

Vitamin D₃ Is More Potent Than Vitamin D₂ in Humans

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Background: Current unitage for the calciferols suggests that equimolar quantities of vitamins D₂ (D2) and D₃ (D3) are biologically equivalent. Published studies yield mixed results.

Objective: The aim of the study was to compare the potencies of D2 and D3.

Design: The trial used a single-blind, randomized design in 33 healthy adults. Calciferols were dosed at 50,000 IU/wk for 12 wk. Principal outcome variables were area under the curve for incremental total 25-hydroxyvitamin D [25(OH)D] and change in calciferol content of sc fat.

Results: Incremental mean (sd) 25(OH)D area under the curve at 12 wk was 1366 ng · d/ml (516) for the D2-treated group and 2136 (606) for the D3 ($P < 0.001$). Mean (sd) steady-state 25(OH)D increments showed similar differences: 24 ng/ml for D2 (10.3) and 45 ng/ml (16.2) for D3 ($P < 0.001$). Subcutaneous fat content of D2 rose by 50 $\mu\text{g}/\text{kg}$ in the D2-treated group, and D3 content rose by 104 $\mu\text{g}/\text{kg}$ in the D3-treated group. Total calciferol in fat rose by only 33 ng/kg in the D2-treated, whereas it rose by 104 $\mu\text{g}/\text{kg}$ in the D3-treated group. Extrapolating to total body fat D3, storage amounted to just 17% of the administered dose.

Conclusion: D3 is approximately 87% more potent in raising and maintaining serum 25(OH)D concentrations and produces 2- to 3-fold greater storage of vitamin D than does equimolar D2. For neither was there evidence of sequestration in fat, as had been postulated for doses in this range. Given its greater potency and lower cost, D3 should be the preferred treatment option when correcting vitamin D deficiency. (*J Clin Endocrinol Metab* 96: E447–E452, 2011)

During the seven decades from the discovery of the D vitamins to the publication of vitamin D reference intakes in 1997, the principal approach to quantifying their potency was bioassay, which is at best a semiquantitative approach. By assigning the same number of international units to molar equivalent quantities of the two calciferols early on, it was presumed that the two were equally potent. With the 1997 vitamin D reference intake publication for vitamin D (1), serum 25-hydroxyvitamin D [25(OH)D], rather than antirachitic activity, was defined as the functional indicator of vitamin D status. Since then several studies have reported on the relative potencies of cholecalciferol and ergocalciferol, as assessed by their

effect on elevating or sustaining serum 25(OH)D concentration. Several reports found cholecalciferol to be superior to ergocalciferol by this criterion (2–5), with the potency of the two compounds varying by factors ranging up to four fold. At least one other study (6) reported that the two calciferols were essentially equipotent. Aside from the fact that the doses and dosing regimens in these studies differed considerably, the reason for this discordance is unclear.

In an attempt to clarify this issue, we designed a study specifically to test the hypothesis of superiority of cholecalciferol (D3) over ergocalciferol (D2) in a randomized, controlled trial, using change in total serum 25(OH)D as

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Abbreviations: AUC, Area under the curve; AUC₈₄, AUC to 84 d; BMI, body mass index; D2, vitamin D₂ (ergocalciferol); D3, vitamin D₃ (cholecalciferol); 25(OH)D, 25-hydroxyvitamin D.

the primary outcome variable, and change in adipose tissue fat content of the calciferols as a secondary outcome.

Subjects and Methods

Design

The study was a parallel, single-blind design, with participants randomly assigned to receive 50,000 IU of one or the other calciferol weekly for a total of 12 doses. The 50,000-IU dose was chosen because it is the most commonly available form for D₂; because that dose, given once or twice weekly, is the one most commonly used by clinicians treating vitamin D deficiency today; and because an input of that size is approximately what is now thought to be required to sustain 25(OH)D levels in the range likely to have prevailed during hominid evolution.

Vitamin D₂ was obtained as 50,000 IU gel caps manufactured by Banner Pharmacaps, Inc. (Highpoint, NC) for PLIVA, Inc. (Pomona, NY) through the Creighton University Medical Center pharmacy. Vitamin D₃ was provided by BTR Group, Inc., as 10,000 IU gel caps. Both products were vegetable oil-based, and the capsules for each were from single production lots. The D₂ capsules were analyzed each to contain 46,800 IU, and the D₃ capsules, 11,100 IU. For most of the discussion that follows, we will use the labeled dose. The dosage was one capsule of D₂ weekly or five capsules of D₃ weekly. The project manager responsible for product administration was not blinded, but the investigators and personnel performing the assays and statistical analysis were unaware of treatment assignment.

