Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol¹–³

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ABSTRACT

Background: The cholecalciferol inputs required to achieve or maintain any given serum 25-hydroxycholecalciferol concentration are not known, particularly within ranges comparable to the probable physiologic supply of the vitamin.

Objectives: The objectives were to establish the quantitative relation between steady state cholecalciferol input and the resulting serum 25-hydroxycholecalciferol concentration and to estimate the proportion of the daily requirement during winter that is met by cholecalciferol reserves in body tissue stores.

Design: Cholecalciferol was administered daily in controlled oral doses labeled at 0, 25, 125, and 250 μg cholecalciferol for ~20 wk during the winter to 67 men living in Omaha (41.2°N latitude). The time course of serum 25-hydroxycholecalciferol concentration was measured at intervals over the course of treatment.

Results: From a mean baseline value of 70.3 nmol/L, equilibrium concentrations of serum 25-hydroxycholecalciferol changed during the winter months in direct proportion to the dose, with a slope of ~0.70 nmol/L for each additional 1 μg cholecalciferol input. The calculated oral input required to sustain the serum 25-hydroxycholecalciferol concentration present before the study (ie, in the autumn) was 12.5 μg (500 IU)/d, whereas the total amount from all sources (supplement, food, tissue stores) needed to sustain the starting 25-hydroxycholecalciferol concentration was estimated at ~96 μg (~3800 IU)/d. By difference, the tissue stores provided ~78–82 μg/d.

Conclusions: Healthy men seem to use 3000–5000 IU cholecalciferol/d, apparently meeting >80% of their winter cholecalciferol need with cutaneously synthesized accumulations from solar sources during the preceding summer months. Current recommended vitamin D inputs are inadequate to maintain serum 25-hydroxycholecalciferol concentration in the absence of substantial cutaneous production of vitamin D. Am J Clin Nutr 2003;77:204–10.

KEY WORDS Vitamin D, cholecalciferol, 25-hydroxycholecalciferol, nutrient requirement, parathyroid hormone, seasonal variation, tolerable upper intake level

INTRODUCTION

It has long been recognized that vitamin D is not a nutrient in the usual sense, because it is not naturally present in most of the foods that our hominid ancestors would have consumed. Also, evolving as it did in equatorial East Africa, the human race would have received very generous solar radiation and had correspondingly high cutaneous synthesis of vitamin D year-round. It has also been recognized that people living and working in northern latitudes or in environments in which they are deprived of solar exposure need supplemental vitamin D. Indeed, it was precisely because of the success of oral fish liver oil supplementation in eradicating rickets that vitamin D came to be considered a nutrient in the first place.

In 1997, when the Food and Nutrition Board (FNB) published recommendations for calcium and related nutrients (1), it established the concentration of serum 25-hydroxycholecalciferol [25(OH)D] as the functional indicator for vitamin D, abandoning the older criterion of absence of disease as the definition of nutritional adequacy. However, because the requisite data were not then available, 3 key questions were left unanswered when the recommendations for vitamin D input were published: 1) what concentration of serum 25(OH)D would constitute the lower limit of adequacy?; 2) how much input of cholecalciferol is required each day to meet or sustain any given concentration of serum 25(OH)D?; and 3) how much of that required input comes from stores of cholecalciferol produced by cutaneous photoconversion from 7-dehydrocholesterol?

During the past 20 y, when the measurement of serum 25(OH)D was routinely available, many reports were published documenting a rise in serum 25(OH)D after treatment with oral vitamin D preparations for various periods (2–9). However, none of these studies attempted a systematic investigation of the dose-response relation. Thus, we do not yet have a satisfactory answer to the second of the questions that confronted the FNB. This report represents an attempt to define this relation and to integrate the results of a formal pharmacokinetic exploration with published but largely anecdotal data—and thus to answer the second question—and to begin to answer the third question.

