Vitamin D_2 Is Much Less Effective than Vitamin D_3 in Humans

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Vitamins D_2 and D_3 are generally considered to be equivalent in humans. Nevertheless, physicians commonly report equivocal responses to seemingly large doses of the only high-dose calciferol (vitamin D_2) available in the U.S. market.

The relative potencies of vitamins D_2 and D_3 were evaluated by administering single doses of 50,000 IU of the respective calciferols to 20 healthy male volunteers, following the time course of serum vitamin D and 25-hydroxyvitamin D (25OHD) over a period of 28 d and measuring the area under the curve of the rise in 25OHD above baseline.

The two calciferols produced similar rises in serum concentration of the administered vitamin, indicating equivalent absorption. Both produced similar initial rises in serum 25OHD over the first 3 d, but 25OHD continued to rise in the D_3-treated subjects, peaking at 14 d, whereas serum 25OHD fell rapidly in the D_2-treated subjects and was not different from baseline at 14 d. Area under the curve (AUC) to d 28 was 60.2 ng·d/ml (150.5 nmol·d/liter) for vitamin D_2 and 204.7 (511.8) for vitamin D_3 ($P < 0.002$). Calculated AUC$_\infty$ indicated an even greater differential, with the relative potencies for D_3:D_2 being 9.5:1.

Vitamin D_2 potency is less than one third that of vitamin D_3. Physicians resorting to use of vitamin D_2 should be aware of its markedly lower potency and shorter duration of action relative to vitamin D_3. (*J Clin Endocrinol Metab* 89: 5387–5391, 2004)

V ITAMIN D DEFICIENCY IS a common problem, especially in older and sick individuals (1, 2). Because most people get most of their vitamin D from sun exposure (3) with a small amount obtained from food and supplements, those at risk for vitamin D deficiency are those with little sun exposure and/or poor dietary intake. Older people are especially at risk because aging lowers the amount of 7-dehydrocholesterol in the skin and the capacity for vitamin D production (4).

Hypovitaminosis D is associated with increased PTH secretion, increased bone turnover, osteoporosis, histological osteomalacia and increased risk of hip and other fractures (5, 6), and, in its most severe expression, clinical osteomalacia (5). Vitamin D deficiency is increasingly being recognized by clinicians and treated, but the treatment guidelines are unclear and available preparations limited. The current adult vitamin D intake recommendation from the Food and Nutrition Board (7) is 200 IU/d up to age 50, 400 IU up to age 70, and 600 IU thereafter. However, it now appears that, if total input were confined to these amounts, only the most severe degrees of vitamin D deficiency would be prevented (3). In any event, these recommendations apply to both ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3).

Since the 1930s it has been generally assumed that vitamin D_2 and vitamin D_3 are equally effective in humans. This conclusion was based mainly on anti-rachitic bioassays. With acceptance of serum 25-hydroxyvitamin D concentration as the appropriate functional indicator of vitamin D status (7), it has become important to reevaluate this assumption of equivalence. Only a few studies have directly compared vitamins D_2 and D_3 using contemporary analytic methods. The limited evidence available indicates that vitamin D_3 is substantially more efficacious than vitamin D_2 (8, 9).

Because ergocalciferol is the only high-dose calciferol on the U.S. market, patients who are severely vitamin D deficient have usually been treated in the U.S. with this form of the vitamin, in a dose of 50,000 IU orally or (in the past) im. Dosing frequencies have varied from one or three times weekly to once every 2 months. Physicians frequently find that such a regimen produces little or no change in serum 25-hydroxyvitamin D (25OHD) concentrations (10). Whether this is because of disease-related abnormalities of vitamin D metabolism in such patients, because of problems with the assay measuring serum 25OHD, or because of nonequivalence of ergocalciferol and cholecalciferol (vitamin D_3) has been unclear. Our sole purpose in this study was to evaluate the relative potency of the two calciferols using research-level assay methods.