At baseline and wk 2, 4, 6, and 8, the dose was personally administered to each participant by the project manager, and for the intervening weekly doses, the participants were contacted by phone and/or e-mail. They were thereby reminded to take the dose(s) that had been provided them at the earlier visit and to respond by e-mail when they had done so. Thus, so far as could be judged, compliance was 100% for participants adhering until study end. Blood samples were drawn at baseline and at wk 2, 4, 6, 8, 12, and 17. The 12-wk sample was by design 1 wk after the last oral dose of a calciferol [approximate T_{max} for 25(OH)D after an oral dose of D₃ of this size (3)]. The 17-wk sample was designed to reflect the posttreatment decline in serum 25(OH)D concentration.

Additionally, the subjects were given the opportunity of contributing a small fat biopsy at wk 0 and 12. The samples were obtained surgically under local anesthesia from lower abdominal sc fat, typically producing 3–5 g fat. Eleven of the enrolled subjects agreed to these paired biopsies, but two withdrew after the first biopsy, leaving nine paired samples. The overall plan for the interventions and blood and fat samples is set forth in Fig. 1.

Subjects

Thirty-five participants were recruited overall, and two dropped out between randomization and completion. There were three males and 30 females in the final group, all Caucasian, with a mean age of 49.5 (± 9.8) yr and a mean body mass index (BMI) of 25.8 kg/m² (± 3.9). None had gastrointestinal or skeletal disorders, and none were taking corticosteroids, anticonvulsants, or other agents known to affect hepatic metabolism of vitamin D. Baseline characteristics, by treatment assignment, are given in Table 1. Both groups were well matched, with no significant difference between them in any of the listed features.

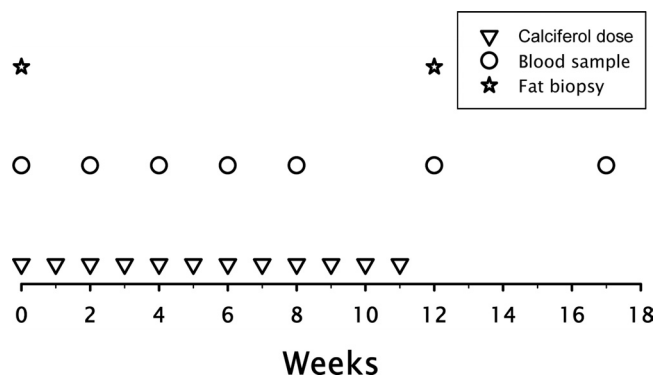


FIG. 1. Dosing and measurement scheme: ▽, 50,000 IU calciferol by mouth; ○, blood sample for 25(OH)D analysis; ☆, fat biopsy. (Copyright Robert P. Heaney, 2010. Used with permission.)

Several individuals were on a regimen of calcium and/or multi-vitamin supplements, many of which contained low doses of vitamin D (median, 400 IU). The participants were instructed to continue this supplementation throughout the study so as to avoid a change in their steady-state input from sources other than the study intervention. The study was approved by the Creighton University Institutional Review Board, and all participants gave written consent, once for the basic study itself, and then separately for the fat biopsies. Subjects were enrolled in late winter 2010, and all completed dosing by late spring 2010.

Analytical methods

Serum 25(OH)D was measured on a Liaison instrument (DiaSorin, Inc., Stillwater, MN) using a chemiluminescent assay, which measures total 25(OH)D [*i.e.* the sum of 25(OH)D₂ and 25(OH)D₃] with a within-assay coefficient of variation of 2.6%. All samples were run in three batches, each against the same reference serum pool, with interbatch adjustments as required to produce the same reference values. D₂ and D₃ content of the administered capsules and of the fat biopsy samples were determined by an HPLC method developed for food (7) and modified for fat.

Unitage

For conversions from mass units to international units, the following factors are involved. By definition, 1 μg cholecalciferol = 40 IU. Because of a slight difference in formula weight, 1 μg of ergocalciferol = 38.8 IU. Using SI units, 1 nmol of either calciferol = 15.4 IU.