SUBJECTS AND METHODS

Subjects

The subjects were 67 men, in good general health, who habitually consumed no more than one serving of milk/d and who did not take a vitamin supplement. Given this pattern, their food sources would have provided <5 μg cholecalciferol/d. In addition, we excluded men who, over the 5-mo course of the study,
when cutaneous vitamin D3 synthesis is minimal (10), both because February or early March. This is a time of year, at Omaha’s latitude, sive years, starting each year in late October and concluding in late
Design
versity, and all subjects gave written informed consent. The project was conducted during the winter months of 2 succes-
were planning a winter vacation to a location at which either the altitude or the latitude would be predicted to result in significant cutaneous vitamin D synthesis from solar radiation (eg, a moun-
tain ski resort or a Gulf Coast locale). Their mean (±SD) age was 38.7 ± 11.2, their weight was 84.8 ± 11.1 kg, and their mean body mass index (in kg/m²) was 26.2 ± 2.4. All subjects resided and worked in Omaha, at a latitude of 41.2° N (approximately the same as that of Boston or southern Italy). The project was approved by the Institutional Review Board of Creighton University, and all subjects gave written informed consent.

Data handling and statistical analysis

It was expected that serum 25(OH)D3 would rise or fall over the course of the winter, depending on whether the daily input was more or less than the daily consumption or utilization. The model describing the expected time course for serum 25(OH)D3 concentrations is set forth in Appendix A. Briefly:

\[ C(t) = C(0) + a \left(1 - e^{-kt}\right) \]  

where \( C(t) \) is the 25(OH)D3 concentration at time \( t \); \( C(0) \) is the serum 25(OH)D3 value at start of the study; \( a \) is the increment (or decrement) at equilibrium produced by a given constant input; \( k \) is the rate constant representing the proportion of the total mass of 25(OH)D3 used (or metabolized) per day; and \( t \) is the time in d. \( C(t), C(0), \) and \( a \) are all expressed in nmol/L. The time course data, both for the aggregate dosage groups and for each subject, were fitted to this equation with the use of the curve-fitting facility of SIGMAPLOT 2001 software (SPSS, Chicago). For 1 of the subjects in the highest-dosage group and for 17 subjects in the 2 lower-dosage groups, the data were erratic and the curve-fitting algorithm would not converge to provide good estimates of the parameters of equation 1. In these cases, the equilibrium increment (\( a \)) was taken as the simple arithmetic difference between the baseline and the final values. For the aggregated data sets, \( r^2 \) exceeded 0.93 for the 2 higher-dosage groups, which showed the overall aptness of the model for the data. Even for the 2 lower-dosage groups, \( r^2 \) averaged 0.690 and 0.810; each was highly significant and each was substantially greater than the result provided by a simple linear fit.

The values for the equilibrium increment (\( a \)), both for the grouped data and for each subject, were compared in regression analysis with the input variables (labeled and actual doses and dose adjusted for body size) by the use of standard statistical tech-niques. No biologic hypotheses were tested; the purpose of the investigation was simply to characterize the quantitative relation between input and the resulting increment in equilibrium serum concentration (ie, \( a \)), for which the regression methods provided the requisite descriptive statistics.

RESULTS

The time course data for each of the 4 dosage groups are presented in Figure 1, which shows both the mean values at each time

FIGURE 1. Time course of serum 25-hydroxycholecalciferol [25(OH)D] concentration for the 4 dosage groups. The points represent the mean values, and error bars are 1 SEM. The curves are the plot of Equation 2, fitted to the mean 25(OH)D3 values for each dosage group. The curves, from the lowest upward, are for 0, 25, 125, and 250 μg cholecalciferol (labeled dose)/d. The horizontal dashed line reflects zero change from baseline.

\[ C(t) = C(0) + a \left(1 - e^{-kt}\right) \]  

\[ a \] is the increment (or decrement) at equilibrium produced by a given constant input; \( k \) is the rate constant representing the proportion of the total mass of 25(OH)D3 used (or metabolized) per day; and \( t \) is the time in d. \( C(t), C(0), \) and \( a \) are all expressed in nmol/L. The time course data, both for the aggregate dosage groups and for each subject, were fitted to this equation with the use of the curve-fitting facility of SIGMAPLOT 2001 software (SPSS, Chicago). For 1 of the subjects in the highest-dosage group and for 17 subjects in the 2 lower-dosage groups, the data were erratic and the curve-fitting algorithm would not converge to provide good estimates of the parameters of equation 1. In these cases, the equilibrium increment (\( a \)) was taken as the simple arithmetic difference between the baseline and the final values. For the aggregated data sets, \( r^2 \) exceeded 0.93 for the 2 higher-dosage groups, which showed the overall aptness of the model for the data. Even for the 2 lower-dosage groups, \( r^2 \) averaged 0.690 and 0.810; each was highly significant and each was substantially greater than the result provided by a simple linear fit.