Subjects and Methods

Subjects

The subjects were 30 men, between ages 20 and 61, in good general health, who habitually consumed less than 16 oz of milk per day and had less than 10 h of sun exposure per week. We excluded those with granulomatous conditions, liver disease, kidney disease, or diabetes and those taking anticonvulsants, barbiturates, or steroids in any form. (There were four subjects who took a multivitamin occasionally, averaging one time per week. They agreed to stop taking this supplement 1 wk before and throughout the study.) Mean ($\pm$ SD) age was 33.06 $\pm$ 11.47, weight was 89.36 $\pm$ 11.59 kg, and body mass index was 27.14 $\pm$ 2.77 kg/m². All subjects were from Omaha, Nebraska, and surrounding communities. The project was approved by the Institutional Review Board of Creighton University, and all subjects gave written informed consent.

Abbreviations: AUC, Area under the curve; DBP, vitamin D-binding protein; 25OHD, 25-hydroxyvitamin D.

5387

Design

The project was conducted during the month of July, 2003. Subjects were randomly assigned to receive 1) no supplement (the seasonal effect, control group), 2) one tablet labeled to contain 50,000 IU (1.25 mg) ergocalciferol (the vitamin D$_2$ group), or 3) 10 tablets labeled to contain 5,000 IU (125 μg)/tablet cholecalciferol (the vitamin D$_3$ group). Because the vitamin D$_3$ preparation was not a marketed product, we asked the supplier to provide a certificate of analysis. [The 50,000-IU D$_2$ tablet preparation was supplied by Sidmak Laboratories, Inc. (High Point, NC). The 5,000-IU D$_3$ tablet preparation was supplied by Tishcon Corp. (Salisbury, MD). The product was assayed on June 12, 2003, and found to contain 5,513 IU/capsule.]

For the control group receiving no vitamin D supplement, serum samples were obtained at d 0 and 28, so as to quantify the midsummer rise in 25OHD that would be expected in all groups. For the two groups receiving a vitamin D supplement, serum samples were obtained at d 0, 1, 3, 5–7, 14, and 28. At the initial visit, each subject's weight and height were measured. Height was measured using a Harpenden stadiometer (Seritex, Inc., Carlstadt, NJ). Blood was obtained for measurement of serum vitamin D and 25OHD. After the baseline blood was obtained, the subjects were observed while they took the assigned vitamin D supplement dose. At each subsequent visit, the subject's weight was measured and blood obtained for measurement of serum vitamin D and 25OHD. The subjects were asked to recall their sun exposure since the previous visit. The subjects were given supplies of sun block lotion, sun protection factor (SPF) 15, to use during out-of-the-ordinary sun exposure.

Analytical methods

Serum ergo- and cholecalciferol concentrations were determined by reversed-phase HPLC, as described elsewhere (11). Serum 25OHD was determined by RIA, using the IDS kit (Nichols Institute, San Clemente, CA). Because it has been reported (12) that the antibody in this kit reacts poorly with 25OHD$_2$, we measured the samples from the vitamin D$_2$-treated subjects using both the IDS and the DiaSorin kits (DiaSorin, Stillwater, MN). However, in this group of subjects, there were no significant differences in analyzed 25OHD increments above baseline between the values produced by the two antibodies. Hence, for the values in the D$_2$-treated participants that we report here, we averaged the results obtained with the two RIAs. Finally, to be certain that the RIAs were adequately detecting both 25OHD$_2$ and 25OHD$_3$, aliquots of the serum samples obtained at 0, 3, and 28 d were assayed by HPLC (10) for both 25OHD$_2$ and 25OHD$_3$. The mean increment in total 25OHD by HPLC at 3 and 28 d was virtually identical with the mean increment measured by RIA.

Statistical methods

The 25OHD signal produced by the 50,000-IU calciferol dose was analyzed as the increment in total 25OHD concentration above baseline, adjusted for the mean rise in serum 25OHD observed in the untreated controls (0.259 nmol/liter·d). Area under the curve (AUC) of serum 25OHD increments at 14 and 28 d was calculated by the trapezoidal method individually for each subject. AUC$_\infty$ was calculated using pharmacokinetic, biexponential models (PK Solutions, Summit Research Services, Ashland, OH) fitted to the mean 25OHD values at each time point. Mean values for AUC$_{14}$ and AUC$_{28}$ for the two calciferols were compared by the usual *t* test for independent samples.

