsies, the bone in stage 1 showed no evidence of osteomalacia. In stage 2 hypovitaminosis D, there was decreased intestinal calcium absorption and decreased bone mass, as in stage 1, but in biopsies there was identifiable early osteomalacia, ie, increased surface coverage by osteoid and decreased mineral apposition rates. Patients with stage 2 disease exhibited no clinical or laboratory chemical signs of osteomalacia. Their only clinical manifestation was reduced bone mass, ie, osteoporosis. In stage 3 hypovitaminosis D, there was continued hypoabsorption of calcium and osteomalacia was evident clinically, biochemically, and histologically.

The importance of this reconceptualization is that it clearly demarcated the traditional index disease for vitamin D deficiency as constituting only the most extreme degree of deficiency. It was proposed that lesser degrees produced osteoporosis, which is silent until fractures occur, as has long been recognized. Therefore, the presence of osteoporosis and its connection to vitamin D status would have gone unrecognized.

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Given the absence of reliable $25(OH)D_3$ values for the patients who contributed biopsy samples for his analysis, Parfitt (2) was unable to relate quantitatively his 3 stages to specific values for what the FNB would designate subsequently as the functional indicator. Nevertheless, Parfitt's work made clear that the then-current recommended dietary allowance for adults (200 IU/d), which was just sufficient to prevent clinical osteomalacia, was insufficient to protect against stage 1 or 2 hypovitaminosis D osteopathy. It is only now possible to assign, at least tentatively, specific serum $25(OH)D_3$ concentrations to the boundary between stage 1 disease and normal conditions and to estimate the input of vitamin D needed to reach such concentrations.

Before we proceed to this demarcation of normal and deficient concentrations, it is worth noting that a growing body of evidence, summarized in other reports in this symposium, points to a role for vitamin D not only in the calcium economy but also in a variety of muscular and/or neuromuscular functions, as well as in control of cellular proliferation and differentiation (with implications for oncogenesis). For the most part, these outcomes cannot yet be linked to specific serum $25(OH)D_3$ concentrations, although there are some indications of those boundaries. Bischoff et al (3), in an analysis of the National Health and Nutrition Examination Survey data, recently showed that lower-extremity muscle function improved with increasing serum $25(OH)D_3$ concentrations, at least to values in the range of 80–100 nmol/L. Moreover, there is at least one prospective study relating prostate cancer risk to serum $25(OH)D_3$ concentrations, showing an inverse risk within the range of serum concentrations usually observed for $25(OH)D_3$ (4). Therefore, better quantitative estimates of optimal serum $25(OH)D_3$ concentrations for such health outcomes may soon be forthcoming.

DEFINITION OF CRITICAL VALUES FOR SERUM $25(OH)D_3$ CONCENTRATIONS

It is generally recognized that serum $25(OH)D_3$ concentrations of < 20 nmol/L are associated with clinical osteomalacia among adults. Most laboratory reference ranges, in contrast, extend from lower limits of 37.5 or 40 nmol/L to somewhat more than 100 or 120 nmol/L. The range between 20 nmol/L (the rickets/osteomalacia threshold) and the lower end of the reference range has usually been termed vitamin D insufficiency, in recognition of its presumed inadequacy for optimal functioning of the vitamin D and calcium economies. (The avoidance of the term deficiency for values in this range reflects the usually implied but common premise in nutritional science that inadequate intake of any nutrient causes only one disease; therefore, if patients did not have osteomalacia, they could not be "deficient.")

The expected physiologic response to insufficient calcium absorption (whether attributable to decreased vitamin D status or low calcium intake) is increased activity of the parathyroid hormone (PTH)-calcitriol axis. Many studies reported the expected inverse association between serum $25(OH)D_3$ concentrations and serum PTH concentrations (5–7). In most of those analyses, PTH concentrations tended to bottom out at serum $25(OH)D_3$ concentrations of 70-110 nmol/L. Only the data of Lips et al (8) indicated a value lower than this range. Elevated PTH concentrations indicate a physiologic response to calcium insufficiency and might therefore be considered an appropriate reaction to a physiologic stressor, rather than an indicator of inadequacy (9). However, PTH is the principal determinant of bone remodeling, and it is now clear that a high remodeling rate is an important and perhaps the principal determinant of osteoporotic bone fragility (10–12). Therefore, this can hardly be considered a benign condition.

