25-Hydroxylation of vitamin D_3: relation to circulating vitamin D_3 under various input conditions[1–3]

Robert P Heaney, Laura AG Armas, Judith R Shary, Norman H Bell, Neil Binkley, and Bruce W Hollis

ABSTRACT

Background: Neither the efficiency of the 25-hydroxylation of vitamin D nor the steady state relation between vitamin D_3 and 25-hydroxyvitamin D [25(OH)D] has been studied in humans.

Objective: We aimed to examine the relation between serum vitamin D_3 and 25(OH)D in normal subjects after either oral administration of vitamin D_3 or ultraviolet-B radiation across a broad range of inputs.

Design: Values for serum vitamin D_3 and $(OH)D_3$ were aggregated from 6 studies—1 acute and 5 near-steady state—at various vitamin D_3 inputs. In 3 of the steady state studies, vitamin D_3 had been administered for 18–26 wk in doses of 0 to 11 000 IU/d; in 2 studies, subjects had received solar or ultraviolet-B irradiation.

Results: In the acute study, subjects receiving a single 100 000-IU dose of vitamin D_3 had a rise in serum cholecalciferol to a mean of 521 nmol/L at 1 d and then a fall to near-baseline values by 7–14 d. Serum 25(OH)D peaked at 103 nmol/L on day 7 and fell slowly to baseline by day 112. In the 5 steady state studies, the relation of serum 25(OH)D to serum vitamin D_3 was biphasic and was well described by a combined exponential and linear function: $Y = 0.433X + 87.81[1 - \exp(-0.468X)]$, with $R^2 = 0.448$.

Conclusions: At physiologic inputs, there is rapid conversion of precursor to product at low vitamin D_3 concentrations and a much slower rate of conversion at higher concentrations. These data suggest that, at typical vitamin D_3 inputs and serum concentrations, there is very little native cholecalciferol in the body, and 25(OH)D constitutes the bulk of vitamin D reserves. However, at supraphysiologic inputs, large quantities of vitamin D_3 are stored as the native compound, presumably in body fat, and are slowly released to be converted to 25(OH)D. *Am J Clin Nutr* 2008;87:1738–42.

INTRODUCTION

Vitamin D deficiency is widespread throughout the United States (1) and the world (2). Deficiency of the vitamin is associated with greater secretion of parathyroid hormone and skeletal remodeling and greater risks of osteoporosis, fractures, and rickets or osteomalacia (1, 3, 4). Vitamin D deficiency has also been implicated in the pathogenesis of certain cancers including breast, ovarian, prostate, and colon cancer and of other diseases, including multiple sclerosis, type 1 diabetes mellitus, lupus erythematosus, and tuberculosis (4–7). In most persons, cutaneous production of vitamin D_3 from sunlight is the primary source of the vitamin (8, 9), and the remainder is obtained from dietary sources and supplements. The elderly are at greatest risk of vitamin D deficiency because of their limited exposure to sunlight and lesser cutaneous synthesis of 7-dehydrocholesterol and, hence, their lower production of vitamin D_3 (10).

It is conventional wisdom that vitamin D_3, a fat soluble molecule, is stored in body fat and that, to become metabolically active, it is first hydroxylated in the 25-position of the sterol molecule. The latter conversion takes place in the liver and is mediated by both microsomal and mitochondrial enzymes (ie, CYP2R1, CYP3A4, and CYP27A) (11–18). However, the partition in the body between the native compound and its 25-hydroxy derivative [$25(OH)D_3$] at various inputs in humans is largely unknown, as are the kinetics of the conversion in vivo. Better understanding of these issues is important both for designing public health strategies to optimize vitamin D_3 nutritional status and for avoiding potential toxicity.

The principal reason for contemporary ignorance of these matters is that few human studies of controlled vitamin D_3 inputs have been performed or published, and essentially none of the studies to date, either cross-sectional or prospective, have provided data for both serum vitamin D_3 and serum $25(OH)D_3$ in the same subjects.

In this report, we attempt to address this deficiency, assembling data from 6 different studies in which values for both vitamin D_3 and $25(OH)D_3$ were measured. The studies fall into 2 groups: 1 acute dosing study permitting semiquantitative description of the partition and conversion and 5 near-steady state studies in which the equilibrium relation between vitamin D_3 and $25(OH)D_3$ can be estimated. The vitamin D_3 values and their relation to $25(OH)D_3$ concentration are published here for the first time.