Results

Serum calciferol concentrations were measured at d 0, 1, and 3. The results are shown in Fig. 1. Baseline values of both calciferols were low, with the D$_3$ concentration higher than the D$_2$, as would be expected. However, the rise by d 1 was essentially identical for both calciferols, and at d 3 the serum levels of the two had fallen close to baseline and were virtually identical. This behavior indicates that absorption of the two calciferols was approximately equivalent.

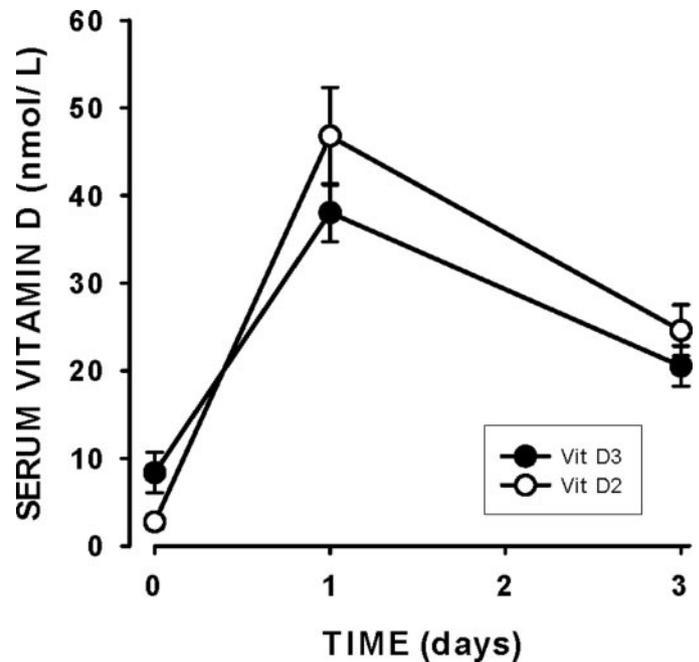The time course for the increment in serum 25OHD is

FIG. 1. Time course of serum concentrations of vitamin D after a single oral dose of 50,000 IU of cholecalciferol (vitamin D$_3$) or ergocalciferol (vitamin D$_2$) in healthy male subjects (n = 10 for each group). The error bars are 1 SEM. [Copyright Robert P. Heaney, 2004. Used with permission.]
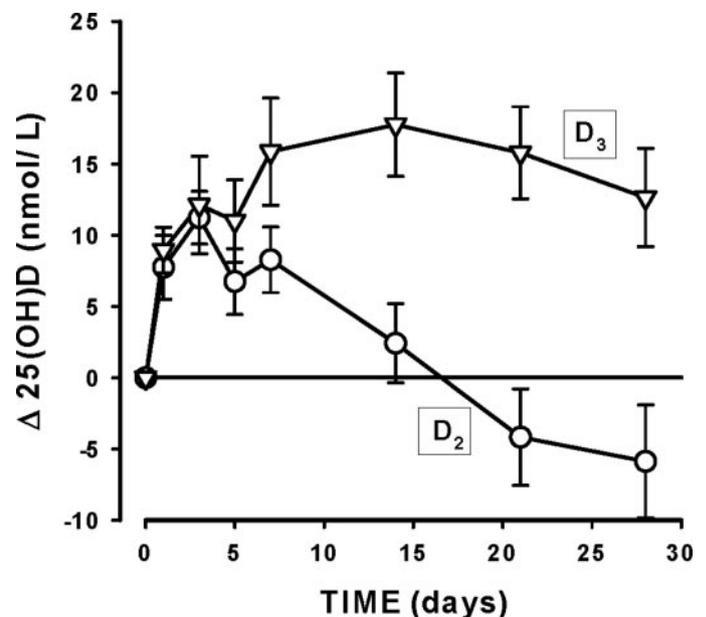