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RESULTS

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Our primary purpose in this investigation was pharmacokinetic, ie, to define mathematically the relation between cholecalciferol input and the induced concentration of its principal metabolite. For this purpose, the mean values provided a better reflection of the reality we were attempting to quantify than did the individual values, affected as they were by analytic imprecision and irregular compliance, both of which are averaged out when we use the means. Nevertheless, the individual data provide useful information in their own right, particularly about interindividual variability. Descriptive statistics for the variables of greatest interest, derived from the application of the model to each subject’s data set, are shown in Table 2. As expected, the means for the principal model variables are close to (but not quite identical to) those derived from the fits to the group means.

The data for the induced rise (ie, \(a\)) in serum 25-(OH)\(_3\)D, graphed as a function of the analyzed oral input by dosage group, are shown in Figure 2, and the induced 25-(OH)\(_3\)D increments (\(a\)) are plotted in Figure 3 for each subject against the corresponding oral inputs calculated in 4 ways: labeled dose (A), actual dose (taking into consideration both analyzed pill content and subject compliance).

### Table 1

<table>
<thead>
<tr>
<th>Model variables by daily cholecalciferol dose (^1)</th>
<th>(C(0)) (^2)</th>
<th>(a)</th>
<th>(k)</th>
<th>(n)mol/L</th>
<th>(n)mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labeled dose ((\mu)g)</td>
<td></td>
<td></td>
<td></td>
<td>70.1 ± 5.8</td>
<td>−11.4 ± 4.4</td>
</tr>
<tr>
<td>0</td>
<td>25</td>
<td>125</td>
<td>250</td>
<td></td>
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</tbody>
</table>

\(^1\) ± SE. \(C(0)\), the best-fit baseline estimate of 25-hydroxycholecalciferol [25(OH)D] concentration; \(a\), the increment in 25(OH)D concentration at equilibrium; \(k\), the rate constant characterizing the approach to equilibrium.

\(^2\) \(r^2\) (time in d).

### Table 2

<table>
<thead>
<tr>
<th>Measured cholecalciferol and 25-hydroxycholecalciferol [25(OH)D(_3)] concentrations of all subjects and calculated model variables for each of the 4 dose groups (^2)</th>
<th>Value (n)mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (all subjects)</td>
<td></td>
</tr>
<tr>
<td>Cholecalciferol (^2)</td>
<td>4.8 (2.1, 11.5)</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>70.3 ± 19.9</td>
</tr>
<tr>
<td>Zero-dose group</td>
<td></td>
</tr>
<tr>
<td>(C(0))</td>
<td>70.2 ± 23.4</td>
</tr>
<tr>
<td>(a)</td>
<td>−11.4 ± 17.7</td>
</tr>
<tr>
<td>20.9- (\mu)g dose group</td>
<td></td>
</tr>
<tr>
<td>(C(0))</td>
<td>72.05 ± 16.0</td>
</tr>
<tr>
<td>(a)</td>
<td>12.0 ± 16.2</td>
</tr>
<tr>
<td>137.5- (\mu)g dose group</td>
<td></td>
</tr>
<tr>
<td>(C(0))</td>
<td>69.3 ± 16.7</td>
</tr>
<tr>
<td>(a)</td>
<td>91.9 ± 37.6</td>
</tr>
<tr>
<td>275- (\mu)g dose group</td>
<td></td>
</tr>
<tr>
<td>(C(0))</td>
<td>65.6 ± 24.2</td>
</tr>
<tr>
<td>(a)</td>
<td>159.4 ± 62.4</td>
</tr>
</tbody>
</table>

\(^1\) ± SD except where indicated otherwise. \(C(0)\), the best-fit baseline estimate of 25(OH)D concentration; \(a\), the increment in 25(OH)D\(_3\) concentration at equilibrium.

\(^2\) Because the values were not normally distributed, the data were log transformed and converted back to decimal values; values in parentheses are 1 SD below and 1 SD above the mean, respectively.