TABLE 1. Baseline characteristics of study participants

	Vitamin D ₂	Vitamin D ₃
n	16	17
Age	49.7 (10.3)	49.3 (9.7)
Sex (males/females)	2/14	1/16
BMI (kg/m ²)	25.5 (4.1)	26.2 (3.7)
Body fat (kg)	25.2 (7.0)	27.0 (8.0)
Menopausal status (pre/post)	6/8	7/9
Vitamin D intake (IU/d) ^a	344 (444)	328 (305)
Serum 25(OH)D (ng/ml)	30.6 (14.8)	26.0 (9.2)

Data are expressed as mean (SD) (except for sex and menopausal status).

^a Exclusive of study intake.

Statistical analysis

Area under the curve (AUC) for the increment in serum 25(OH)D was computed by the trapezoidal method for each participant's set of blood values. Additionally the participant's serum 25(OH)D increment values were fitted individually (using SigmaPlot 11; Systat Software, Inc. San Jose, CA) to the following equation, modified from an approach derived and justified in an earlier publication (8): $y = a(1 - e^{-bx})$ [Eq. 1], where y is the measured increment in serum 25(OH)D above baseline, and x is the time in days from baseline. The a parameter in this equation is the equilibrium increment, *i.e.* the best estimate of the steady-state change from baseline that would have been reached had dosing continued without change in nonstudy inputs; b is the rate constant, characterizing the rapidity with which the steady state was reached. The goodness of fit to this model was excellent, with r^2 averaging 0.913 for the individual D2 curves and 0.978 for the individual D3 curves. Additionally, Eq. 1 was integrated from zero to 84 d as a separate estimate of the AUC for each individual.

Body fat mass was estimated separately for men and women, using the empirical formulas of Deurenberg *et al.* (9) to calculate percentage body fat and then applying that value to measured total body weight. The Deurenberg formula had been independently calibrated in our unit against total body fat measured by dual-energy x-ray absorptiometry, with a coefficient of correlation of approximately 0.80. Total body calciferol content was estimated by extrapolating the sc biopsy samples to the total body fat mass, assuming a similar steady-state calciferol concentration in all fat deposits.

The difference between mean AUC values was tested by the two-sample t test. As an independent test of the main hypothesis for the primary outcome variable, the a and b values from the fitted curves were compared between the two calciferol groups, also by a simple two sample t test. Differences in calciferol content of fat between groups were also tested by the two-sample ranks test. Pearson correlation and linear regression were used to explore relationships between variables.

Results

Serum 25(OH)D response

The time course for the rise in serum 25(OH)D for both calciferol regimens is shown graphically in Fig. 2 for the mean values at each sampling point. The corresponding equation parameters are set forth in Table 2. The AUC to 84 d (AUC_{84}), the integral of Eq. 1 to 84 d, and the a parameter from Eq. 1 all capture this difference between the two calciferols, with AUC_{84} being 56% higher for D3 than for D2, and the equilibrium value (a) 87.5% higher ($P < 0.001$ for both). As the figure shows clearly, neither calciferol had reached a new steady state by 84 d. [This was as expected because we had shown previously (8) that dosing with D3 required on the order of 150–180 d to reach something approaching a steady state.] However, not only was the equilibrium value for D2 lower than for D3, but by 84 d, as the figure suggests visually, the curve was flatter for D2 than for D3. This is reflected numeri-

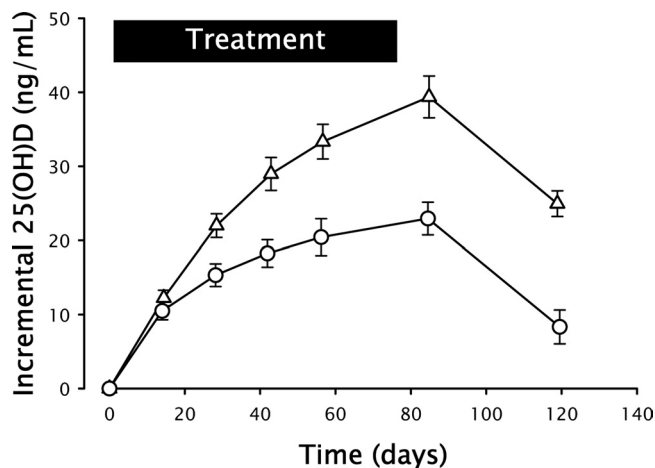


FIG. 2. Time course of mean total serum 25(OH)D in the two calciferol treatment groups. \circ , D2 ($n = 16$); \triangle , D3 ($n = 17$). Error bars are 1 SEM. Horizontal bar indicates treatment duration. (Copyright Robert P. Heaney, 2010. Used with permission.)

cally in the rate constant, (b) (from Eq. 1), presented in Table 2. That constant, for D2, was significantly higher than for D3 ($P = 0.003$), indicating an inherently more rapid approach to equilibrium. This is the reason the a values differed by more than the AUC_{84} values.