FIG. 2. Time course of the rise in serum 25OHD after a single oral dose of 50,000 IU of either cholecalciferol (vitamin D$_3$) or ergocalciferol (vitamin D$_2$) to two groups of 10 normal men each. Error bars are 1 SEM. The zero-change line incorporates a correction for the seasonal rise in 25OHD occurring at the time this study was performed. (To convert from nmol/liter to ng/ml, multiply by 0.4.) [Copyright Robert P. Heaney, 2004. Used with permission.]

shown in Fig. 2, which presents the mean changes in values at each visit for total 25OHD (*i.e.* the sum of 25OHD$_2$ and 25OHD$_3$). These values were corrected for the change we measured in 25OHD levels in our control population because

of summer sun exposure. Both vitamin D$_2$ and vitamin D$_3$ produced initial rises in 25OHD levels during the first 3 d that did not differ significantly from one another. The mean 25OHD concentration in the D$_2$-treated subjects then began to fall until, by d 14, it was not different from baseline. By contrast, 25OHD concentration in the D$_3$-treated subjects continued to rise through d 14 and by d 28 was still higher than the peak value for the D$_2$-treated group.

The best measure of total exposure of the organism to an administered agent is given by AUC of the serum concentration against time. Here the greater potency of D$_3$ was dramatically evident. AUC$_{28}$ was 60.2 ± 23.4 ng·d/ml (150.5 ± 58.5 nmol·d/liter) for D$_2$ and 204.7 ± 32.4 ng·d/ml (511.8 ± 80.9 nmol·dl) for D$_3$ ($P < 0.002$). This is a more than 3-fold difference in potency. AUC$_\infty$ is actually the preferable pharmacokinetic measure of total exposure, and if AUC$_\infty$ is used instead, the values for D$_2$ and D$_3$ are, respectively, 112.8 and 1072.8 ng·d/ml (282 and 2682 nmol·d/liter), for a nearly 10-fold difference in potency. Because, as it turned out, 28 d was not long enough to get a firm estimate of the elimination phase in the D$_3$-treated subjects, the AUC$_\infty$ for D$_3$ must be considered uncertain. In any event, it is clear that the AUC$_{28}$ values understate the contrast and that the potency difference must lie somewhere between 3- and perhaps 10-fold.

An initially unanticipated finding was the decline in 25OHD$_3$ concentration in the ergocalciferol-treated men, as shown by HPLC (Fig. 3). Whereas 25OHD$_3$ in the untreated

FIG. 3. Changes in serum 25OHD$_3$ in the subjects treated with vitamin D$_2$ and in those in the untreated control group over the 28 d of follow-up after a single oral dose of 50,000 IU vitamin D$_2$. The error bars are 1 SEM. The mean 28-d value in the D$_2$-treated subjects was significantly lower than both their own baseline values and the corresponding values in the control group (which exhibited the expected summer rise in serum 25OHD). (To convert from nmol/liter to ng/ml, multiply by 0.4.) [Copyright Robert P. Heaney, 2004. Used with permission.]

control group rose by 3 ng/ml (7.5 nmol/liter), presumably because of ongoing sun exposure, the vitamin D$_2$-treated group experienced a fall in 25OHD$_3$ of nearly 4 ng/ml (10 nmol/liter) ($P < 0.01$).

Discussion

To our knowledge, this is the first study comparing vitamins D$_3$ and D$_2$ by mapping the time course of serum 25OHD after a single dose. We showed that vitamin D$_3$ raises and maintains 25OHD levels to a substantially greater degree than does vitamin D$_2$, with a differential potency of at least 3-fold, and more likely closer to 10-fold.