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**FIGURE 2.** Regression of the equilibrium increment (\(\Delta\)) in serum 25-hydroxycholecalciferol [25(OH)D\(_3\)] on analyzed doses for the means of each treatment group.

**FIGURE 3.** Regression of the equilibrium increment (\(\Delta\)) in serum 25-hydroxycholecalciferol [25(OH)D\(_3\)] in individual subjects on labeled dose (A), on actual dose (B), on actual dose expressed/kg body weight (C), and on actual dose expressed/kg body fat (D). The dashed lines represent the 95% CIs for the regression lines.
(B), actual dose/kg body wt (C), and actual dose/kg fat mass (D). The slope for actual dose, as can be seen, was 0.70, which means that, for every milligram of the increment in cholecalciferol input per day, serum 25(OH)D₃ at equilibrium was higher by 0.70 nmol/L. The 2 regressions for the dose when expressed per unit of body size (or fat mass) produced no perceptible improvement in the fit of the data. The slopes for the regression of the equilibrium increment on dose for the labeled and actual values were nearly identical, and, in any case, they did not differ significantly. In calculating results from these data, below, we shall use the slope based on the actual dose.

At the zero dose (see Figure 1), measured serum 25(OH)D₃ fell slightly, and, at the 25-μg labeled dose (20.9 analyzed), it rose by nearly the same amount. The 2 higher doses produced predictably greater increases, almost reaching their respective plateaus by the end of the study. The drop at the zero dose from a basal value of 70.1 nmol/L was measured at 11.4 nmol/L with the use of the model parameters derived from the grouped data. By contrast, those assigned to the 1000-IU (25 μg) dosage group exhibited a measured change in the opposite direction of nearly the same magnitude, ie, a rise of 12.0 nmol/L with the individual values and of 9.0 nmol/L with the use of the model parameters for the grouped data. These sets of values straddle zero change, which, by interpolation, can be estimated to occur at a dose of < 12.5 μg (500 IU)/d. This is the amount of cholecalciferol that these men would have needed, in addition to the daily contribution from food and tissue stores, to maintain their starting serum 25(OH)D₃ concentrations through the winter.

Serum PTH rose by 6% during the winter in the zero-dosage group (from a beginning value of 32 pg/mL; P = 0.05), was unchanged in the intermediate-dosage groups, and fell by 24% in the highest-dosage group (from 29.5 pg/mL; P < 0.001). Across all groups, the change in PTH was inversely correlated with the actual cholecalciferol dose (P < 0.01). Serum calcium was measured at each visit in the 2 higher-dosage groups, but it did not change significantly from baseline (x̄: 9.6 mg/dL) at any time point at either dose. The first and last serum calcium values for the 31 participants in the higher-dosage groups are shown in Figure 4. No value rose above the upper limit of normal, and, as is visually evident, treatment did not increase the dispersion of serum calcium values.

**DISCUSSION**

To our knowledge, this study represents the first formal attempt to estimate the steady state cholecalciferol input required to achieve or maintain any given serum 25(OH)D₃ concentration. We found, by using a broad range of controlled oral doses in subjects with low inputs of vitamin D from food sources and by performing these measurements during the winter season when solar vitamin D input is minimal, that the slope of the relation is 0.70 nmol·L⁻¹·μg input⁻¹.

Byrne et al (2), in a meta-analysis of 10 published studies reporting 25(OH)D₃ increments produced by various doses of vitamin D (all within the range of current dietary recommendations), provided data for serum 25(OH)D₃ that can be calculated at 2.2 nmol·L⁻¹·μg⁻¹. The data of Chapuy et al (3), a study not included in the meta-analysis, yield an estimate of 1.9 nmol·L⁻¹·μg⁻¹. Krall et al (4) reported data exhibiting a slope of serum 25(OH)D₃ on calculated dietary input of 2.1 nmol·L⁻¹·μg⁻¹, very close to the meta-analytic estimate of Byrne et al (2). However, more recent work from a group in Lyon, France (5) contained data with a slope of 1.6 nmol·L⁻¹·μg⁻¹, which is lower than their earlier estimate. In the same range of values, Kyriakidou-Himonas et al (6) reported data yielding a slope of 1.95 nmol·L⁻¹·μg⁻¹ in older black women with low starting values for 25(OH)D₃. In most of these studies, total vitamin D input was not well controlled, adjustment was not made for the season, and the inputs did not extend beyond the range of current recommendations. Our own earlier study (7) used a much broader range of vitamin D inputs, but it also lacked seasonal control and found a slope of only 0.57 nmol·L⁻¹·μg⁻¹.