[1] From the Osteoporosis Research Center, Creighton University, Omaha, NE (RPH and LAGA); the Departments of Pediatrics (BWH) and Medicine (JRS and NHB), Medical University of South Carolina, Charleston, SC; and the Department of Medicine, University of Wisconsin, Madison, WI (NB).

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[3] Reprints not available. Address correspondence to RP Heaney, Creighton University, 601 North 30th Street, Suite 4841, Omaha, NE 68131. E-mail: rheaney@creighton.edu.

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TABLE 1
Studies providing serum data for both vitamin D_3 and 25-hydroxyvitamin D_3 concentrations

Study and reference	Type of study	Subjects	Age	Sex	Input method	Vitamin D_3 dose[1]
		n	*y*	*M/F*		
A (23)	Acute	30	59.8 ± 17[2]	9/21	Oral	100 000 once
B[3]	Steady state	11	28.2 ± 2.2	11/0	Oral	4000
C (20)	Steady state	9	28.3 ± 5.9	0/9	Oral	6400
D (19)	Steady state	67	38.7 ± 11.2	67/0	Oral	0, 1000, 5500, and 11 000
E (21)	Steady state	20	24.0 ± 3.1	13/7	Cutaneous	—
F (22)	Steady state	69	33.8 ± 9.3	30/42	Cutaneous	—

[1] Doses are IU/d unless indicated otherwise.
[2] $\bar{x}$ ± SD (all such values).
[3] NH Bell, et al, unpublished observations, 2005.

MATERIALS AND METHODS

Design

The studies providing data for this analysis are listed in **Table 1**, and pertinent descriptive information from each study is provided. Further details on the 5 published studies (studies A and C-F), including identification of the principal investigators and institutions, are contained in their respective reports (19–23). The other study (study B), whose data have not been completely published, was conducted at the Medical University of South Carolina, with Normal Bell as the principal investigator.

Treatment duration in studies B, C, and D ranged from 18 to 26 wk, and the serum concentrations of vitamin D_3 and 25(OH)D were measured throughout the treatment period. Study E was performed in Hawaiian sports participants going about their customary outdoor activities, and study F, conducted in participants who previously had little solar exposure, involved 3 sessions/wk, for 4 wk, of controlled ultraviolet-B (UV-B) exposure in a dermatology light box (HOUVA-A II; National Biological Corp, Twinsburg, OH). For studies of the relation between vitamin D_3 inputs or concentrations and serum 25(OH)D_3 concentrations, values were taken from the end of the period of treatment, so as to approximate the true equilibrium status.

For all studies, participants gave written informed consent. All of the study designs had been approved by the respective institutional review boards.

Analytic methods

The analytic methods were described in detail in the primary reports of the studies concerned [(19–23) also: N Bell, et al, unpubished observations, 2005]. Serum vitamin D_3 in study D was measured by Tai Chen (Boston University). For all of the other studies, the measurements of serum vitamin D_3 were performed in the laboratory of one of us (BWH) by using methods described elsewhere (24–26). Briefly, serum vitamin D_3 and 25(OH)D_3 were determined by reverse-phase HPLC and radioimmunoassay, respectively, as previously described (19–26). For 25(OH)D_3, the methods used employed an external vitamin D quality assurance survey [eg, DEQAS (27)].

Statistical analysis

The area under the curve (AUC) for the data of study A was calculated by using the trapezoidal method. Data from studies B through F were analyzed by fitting to a combination of exponential and linear functions with the use of the curve-fitting routine of SIGMAPLOT software (version 10; Systat Inc, Richmond, CA). Because the methods for vitamin D_3 assay in study D differed from those in studies B, C, E, and F, the data from study D were not pooled with the others. Routine descriptive statistics and linear regressions were computed by using the statistical functions of EXCEL software (version 2003; Microsoft Inc, Redmond, WA). Results are expressed as means ± SDs or SEMs, as appropriate.