The two treated groups had the same baseline 25OHD levels. With the dose of 50,000 IU of vitamin D$_2$ and vitamin D$_3$, the respective vitamin D levels rose in parallel, showing that both forms of vitamin D were absorbed comparably. And the rise in serum 25OHD was virtually the same for the first 3 d of the study for both vitamins D$_2$ and D$_3$, indicating comparable conversion to the 25-hydroxy metabolite. The much more rapid decline of serum 25OHD in the vitamin D$_2$-treated subjects after 3 d would seem to reflect substantially more rapid metabolism or clearance of the vitamin D$_2$ metabolite. Other studies (13, 14) have suggested either differences in affinity of the vitamin D-binding protein (DBP) for the two calciferols or higher affinity of the hepatic 25-hydroxylase for vitamin D$_3$ than vitamin D$_2$. The latter seems improbable from our data, because the initial rise in 25OHD concentration was the same for the two calciferols. The former offers a more plausible explanation. 25OHD$_2$ has been shown to have a lesser affinity for DBP than does 25OHD$_3$ (15), which would result in a shorter circulating half-life for 25OHD$_2$ *vs.* 25OHD$_3$. The relative binding of vitamin D and its metabolites to DBP determines the circulating half-lives of these substances (16). [That is why vitamin D and 1,25(OH)$_2$D possess much shorter circulating half-lives than 25OHD (17). Similarly, the reason birds cannot use vitamin D$_2$ as a feed supplement is because 25OHD$_2$ will not bind to the avian DBP and is thus rapidly eliminated from the circulation (18).]

This study complements the findings of Trang *et al.* (9) who, using daily dosing of 4000 IU for 2 wk, reported an increase in 25OHD 70% greater with vitamin D$_3$ than for vitamin D$_2$. (After adjustment for concomitant changes in the control group, the difference between the two groups can be shown to have been approximately 2-fold.) The reason for the larger differential found in our study is unclear. In any case, both studies show that there is a substantial difference in serum 25OHD achieved by the same dose of the two calciferols.

There can be little doubt that the demonstrated lower potency of vitamin D$_2$ is physiologically/pharmacologically meaningful. Serum 25OHD is the recognized functional status indicator for vitamin D nutrition (7). Recent studies have shown that raising serum 25OHD improves calcium absorption (19), reduces fall frequency (20), and lowers osteoporotic fracture risk (6). Furthermore, lower extremity muscle function improves across virtually the entire range of prevailing serum 25OHD concentrations (21).

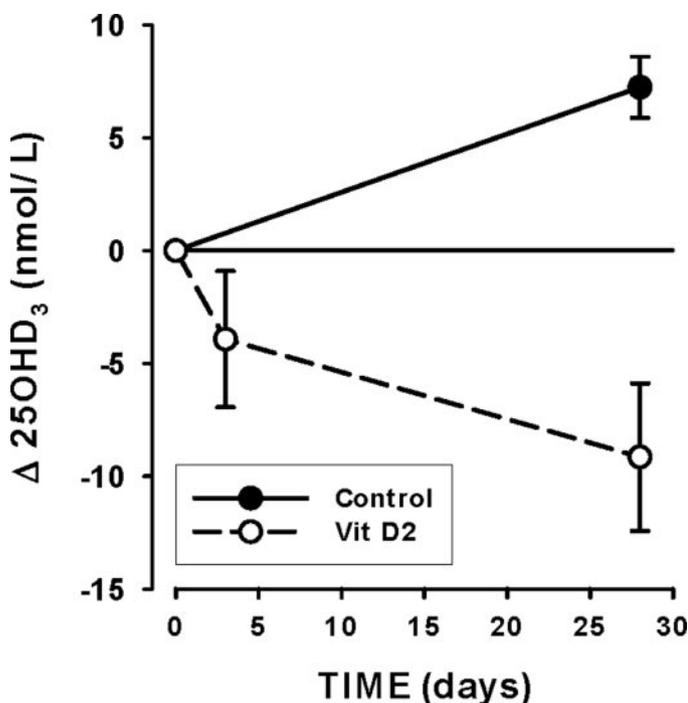It would be desirable to have long-term dosing data as

well, because such information would more closely approximate the situation of actual treatment. However, such information would serve only to define more precisely the magnitude of the difference. It would not be expected to alter the finding of a substantial differential in potency between the two calciferols, because by standard pharmacokinetics, the concentration achieved by multiple doses of a short half-life substance is virtually always lower than the concentration achieved by comparable doses with a long half-life compound. Continuous dosing studies would need to be of several months' duration because Heaney *et al.* (3) have shown, at least for vitamin D$_3$, that time to equilibrium is approximately 5 months. At 14 d of continuous dosing, Tjelleson *et al.* (8) found a potency difference of nearly three times, which, for the reason just given, must understate the differential.