Both groups had lost an appreciable amount of their gain in serum 25(OH)D by 6 wk after the last dose [mean change, 14.5 ng/ml (± 7.5); $P < 0.001$], and there was no difference in absolute magnitude of decline between D2 and D3. However, because the D2 group exhibited a substantially lower equilibrium value, the 6-wk decline was proportionately much greater for D2 than for D3 (-64 vs. -37%). Because the pharmacokinetics of vitamin D postulate an exponential decline from an induced peak, this suggests, as above, that D2 induced an increase in degradation of 25(OH)D. In fact, several of the D2-treated group fell below their individual baseline values by the 17-wk time point.

Adipose tissue calciferol content

Figure 3 sets forth the D2 and D3 contents of the paired fat samples obtained at baseline and then at d 84. As would be expected, most of the stored calciferol at baseline was D3 because nonprescription D2 sources are uncommon in the United States. There were no significant differences in baseline content of either D2 or D3 for the two treatment groups. Mean baseline total calciferol was 76.1 $\mu\text{g}/\text{kg}$ (± 15.9), or 3036 IU/kg.

Fat content of the cognate calciferol rose across the 12 wk of treatment for both D2 and D3. However, the rise in D2 averaged only 57 $\mu\text{g}/\text{kg}$ (± 10) in the D2-treated individuals, whereas in the D3-treated individuals the rise in D3 content was nearly twice as great, averaging 104 $\mu\text{g}/\text{kg}$ (± 77.2). Because of the small sample sizes for the paired biopsies ($n = 2$ and 7), the difference between the two was

TABLE 2. Serum total 25(OH)D response by treatment assignment

	Vitamin D ₂ group	Vitamin D ₃ group	P
AUC ₈₄ (ng · d/ml)	1366 (516)	2136 (606)	0.005
Integral (ng · d/ml) ^a	1385 (544)	2121 (656)	0.001
Equilibrium value– <i>a</i> (ng/ml)	24.05 (10.30)	44.95 (16.18)	0.0001
Rate constant– <i>b</i>	0.0435 (0.0193)	0.0257 (0.0095)	0.003
Change on stopping (ng/ml)	–14.6 (6.61)	–14.4 (8.39)	0.85

Data are expressed as mean (SD).

^a Integral of Eq. 1 to 84 d.

not statistically significant. The rise in content of total calciferols (D2 + D3) in fat was 104 ng/g for the D3-treated group, but only 32.5 ng/g for the D2-treated—a better than 3-fold greater rise for D3 than for D2 ($P = 0.20$).

Interrelationships

There was no relationship between the 12-wk increment and the baseline value for 25(OH)D, indicating that the measured increases were substantially free of distortion from regression to the mean. However, the measured increment at 12 wk and the *a* parameter are, as expected, highly correlated ($r^2 = 0.91$; $P < 0.001$), and when regressed through the origin, the slope of *a* on the 12-wk increment was +1.15, indicating that the calculated equilibrium increment was about 15% higher than had been achieved at 12 wk (or conversely, that the 12-wk value was about 87% of the ultimate steady-state value).

Baseline 25(OH)D was inversely correlated with body weight ($r^2 = 0.16$; $P < 0.02$), and weakly inversely correlated to body fat ($r^2 = 0.088$; $0.10 > P > 0.05$). However, neither for D2 nor for D3 was there a statistically significant relation between either measure of the increase (the 12-wk increment or the *a* parameter) and either body weight or body fat. For

D2, the slopes of the fitted regression lines were quite flat; for D3, the slopes, although negative as hypothesized, were of borderline significance ($r^2 = 0.11$ and 0.12 for 12-wk increment and *a*, respectively; $P \cong 0.20$).

Discussion

These results show clearly that D3 produces a substantially larger effect than D2, as measured both by serum 25(OH)D and by fat storage of the vitamin. By the various measures employed, D3 was from 56 to 87% more potent than D2 in raising serum 25(OH)D, and more than three times as potent in increasing fat calciferol content. When computed on the basis of analyzed, rather than labeled intake, the difference, although somewhat less than the foregoing figures indicate, is still substantial. Moreover, the observed differential is likely to be reflective of what would be found in practice, using similarly labeled products, because the dosage regimen employed in this study is similar to that commonly used by clinicians today. Also, these results are concordant with several other studies, using different doses and dosing regimens (2–5). Hence, these results can, we believe, not only safely be generalized to routine clinical practice, but also are pertinent to what clinicians are actually prescribing.