RESULTS

Conversion of vitamin D_3 to 25-hydroxyvitamin D_3

The time course for serum vitamin D_3 and 25(OH)D_3 after a single oral dose of 100 000 IU cholecalciferol (study A) is plotted in **Figure 1**. Serum vitamin D_3 peaked on day 1 at a mean concentration of 521 nmol/L, whereas serum 25(OH)D_3 rose more slowly, peaking on day 7. The increment above baseline at the maximal concentration (C_{max}) for vitamin D_3 was 515 nmol/L, and that for 25(OH)D_3 was 34 nmol/L. Whereas the vitamin D_3 concentration fell rapidly, being close to baseline by 7 d, the concentration of 25(OH)D_3 fell slowly, reaching baseline by 112 d. The AUC for the increment above baseline for vitamin D_3 calculated to day 14 was 1493 nmol·d/L, and that for 25(OH)D_3 was 1911 nmol·d/L. The lower AUC for vitamin D_3

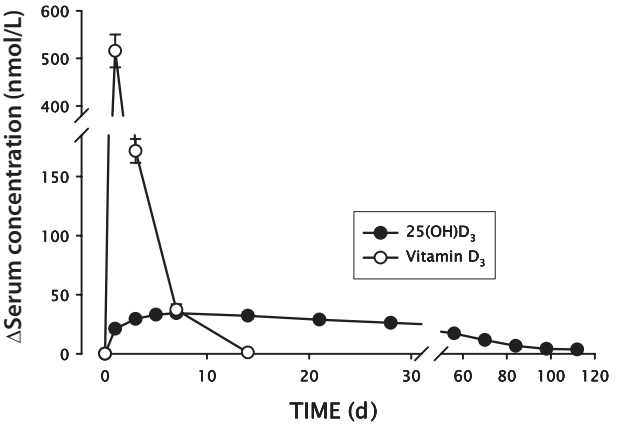

FIGURE 1. Time course of the rise from baseline of serum vitamin D_3 and 25-hydroxyvitamin D_3 for study A (23) after a single oral dose of 100 000 IU cholecalciferol to 30 healthy adults of both sexes. Baseline serum vitamin D_3 was 5.1 ± 1.3 nmol/L, and baseline 25-hydroxyvitamin D_3 was 67.6 ± 3.5 nmol/L.

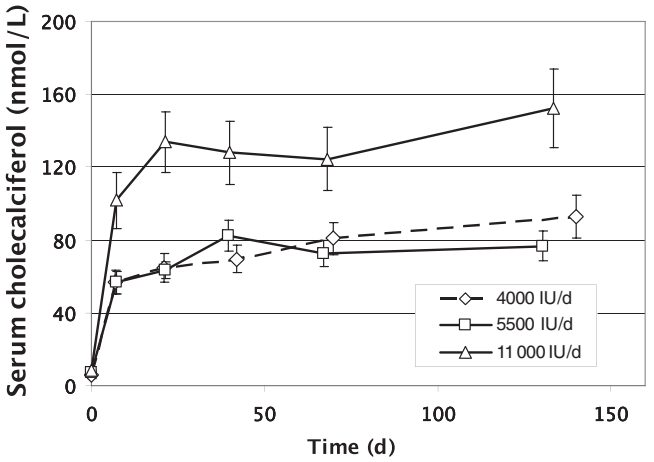

FIGURE 2. Time course of serum cholecalciferol concentration after oral vitamin D_3 doses of 4000, 5500, and 11 000 IU/d in healthy human subjects. Serum vitamin D_3 increased rapidly during the first week, and the rate of increase then declined, plateauing by ≈3 wk.

would not have been predicted, and it probably reflects a failure to capture the actual C_{max} with the 0-, 1-, and 3-d sampling frequencies used in the present study. Nevertheless, the similar magnitudes of the values for vitamin D_3 and for 25(OH)D_3 suggests complete conversion of the dosed vitamin D_3 to 25(OH)D_3 by day 112 at, therefore, an average rate approximating 1000 IU/d.

Serum vitamin D and orally dosed vitamin D

The time course of the relation of serum vitamin D_3 concentration as a function of continuing intake (4000 IU/d from study B and 5500 and 11 000 IU/d from study D) is shown in **Figure 2**. As is evident from inspection, vitamin D_3 concentration plateaued after ≈3 wk of daily dosing. The regression of vitamin D_3 dose on the equilibrium concentration of vitamin D_3 in the 78 subjects of studies B and D is shown in **Figure 3**. (The usual *x*- and *y*-axes have been reversed to facilitate estimation of effective dose from measured steady state concentration.) Basal, unsupplemented serum vitamin D_3 concentration in studies A–D and F (the 5 studies for which such data were available) averaged 10.6 ± 9.3 nmol/L (4.1 ± 3.6 ng/mL). Using the regression equation of Figure 3, that value is what would be produced by an input from all sources of ≈39 μg/d, or just shy of 1600 IU/d.