Chel et al (8), in a study of ultraviolet radiation in 85-year-olds, also provided data for serum 25(OH)D₃ concentrations after starting a steady input of 400 IU (10 μg/d) by mouth. Serum 25(OH)D₃ rose from a very low concentration of ≈20 nmol/L to ≈60 nmol/L. When their time course data were analyzed by us, using the model of equation 1, the estimate of the equilibrium increment was 55 nmol/L, for a slope of 25(OH)D₃ with a vitamin D₃ dose of 5.5 nmol·L⁻¹·μg⁻¹. This is one of the largest increments known to us. This value, as well as several of those cited above, indicate either that the slope is much steeper than we report here when starting from a status of depletion or that the 25(OH)D₃ compartment is substantially smaller in elderly subjects than in the healthy younger men in our study. Also, assay analytic factors (13) may explain some of the differences in slope estimates among the various studies cited.

Vieh et al (9), in a recent study with a design similar to ours, reported values for the equilibrium increment of 1.15 nmol·L⁻¹·μg⁻¹ for a 25-μg/d dose and of 0.56 nmol·L⁻¹·μg⁻¹ for a 100-μg/d dose. Why these investigators did not find the same slope for both dosage concentrations (as was observed in our data) is unclear. Nevertheless, their estimates of slope bracket our value (0.70 nmol·L⁻¹·μg⁻¹) and hence constitute independent confirmation of the general magnitude of the effect we describe here.
Without attaching particular significance to any given serum 25(OH)D concentration, it is nevertheless useful to select one such concentration so as to estimate the daily vitamin D input needed to achieve or sustain it. For this purpose, we selected 80 nmol/L, a figure near which serum PTH bottoms out (5, 14, 15). For a person with a starting value such as 50 nmol/L, the target increase would be 30 nmol/L, and, as the data in Figures 2 and 3B make clear, this increase would in turn require an additional input from all sources of \( \approx 43 \mu g (\approx 1700 \text{ IU})/\text{d} \). The standard error of the slope of the relation, derived from the data plotted in Figures 2 and 3B, produces a 95% CI for this estimate of 33 to 65 \( \mu g \) (1320, 2600 IU)/d. If maintenance of the assumed basal concentration of 50 nmol/L required input in the same proportion, the total daily input needed to maintain a concentration of 80 nmol/L would be about 114 \( \mu g \) (4600 IU)/d. The 95% CI around this estimate extends from 88 to 175 \( \mu g \) (3520, 7000 IU). Even at the lower bound of the CI, this estimate is high in relation to current assumptions about vitamin D. Nevertheless, our point estimate is very close to the one produced by Vieth (16) in a recent analysis of the vitamin D literature.

As noted in the Results section, 12.5 \( \mu g/d \) is the approximate oral input required to maintain serum 25(OH)D at zero change, at least from starting values in the range we observed (\( \approx 70 \) nmol/L). If one assumes input of \( \leq 5 \mu g/d \) from food sources, these 2 sources combined would predict a serum 25(OH)D concentration of only 12 nmol/L, whereas the actual concentration was \( \approx 70 \) nmol/L, which leaves an unaccounted-for difference of \( \approx 58 \) nmol/L. With the use of our measured slope of serum concentration on actual daily input (ie, \( 0.7 \text{ nmol·L}^{-1} \cdot \mu g^{-1} \cdot \text{d}^{-1} \)), it follows that about 85 \( \mu g (\approx 3400 \text{ IU}) \) cholecalciferol in body tissue stores must have been converted to 25(OH)D Each day in these healthy men. The identification of the relative magnitude of the contribution to 25(OH)D concentrations coming from cholecalciferol stores may help explain the broad range of dose response relations reported by others and summarized above.