It is important to be clear about what “potent” means in this context. We do not suggest that the 1,25-dihydroxyvitamin D derivatives of the two calciferols differ in molar potency. However, the autocrine synthesis of the 1- α -hydroxyl derivative operates below the K_M of the various tissue hydroxylases, and accordingly, the rate of 1,25-dihydroxyvitamin D production in a tissue is strictly dependent on the serum level of the 25(OH)D precursor. The lower tissue exposure produced by D2 will inevitably result in lower production of the di-hydroxylated, active form of the vitamin within the tissues concerned. Thus, the several studies showing lower 25(OH)D response to the same oral dose strongly suggest that equimolar systemic doses of the two calciferols will not produce equal tissue-level biological effects.

Although there remains some controversy in this field as to the relative potencies of the two calciferols, it ought

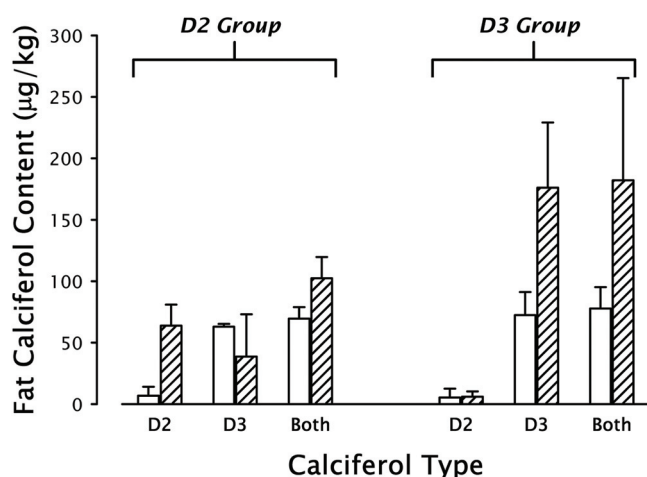


FIG. 3. Calciferol content of sc fat. Bars on left depict the values obtained in the samples from D2-treated participants ($n = 2$), and bars on right depict the D3-treated participants ($n = 7$). The open bars in each case are the baseline content values, and the striped bars, the 12-wk values. Error bars are 1 SEM. (Copyright Robert P. Heaney, 2010. Used with permission.)

not be surprising today to find a difference. Nearly 30 yr ago, Horst *et al.* (10), in a careful analysis of calciferol metabolism in pigs, rats, and chicks, found substantial interspecies differences. In pigs, for example (with metabolism closer to human than that of the other species), D3 was four times as potent as D2. Holick *et al.* (6) provide the sole published evidence of effective equivalence in humans, but their doses were much smaller than those used by either Trang *et al.* (2) or Tjellesen *et al.* (11), both of whom used 4000 IU/d and found substantial discrimination in favor of D3. The package label dose used in this study, which translates to approximately 7100 IU/d, also favored D3 over D2. Depending upon the 25(OH)D level one considers “adequate,” the doses in these three studies are by no means pharmacological, but are of the approximate magnitude needed for maintenance of steady-state vitamin D status at values commonly found in outdoor workers. By contrast, at the doses used in Holick’s study (6), the increase in serum 25(OH)D would be relatively small (approximately 6–10 ng/ml · 1000 IU/d) and might well not be sufficient to permit detection of differing potencies between calciferols because the induced change would tend to be swamped by analytical and biological variability.

The mean value of the *a* parameter in the D3-treated group (44.95 ng/ml) amounts to 0.57 ng/ml per 100 IU/d of additional D3, with a 95% confidence interval of 0.47 to 0.69, slightly lower than, but not significantly different from, our previously published estimate of 0.70 ng/ml · 100 IU/d (8). However, both values are below the often-quoted value of 1.0 ng/ml per 100 IU/d (which may nevertheless be applicable for lower starting 25(OH)D values than the studied participants exhibited).