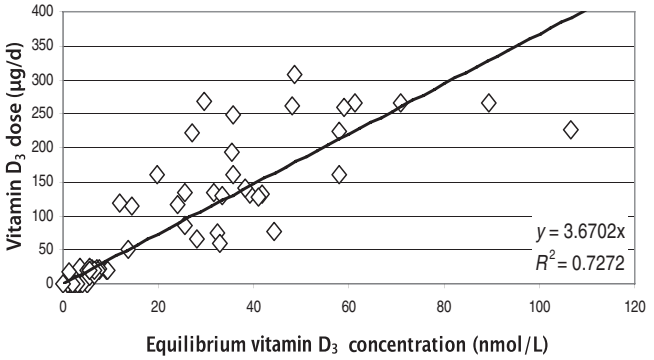

FIGURE 3. Relation of equilibrium serum vitamin D_3 concentration to oral vitamin D_3 doses of 4000, 5500, and 11 000 IU/d in the subjects from studies B and D.

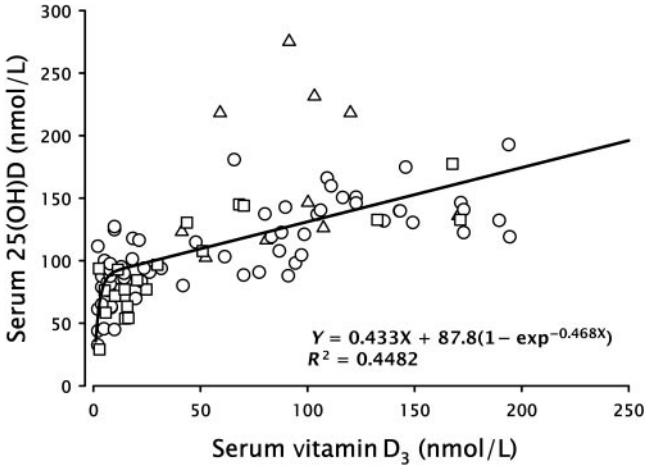

FIGURE 4. Plot of the relation between serum concentrations of vitamin D_3 and 25-hydroxyvitamin D after 18–20 wk of treatment with various doses of vitamin D_3. Δ, subjects from study B; ○, subjects from study C; □, subjects from study F. The regression line is a least-squares fit of the data to a combination exponential and linear function.

Serum vitamin D_3 and 25-hydroxyvitamin D_3

The relation between steady state concentrations of vitamin D_3 and 25(OH)D_3 is shown in **Figure 4** and **Figure 5**. Figure 4 plots the data of studies B, C, E, and F, and Figure 5 is based on the data of study D. Both graphs show the replicability of the pattern of the relation. In both, the mean concentration of 25(OH)D rises very steeply from values close to zero to values of ≈100 nmol/L (40 ng/mL) and even higher. As is suggested visually, the relation is biphasic, with serum 25(OH)D rising very rapidly at low serum vitamin D_3 concentrations and then more slowly, but with no apparent tapering off at progressively higher serum vitamin D_3 concentrations. This slow phase begins at serum vitamin D_3 values of ≈15 nmol/L (5.8 ng/mL) and at 25(OH)D concentrations of ≈80–100 nmol/L. Consistent with standard enzyme kinetics, involving a first-order reaction at low substrate concentrations and then a zero-order reaction at higher concentrations, the data were fitted to a combination exponential and linear function, according to the following equation:

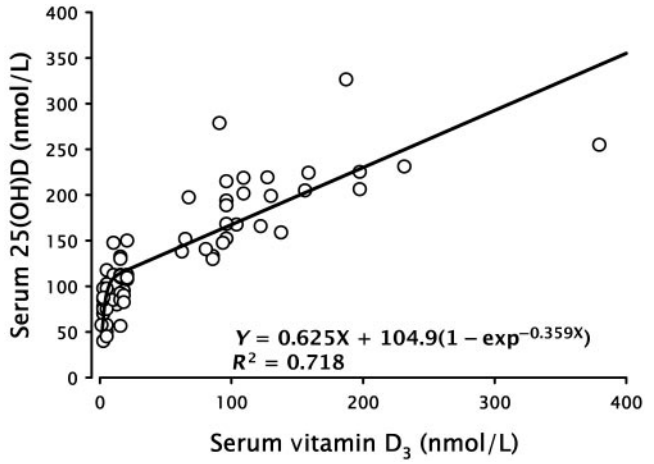

FIGURE 5. Plot of the relation between serum vitamin D_3 and 25-hydroxyvitamin D in study D only. As in Figure 4, the regression line is a least-squares fit of the data to a combination exponential and linear function.