The fall in 25OHD$_3$ that we observed in the D$_2$-treated subjects has been reported previously. Using a design similar to that of Trang *et al.* (9), Tjellesen *et al.* (8) described a nearly 70% drop in 25OHD$_3$ in subjects treated for 2 wk with 4000 IU/d of vitamin D$_2$. This fall may reflect either competition by D$_2$ for the 25-hydroxylase or increased metabolic degradation of 25OHD$_3$ by the mechanisms up-regulated to metabolize vitamin D$_2$ and its metabolites (or both).

It is worth noting in passing that our subjects were all healthy young men with some sun exposure (not homebound as a nursing home resident or elderly person might be). Their mean baseline 25OHD level was 31.7 ng/ml ± 8.45 (79.19 nmol/liter ± 21.13), nearly at the optimal level of 32 ng/ml (80 nmol/liter) or higher [where calcium absorption plateaus and PTH levels become minimal (22)]. Even so, individual baseline 25OHD levels ranged from 15.2–58.7 ng/ml (37.9–146.8 nmol/liter). Thus, approximately half of the subjects, who would not usually be considered at risk for vitamin D deficiency, nevertheless exhibited suboptimal vitamin D status during the summer. Presumably, their vitamin D levels would be even lower at midwinter. The finding of suboptimal vitamin D levels in those without obvious risk is consistent with other studies that report high prevalence of vitamin D deficiency in general medicine patients at no particular risk for vitamin D deficiency (23).

It is important to note that even in those subjects with high baseline serum 25OHD values, one large dose of vitamin D$_3$ produces serum 25OHD values well within the safe range of 25OHD [*i.e.* <88 ng/ml (220 nmol/liter) (1)]. The mean rise was only approximately 7 ng/ml (~18 nmol/liter), and the highest observed 25OHD rise was 10.4 ng/ml (26 nmol/liter); the latter produced a value of 69.2 ng/ml (173 nmol/liter) and occurred in the subject with the highest starting value.

As the medical community is becoming more aware of vitamin D deficiency and its effects, both on bones and other body tissues (24–26), there will be more testing of vitamin D levels and interest in treating the deficiency. The goal should be standardized methods of testing and clear recommendations on the level of 25OHD that should be achieved and what form of vitamin D to use, in what amount, and how often (27).

This study addresses some of these issues. Clearly, vitamin D$_3$ is the preferable form of vitamin D. This is in contrast to the long-held belief that vitamin D$_2$ and vitamin D$_3$ are seen by the body as identical. This nonequivalence makes sense, because the two calciferols are known not to be equivalent in other species, and vitamin D$_3$ is the form that animals make in response to sunlight.

There are several barriers to the clinician in treating vitamin D-deficient patients. Most published studies were performed using vitamin D$_3$, and application of their results to patients using vitamin D$_2$ is not easily possible, as we have shown here. This is not to suggest that vitamin D$_2$, in the 50,000-IU dosage form, is not efficacious in treating severe vitamin D deficiency. A large body of experience indicates that it can be quite effective. But, as the unitage of the two forms of the vitamin is clearly not equivalent, thinking about dosing must be adjusted to match the product used. The data presented in this paper indicate that the 50,000-IU dosage form of vitamin D$_2$ should be considered to be equivalent to no more than 15,000 IU of vitamin D$_3$ and perhaps closer to only 5,000 IU. In any event, the tolerable upper intake level, 2,000 IU/d, published for vitamin D$_3$ (7), and already judged to be set too low (3), ought not be applied to vitamin D$_2$.

Another barrier is the lack of a high-potency therapeutic vitamin D$_3$ preparation in the United States. In Europe, several high-potency preparations are available, some used for "stoss" therapy in clinical trials (6, 28, 29). With the vitamin D$_3$ that is available mainly by special order in the United States, it would require 25–50 pills (of 1,000 or 2,000 IU each) to achieve a 50,000-IU dose, a regimen that would not be practical or acceptable to most patients.

More needs to be done both to standardize methods of testing 25OHD and to provide a high-potency vitamin D$_3$ preparation available for clinical use (27). Additional studies are also needed to establish optimal dosing recommendations.

Acknowledgments

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