Although every participant experienced a rise in serum 25(OH)D during treatment and all fell on cessation, four of the 16 subjects in the D2 group had fallen below 32 ng/ml by wk 17, and three were actually below their own starting values. This is similar to what we had seen previously with a single 50,000-IU dose of D2 (3) and what Tjellesen *et al.* (11) had earlier reported for 4,000 IU/d. By contrast, none in the D3 group fell below 32 ng/ml at wk 17, nor had any of the D3-treated group fallen below the participant’s own starting value. Also, as we have reported previously, the response range to the same oral dose was very broad (12). In this study, the range of equilibrium increases in serum 25(OH)D (the *a* parameter) extended from 31 to 96 ng/ml, with no statistically significant correlation with body size, suggesting that interindividual differences in 25-hydroxylation capacity or 24-hydroxylase degradation were more powerful determinants of 25(OH)D concentration than the distributional effect of body size (3, 11).

Because the rise in 25(OH)D with time is the algebraic sum of continuing inputs and countervailing metabolic

degradation or consumption, an increase in the latter would produce both a smaller increment and a more rapid approach to equilibrium. The higher value of the *b* parameter (Eq. 1) for the D2 group is consistent with precisely such enhanced metabolic 25(OH)D degradation in those supplemented with D2. This conclusion is supported by the proportionately greater decline for the D2-treated group on stopping supplementation, by the decline to substarting values in some of them, and by the lower level of D2 storage in fat. Together, these facts all point toward D2’s having a much shorter body half-life than D3.

The fat content data and fat storage components of our study are essentially new; their interpretation relies on extrapolation of biopsy values to total body fat mass. Very little prior information can be found in regard to fat content of vitamin D in healthy humans. Although the number of biopsy samples in this study is small, the data provided are virtually the only such data available for healthy adult humans to date. What is particularly noteworthy from these data is the relatively small magnitude of fat storage of vitamin D. Baseline total calciferol content, across both groups, averaged 76.1 $\mu\text{g}/\text{kg}$ (± 15.9) for a group of volunteers with a mean serum 25(OH)D concentration of 28.3 ng/ml. This is just slightly more than 3000 IU/kg fat. Blum *et al.* (13), in sc fat specimens removed at bariatric surgery from morbidly obese patients (mean BMI, 50.6 kg/m^2), reported a mean D3 content of 102.8 nmol/kg or 1583 IU/kg. In contrast, our mean baseline D3 content was 70.3 $\mu\text{g}/\text{kg}$ (*i.e.* 182.8 nmol/kg or 2812 IU/kg) in participants with a mean BMI of 25.5 kg/m^2 . This difference between studies is consistent with the previously reported low vitamin D status in obesity (13–18).

Extrapolating from sc fat, mean total body fat content of total calciferols at baseline in these individuals amounted to approximately 81,000 IU, or only a 20-d supply at the consumption rate needed to sustain a serum concentration of 32 ng/ml (8). Based on analyzed supplementation at 55,400 IU/wk, total body fat stores more than doubled to approximately 197,000 IU. Still, that amounted to less than a 50-d supply at a serum level of 32 ng/ml (8).

Using the foregoing assumptions, the increase in estimated total body calciferol came to 116,000 IU (197,000 less 81,000), from a dosing regimen that provided an analyzed total of 665,000 IU. In brief, about 17% of the ingested D3 input was stored, whereas the rest was presumably consumed and/or metabolized. This is a daily utilization rate of about 6500 IU at a serum 25(OH)D concentration of 50 ng/ml at the end of treatment. This is within 12% of our previous estimate of 4000 IU/d for a serum 25(OH)D of 32 ng/ml, taking into consideration the difference in utilization rates at different serum concentrations. These calculations (and the data on which they are

based) provide strong additional evidence concerning D₃ inputs needed for particular target values of serum 25(OH)D. They also bear directly on the frequently raised issue of sequestration (13) because the rise in storage we measured, while real, was not very large. At the doses used in this study, most vitamin D is metabolically consumed. This is the first study, to our knowledge, to have quantified this issue.

Limitations of this study include its relatively short duration and the need for extrapolation of fat content data to total body fat. Although the 25(OH)D curve parameters are unlikely to change with a longer study [especially because they are highly concordant with our previous study using treatment durations nearly twice as long (8)], the relationship of serum 25(OH)D to fat stores of the calciferols cannot be fully determined from this two-point design. Hence, a longer study with multiple fat biopsies would be in order. Additionally, for estimation of total body calciferol content, we assumed that sc fat content was representative of total body fat content. Hence, our estimates of the disposition of the administered calciferols must be considered approximations.

Acknowledgments

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Disclosure Summary: R.R.R., R.L.H., and L.A.G.A. have nothing to declare. R.P.H. received grant support from BTR Group, Inc., and J.G. has equity interest in BTR Group, Inc. (manufacturer of Maximum D₃).

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