$$Y = 0.433X + 87.8[1 - \exp(-0.468X)] \quad (1)$$

where Y = serum 25(OH)D (nmol/L) and X = serum vitamin D_3 (nmol/L). The R^2 value (0.443) indicated a good fit ($P < 0.001$) to the selected function. In Figure 5, the fit was even better ($R^2 = 0.718$, $P < 0.001$). The data in study D (Figure 5), although identical in pattern, produced slightly different equation variables. Because of likely systematic differences in the assay results of one or both of the 2 measured variables in study D, we did not attempt to use study D to define the values of those variables, only to confirm that the pattern of the relation was the same as for the aggregate of studies B, C, E, and F.

That the relation is truly biphasic is supported in 3 ways. First, the 2 datasets depicted in Figures 4 and 5 each showed the identical biphasic pattern; second, separate analysis of the lower end of the 2 continua [ie, serum vitamin D_3 values <15 nmol/L (5.8 ng/mL)] showed a significant upward trend with a linear slope that was nearly 10 times as great as the corresponding best fit slope for the slower, linear phase above vitamin D_3 concentrations of 15 nmol/L (5.8 ng/mL). Third, the early and late phase slopes were significantly different from each other ($P < 0.01$).

The slope of the linear portion of the curve in Figure 4—ie, 0.433—indicates that serum 25(OH)D rose by 0.433 nmol/L (0.164 ng/mL) for every 1-nmol/L (0.385-ng/mL) rise in serum vitamin D_3. This finding may be interpreted to mean that ≈43% of the daily vitamin D_3 input supporting the circulating D_3 concentration is being converted to 25(OH)D_3 and that the remainder is building up, both in blood and in storage depots (presumably, fat depots). In contrast, the much steeper slope at the low end of the curve indicates near-quantitative conversion of vitamin D_3 to 25(OH)D_3. That such was the case is strongly suggested by results in several of the subjects in study F who showed a median rise of 21 nmol/L (8.1 ng/mL) in serum 25(OH)D_3 with UV-B exposure but no rise whatsoever in serum vitamin D_3 (which remained below the detection limit of the assay after 4 wk of controlled UV-B radiation).

DISCUSSION

The present findings clearly establish for the first time that the concentration of serum 25(OH)D in response to input of vitamin D_3 in humans is biphasic: a rapid increase occurs at low vitamin D_3 concentrations and a slower response occurs at higher concentrations. The ability to elevate serum 25(OH)D to a meaningful extent by additional input of vitamin D_3—with little and, in some persons, no appreciable change in serum vitamin D_3 itself—suggests essentially complete conversion of the vitamin D_3 to 25(OH)D without an appreciable buildup in the concentration of the precursor. But, as Figures 4 and 5 show graphically, above a serum vitamin D_3 concentration of ≈15 nmol/L (5.8 ng/mL), the relation shifts dramatically. Serum 25(OH)D concentration continues to rise, but D_3 now rises as well, at better than twice the rate of the product molecule; ie, with continuing increases in input, the precursor is accumulating faster than it can be converted. We may calculate from Figure 3 that a serum vitamin D_3 concentration of 15 nmol/L would be produced by continuing daily total inputs (from all sources) of ≈50 μg (ie, 2000 IU). Whether the same biphasic response of serum 25(OH)D also occurs with vitamin D_2 is not known. In lactating women given vitamin D_2 at doses of 1600 or 3600 IU/d for 3 mo, increments in serum vitamin D_2 and serum 25(OH)D_2 were modest, and only a steep response was observed (28).

We suggest that these observations point to 3 conclusions. First, at typical inputs of vitamin D_3 (whether cutaneous or oral), there is rapid and near-quantitative conversion of vitamin D_3 to 25(OH)D, which then serves not only as the functional status indicator of the nutrient but, more important, as its principal storage form in the body. Second, above typical serum vitamin D_3 concentrations (ie, above ≈15 nmol/L), which are probably equivalent to a daily input of 2000 IU, the hepatic 25-hydroxylases become saturated and the reaction switches from first order to zero order. Third, the constant (maximal) production of 25(OH)D, irrespective of precursor concentration of vitamin D_3, must be in excess of metabolic consumption, which is the reason that serum 25(OH)D continues to rise as vitamin D_3 concentrations rise.