It should be noted that the amount of cholecalciferol that we found to be required to maintain a constant concentration over the winter months is very similar to the amount that Holick (17) found was required for atomic submariners across the quasi-winter of a tour of duty. However, it is also clear from our data (and from his) that this value presumes a very substantial input from previously acquired tissue stores. Thus, it would not be correct to extrapolate this estimate of the maintenance requirement to populations without substantial body stores of cholecalciferol.

Both Vieth (16) and Holick (18) estimated that a single wholebody minimum erythema dose of solar radiation to the skin produces an input of 10000 IU (250 \( \mu g \)) cholecalciferol, and, hence, frequent exposure, particularly during the summer months, would be expected to produce inputs close to or even exceeding the upper end of the dosage range used in this investigation. We previously noted that the slope of the relation in various reports is inversely related to the size of the dose (7). Because our range of doses was greater than that previously reported, our slope may have been spuriously lowered by inefficient conversion of cholecalciferol to 25(OH)D at the higher dosages. There is, in Figure 2, a hint of curvilinearity in the relation, because the increment from zero to 20.9 \( \mu g/d \) is slightly larger than the corresponding increments at higher dose concentrations. But, as is visually evident, the difference is extremely small.

Since 25(OH)D measurement became available clinically, it has been the common experience of clinicians and investigators that the standard multivitamin preparation (nominally containing 400 IU/dose unit) produced often imperceptible changes in measured serum 25(OH)D. Our present results confirm, in a general way, the relative smallness of the response to inputs in the range of the currently recommended oral vitamin D inputs (1). Using the slope for actual dose (ie, \( 0.7 \text{ nmol·L}^{-1} \cdot \mu g^{-1} \cdot \text{d}^{-1} \)), one can readily calculate that a dose such as 400 IU/d would elevate serum 25(OH)D by 7.0 nmol/L. Given between assay variability of the methods for measuring serum 25(OH)D, it is unlikely that changes this small would be regularly detectable in individual persons.

Our calculations assume the effective equivalence of oral and cutaneous sources of cholecalciferol. If this assumption is approximately correct, it follows that typical food and supplement inputs provide <15% of the amount required to maintain, for example, a serum 25(OH)D concentration of 70–80 nmol/L. Thus, the recommendations of the FNB with respect to oral vitamin D input (1) fall into a curious zone between irrelevance and inadequacy. For those persons with extensive solar exposure, the recommended inputs add little to their usual daily production, and for those with no exposure (or those, such as the elderly, with reduced cutaneous synthesis), the recommended doses are insufficient to ensure desired 25(OH)D concentrations. For example, if a 70-y-old person’s sole source of vitamin D were the 600 IU/d recommended by the FNB (1), the data presented in this paper indicate that such an amount would be sufficient to sustain a 25(OH)D concentration in the range of only 12.5 nmol/L, a value generally recognized as subnormal and probably consistent with osteomalacia. Even if the 25(OH)D response slope from a concentration of severe depletion were 3 times that reported here, following the FNB’s recommendation in those aged > 70 y would produce a serum 25(OH)D concentration of only 37.5 nmol/L, which remains far from adequate. Several groups have reached the same conclusion, namely that, without appreciable cutaneous synthesis, current cholecalciferol input recommendations are inadequate (19, 20).

None of this is to be taken as a criticism of the 1997 recommendations. Two of us (MFH and RPH), in fact, served on the Calcium and Related Nutrients Panel of the FNB. As noted in the Introduction, the data needed for the calculations presented here simply did not exist at the time the current recommendations were formulated. However, given the general consistency of the findings from both this investigation and the others cited above, it may be that the FNB should reopen this issue.

Widespread supplementation with vitamin D has important public health implications. The FNB proposed estimates, for the first time, of tolerable upper input levels for nutrients, particularly for the micronutrients that can be incorporated into foods as fortificants or ingested as nutritional supplements (1). Evidence available to the FNB with regard to vitamin D toxicity at inputs of the magnitude employed in this study was scant in the extreme. Nevertheless, on the basis of sporadic reports (of uncertain quality) of hypercalcemia and hypercalciuria, the panel settled on a conservative tolerable upper input level of 2000 IU/d for vitamin D (1), recognizing that many persons, especially those who work outdoors in the summer, almost certainly had higher inputs without apparent adverse effect.