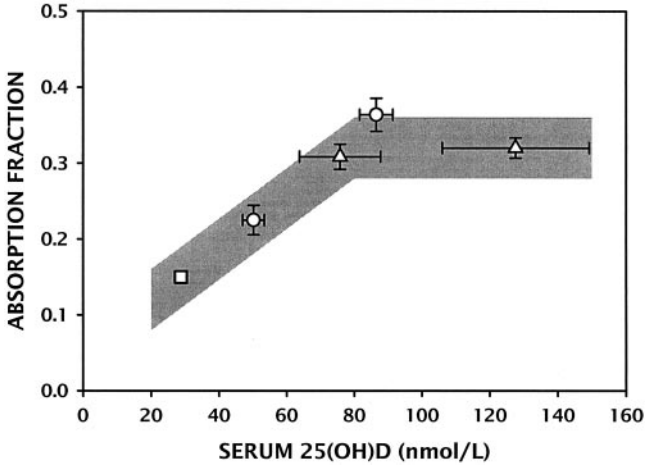

FIGURE 1. Calcium absorption fraction as a function of serum $25(OH)D_3$ concentrations, from 3 published reports [□, study by Bischoff et al (15); ○, study by Heaney et al (13); △, study by Barger-Lux et al (14)]. Error bars indicate 1 SEM.

Clear quantitative evidence of the relationship of serum $25(OH)D_3$ concentrations to calcium absorptive function has emerged only recently. Heaney et al (13) and Barger-Lux and Heaney (14), in 2 companion studies, showed that fractional calcium absorption increased with serum $25(OH)D_3$ concentrations within the reference range, up to ~80 nmol/L, and plateaued above that level. Those studies demonstrated that the reference range should not be taken to indicate the physiologic normality of measured values. Bischoff et al (15, 16), in an article relating vitamin D status to fall propensity, provided data indicating even lower absorption among individuals with serum $25(OH)D_3$ concentrations below the reference range. **Figure 1** presents data from these 3 studies and suggests an apparent threshold response, with absorptive efficiency being maximized at concentrations at or above ~80 nmol/L.

Such physiologic evidence, although strongly suggestive, does not prove a connection with morbidity. The publication in 2003 of a large British vitamin D intervention study provided a crucial piece of needed evidence. With a placebo-controlled, randomized design, Trivedi et al (17) administered 100,000 IU of vitamin D_3 every 4 mo (averaging ~800 IU/d), for 5 y, to 2686 healthy British participants 65–85 y of age. Serum $25(OH)D_3$ concentrations were measured for a subset of the cohort and averaged 53 nmol/L for the placebo group and 74 nmol/L for the vitamin D-treated group. The risk of all fractures was reduced by 22% among the supplement-treated individuals, and typical osteoporotic fractures, taken as a group, were reduced by 33%. As in the absorptive study by Heaney et al (13), the mean treated and untreated serum $25(OH)D_3$ concentrations in the British study were well within the reference range; in fact, the 2 studies spanned nearly the same range of values (50 and 53 nmol/L for the untreated subjects in the 2 studies and 74 and 86 nmol/L for the treated subjects).

These recently published studies clearly establish that there is malabsorption of calcium and increased fracture risk at serum $25(OH)D_3$ concentrations below ~80 nmol/L. These findings

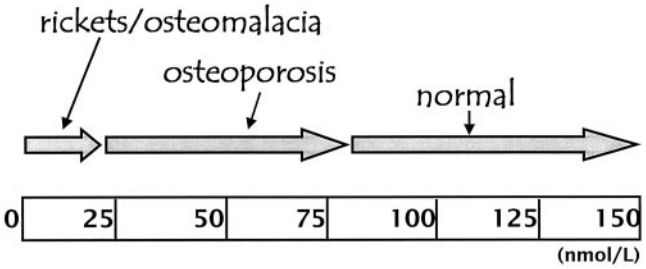

FIGURE 2. Suggested mapping of the principal vitamin D-related bone diseases onto the serum $25(OH)D_3$ concentration continuum. (To convert values to nanograms per milliliter, divide values by 2.5.)

are precisely what would be predicted for Parfitt's hypovitaminosis D osteopathy, stages 1 and 2, and provide quantitative referents not available to Parfitt when he proposed his classification scheme for vitamin D-related disease. Furthermore, these findings underscore the disturbing implications of elevated PTH concentrations at $25(OH)D_3$ concentrations of < 80 nmol/L. A tentative mapping of adult bone disease to serum $25(OH)D_3$ concentrations is presented in **Figure 2**.

REPAIR OF MEASURED VITAMIN D DEFICITS

It has been a common professional experience that administration of vitamin D in amounts in the range of the current adequate intakes (defined by the FNB as 200–600 IU/d) does not produce an appreciable increase in measured serum $25(OH)D_3$ concentrations, which suggests either inadequate potency of the preparations used or greater need than implied in the concept of adequate intakes. Therefore, my colleagues and I (18) attempted to quantify both the daily utilization of vitamin D and the amount required to produce any desired increase in serum $25(OH)D_3$ concentrations. In this analysis, which was performed among healthy adults with average serum $25(OH)D_3$ concentrations in the middle of the reference range, we ascertained that daily utilization of vitamin D approximated 4000 IU (100 μg) and that, at equilibrium, serum $25(OH)D_3$ concentrations increased by 0.7 nmol/L for every 1 μg (40 IU) of vitamin D_3 taken orally as a regular daily dose.