If correct, this explanation may help to clarify many of the uncertainties surrounding vitamin D physiology, one of which is the determination of the approximate concentration of serum 25(OH)D that may be considered optimal for health. Our data offer a different approach to estimating this value. One could plausibly postulate that the point at which hepatic 25(OH)D production becomes zero-order constitutes the definition of the low end of normal status. This value, as suggested from the equation in Figure 3, is at a serum 25(OH)D concentration of ≈88 nmol/L (35.2 ng/mL) (the y-axis intercept of the linear portion of the equation in Figure 3). It is interesting that this estimate is very close to that produced by previous attempts to define the lower end of the normal range from the relations of serum 25(OH)D to calcium absorption (29) and to serum parathyroid hormone concentration (ie, ≈75–85 nmol/L, or 30–34 ng/mL) (30).

In study A, with a supraphysiologic input, slow release from storage depots is indicated by the slow fall in 25(OH)D_3 from its C_{max}. The half-time of 25(OH)D is typically on the order of 20–30 d, whereas the approximate half-time in study A for the increment above baseline was >50 d. Fat is the most likely storage depot, although muscle storage cannot be ruled out. Fat storage of vitamin D_3 is certainly the case in the rat (as well as in humans) when serum vitamin D_3 concentrations are high. Analysis of body distribution in rachitic animals given ^{14}C-labeled vitamin D_3 every day for 2 wk showed that the largest amount, ≈10%, appeared in body fat and was slowly released into the circulation over the next several months along with a more polar metabolite—probably 25(OH)D, which had not been identified at that time (31). In obese human subjects, serum 25(OH)D is lower, serum vitamin D may be very low, and rises in serum vitamin D and 25(OH)D after either UV-B irradiation or oral administration of vitamin D_2 are significantly lower in obese than in nonobese persons (32, 33).

Deposition in body fat almost certainly occurs in cases of vitamin D intoxication, and persistence of hypercalcemia for months has been attributed to sustained release of vitamin D from such body stores. Fat storage is also the best explanation for the seeming disappearance of vitamin D_3 from the serum in the acute dosing experiment (study A). We cannot rule out some excretion of the large dose of vitamin D_3, either directly or by various catabolic reactions; however, the fact that the AUC for the increment in serum 25(OH)D was not lower than that for the increment in serum vitamin D_3 suggests little or no wastage of the ingested 100 000 IU.

Taken together, these results show that, as is typical for enzyme systems, there is a practical limit to the first-order 25-hydroxylation of vitamin D_3 and that, when vitamin D_3 input exceeds that limit, vitamin D_3 itself accumulates within the body, both in serum and probably in body fat. From the data presented in Figure 4, it would seem that that threshold occurs at a serum vitamin D_3 concentration of ≈15 nmol/L. In turn, such a concentration, from the data of Figure 3, is reached on average at a vitamin D_3 input of 2000 IU/d. We suggest that, below this input (whether cutaneous or oral), near-quantitative conversion of vitamin D_3 to 25(OH)D_3 occurs. Thus, at typical inputs, 25(OH)D_3 would constitute the principal storage form of the vitamin.

The strengths of the present study are several. First, it includes both sexes and a broad range of ages and races in the 206 participants of the component studies, which enhances the generalizability of the findings to the adult population. Second, the measurements in most of the component studies were made in a single laboratory by using research-quality assay methods. Third, these studies provide the largest body of simultaneous human vitamin D_3 and 25(OH)D_3 serum values ever published, which permits for the first time an estimation of the quantitative dynamics of the system. The principal weakness of the study lies in the lack of measurement of tissue (eg, fat) vitamin D metabolite content. For the latter reason, all conclusions about fat (or other tissue) storage must be considered tentative.

In summary, results of studies in which vitamin D_3, in doses of 0 to 11 000 IU/d, was given to normal subjects and subjects receiving exposure to either sunlight or UV-B irradiation showed that the response of 25(OH)D to increases in serum vitamin D_3 is biphasic: ie, an initial steep slope is followed by a more gradual rise. These findings are interpreted to mean that the conversion of substrate to 25(OH)D is nearly quantitative at low vitamin D_3 inputs but less than half-quantitative at supraphysiologic intakes.

The authors' responsibilities were as follows: RPH: data generation, data analysis, and manuscript preparation; LAGA: data generation and manuscript review; JRS: data generation and manuscript review; NHB: data generation, data analysis, and manuscript preparation; NB: data generation and manuscript review; and BWH: laboratory analysis, data generation, and manuscript review. None of the authors had a personal or financial conflict of interest.

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