We agree completely with Vieth (9) that the evidence available today indicates that a value of 2000 IU/d for the tolerable upper input level is too low. As already noted, the data presented here indicate an average daily need perhaps twice that amount. Note
that, in our study, 20 wk of supplementation at 5500 and 11 000 IU/d, starting from a status of relative vitamin D repletion, produced no elevation of serum calcium above the upper limits of normal in any subject. Note also that the highest mean 25(OH)D3 values reached were \( \approx 160 \) and \( \approx 220 \) nmol/L, respectively. By contrast, a case report (21) suggested that serum 25(OH)D concentrations between 125 and 225 nmol/L produce bone loss. Although that study was almost certainly in error on technical grounds, the fact that it could be published with an accompanying, supportive editorial (22) underscores both the almost complete absence of basic data in this field and the need to obtain such data. This present report constitutes one step in providing this missing information.

REFERENCES

APPENDIX A

Because serum 25-hydroxycholecalciferol [25(OH)D] concentrations tend to be stable (albeit with some degree of superimposed annual cyclicality), it seemed reasonable to propose a steady state system in which input equals output, ie, a pool or compartment of 25(OH)D1 that is replenished by hepatic hydroxylation of circulating cholecalciferol at the same rate at which 25(OH)D itself is metabolized or consumed. Diagrammatically, the system can be represented as in **Figure A1**. For both compartments at equilibrium, \( dQ/dt = 0 \) and, effectively, \( \rho_{12} = \rho_{23} \). The corresponding rate constants (eg, \( k_{12} \) and \( k_{23} \)), not shown, are given as \( \rho/Q \).

For the purposes of this analysis, this 2-compartment system can be usefully simplified to a single compartment by focusing only on \( Q \), the 25(OH)D compartment (**Figure A2**). At equilibrium, \( Q \) is constant, ie, \( dQ/dt = 0 \), and \( I = kQ \). When \( I \) is increased, \( Q \) rises for a time, until \( kQ = I \), and \( dQ/dt \) once again becomes zero. In brief,

\[
dQ/dt = I - kQ \tag{A1}
\]

and

\[
dQ/(I - kQ) = dt \tag{A2}
\]

Multiplying both sides of equation \( A2 \) by \( I \) and rearranging yields

\[
dQ/(I - [Q/(I/k)]) = I dt \tag{A3}
\]

Then, integrating yields

\[
(I/k)\ln[1 - ([Q/(I/k)])] = It + A \tag{A4}
\]

where \( A \) is a constant of integration; rearranging yields

\[
\ln[1 - ([Q/(I/k)])] = -kt - Ak/I \tag{A5}
\]

Taking the antilog,

\[
[1 - ([Q/(I/k)])] = e^{-kt} \cdot e^{-Ak/I} \tag{A6}
\]

But, \( A, k, \) and \( I \) are constants, and hence the second factor on the right side of equation \( A6 \) is also a constant (eg, \( B \)). Thus, equation...
explicitly in mass terms (oversimplified in several respects. First, the model is expressed only a constant divisor. Hence the kinetics of the 2 situations will be the same, and the relation between dose and equilibrium concentration will be the same as that between dose and equilibrium content. 

Of concentration (I) over time after experimental alteration of input. However, the administered doses of vitamin D do not constitute the only input to the 25(OH)D compartment. Instead, 

Further, the model assumes constancy of I over time after experimental alteration of input. However, the administered doses of vitamin D do not constitute the only input to the 25(OH)D compartment. Instead, 

that most or all our subjects were vitamin D replete, and thus the assumption may not be valid for persons with low serum 25(OH)D concentrations, in whom elevated PTH secretion may drive increased 1-α-hydroxylation of 25(OH)D, thereby increasing k. Similarly, for low serum cholecalciferol concentrations, 25-hydroxylation may be more efficient, causing a given cholecalciferol dose to produce a higher I value. 

Figure 2). Hence, we conclude that this oversimplification does not adversely affect our ability to use the model to analyze the data in the way we have done. 

Finally, the one-compartment simplification is just that—a simplification. It assumes that input to Q2 (ie, 25-hydroxylation) is a constant proportion of the input to Q1 (cholecalciferol dose). That may not be true for all states of vitamin D repletion. The ratio of p12 to p14 may well be higher when Q1 is small. The available literature is of little help in this regard, so we can only note this limitation as a theoretical possibility.