Several other studies provided data that permitted calculation of this rate of increase; they generally yielded similar slope values, ie, between 0.6 and 1.2 nmol/L per 1 μg/d (17, 19, 20). The antifracture trial by Trivedi et al (17) demonstrated an increase of almost exactly 1 nmol/L per 1 μg/d. By taking a value in the middle of the observed range of slopes (eg, 0.9 nmol/L per 1 μg/d), it can be calculated that the recommended daily intake for adults 50–70 y of age (400 IU) would be expected to increase serum $25(OH)D_3$ concentrations by only 9 nmol/L (3.6 ng/mL). Because this increase is within the error range for most laboratory methods, it is now clear why administration of such doses fails to produce appreciable increases in serum $25(OH)D_3$ concentrations.

There is reason to think that the rate of increase may be much more rapid for individuals with more severe depletions than those involved in either our study or the British trial, and my colleagues and I (20) previously published a summary of several studies that indicated that the response to a given dose may well be an inverse function of the starting $25(OH)D_3$ concentration. However, once even modest vitamin D repletion has been achieved, a slope in the range just noted seems to be applicable and governs the quantity of vitamin D that must ultimately be administered to reach desired values.

Implicit in this quantitative analysis of vitamin D utilization is the fact that, with a daily consumption of 4000 IU, most of the vitamin D on which the body depends must come not from dietary sources but from cutaneous sources, presumably synthesized mostly in the summer months and used during winter. Because both skin pigment and age decrease the skin's synthetic capacity (21, 22), these estimates of daily utilization have important implications for what health professionals must do with respect to ensuring adequate vitamin D status among persons of color and older adults. The initial step is to establish the vitamin D status of the individual (or group of individuals) being treated; only measurement of serum $25(OH)D_3$ concentrations can provide the baseline data on which prophylactic or therapeutic dosing can be based. For example, with 0.9 nmol/L per 1 μg/d being taken as the approximate operative slope, a patient with an untreated serum $25(OH)D_3$ concentration of 50 nmol/L would require a daily dose of 33 μg ($\sim$1300 IU) to reach and maintain a serum $25(OH)D_3$ concentration of 80 nmol/L. This hypothetical starting value, 50 nmol/L, is almost exactly the measured concentration for the untreated subjects in our absorption study (13) and in the antifracture trial by Trivedi et al (17).

MEASUREMENT OF SERUM $25(OH)D_3$ CONCENTRATIONS

The measurement of serum $25(OH)D_3$ concentrations, which was once a research procedure, has moved into the clinical laboratory setting in the past 10 y. Several fundamentally different methods are used, with or without prior extraction of the serum. Several attempts have been made to standardize, or at least harmonize, the respective results (23, 24). However, different laboratories, using different methods, currently yield radically different results for the same specimens. Binkley et al (25) sent a group of multiple samples to 6 different laboratories, which all used different methods. Results for the same specimens varied by as much as 2-fold.

In theory, such disparities could be addressed simply by interpreting a given set of results in terms of the respective normal range for the method used. However, a more troubling aspect of the study by Binkley et al (25) was that those authors also sent duplicate samples of the same specimens to the same laboratories, changing the names and spiking the samples with sufficient $25(OH)D_3$ to increase the concentration by 20 ng/mL (50 nmol/L). Only 2 of the laboratories obtained what could be considered good recovery; the poorest performer found only 10% of the added $25(OH)D_3$.

To complicate the matter further, several authors (26, 27) pointed out that some of the better-established methods failed to detect $25(OH)D_2$ with the same efficiency as $25(OH)D_3$. Because vitamin D_3 (cholecalciferol) is the normal animal form of the vitamin and because cutaneous synthesis is the predominant source of total vitamin D for most individuals, this analytic defect is not likely to have serious consequences for assessment of vitamin D status among untreated patients. However, the only high-potency therapeutic vitamin D preparation available in the United States is ergocalciferol (vitamin D_2), and no vitamin D_3 preparation with a unit strength of > 2000 IU is available. The

existing 25(OH)D_3 assays may therefore be unable to help clinicians monitor responses to treatment with therapeutic vitamin D_2.

Given the current chaotic situation, with varying sensitivities and specificities of the existing assays, there is great need for both standardization and harmonization of existing methods. With the increasing and necessary emphasis on assessment of vitamin D status and the recognition that vitamin D inadequacy may be endemic, it becomes even more important to address this problem without delay.

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