Vitamin D2 Is as Effective as Vitamin D3 in Maintaining Circulating Concentrations of 25-Hydroxyvitamin D

Michael F. Holick, Rachael M. Biancuzzo, Tai C. Chen, Ellen K. Klein, Azzie Young, Douglass Bibuld, Richard Reitz, Wael Salameh, Allen Ameri, and Andrew D. Tannenbaum

Endocrine Section (M.F.H., R.M.B., T.C.C., E.K.K., A.A., A.D.T.), Department of Medicine, Boston University School of Medicine, Boston, Massachusetts 02118; Mattapan Community Health Center (A.Y., D.B.), Mattapan, Massachusetts 02126; and Quest Diagnostics Nichols Institute (R.R., W.S.), San Juan Capistrano, California 92675

Context: Two reports suggested that vitamin D2 is less effective than vitamin D3 in maintaining vitamin D status.

Objective: Our objective was to determine whether vitamin D2 was less effective than vitamin D3 in maintaining serum 25-hydroxyvitamin D levels or increased the catabolism of 25-hydroxyvitamin D3.

Subjects and Design: This was a randomized, placebo-controlled, double-blinded study of healthy adults ages 18–84 yr who received placebo, 1000 IU vitamin D3, 1000 IU vitamin D2, or 500 IU vitamin D2 plus 500 IU vitamin D3 daily for 11 wk at the end of the winter.

Results: Sixty percent of the healthy adults were vitamin D deficient at the start of the study. The circulating levels of 25-hydroxyvitamin D (mean ± SD) increased to the same extent in the groups that received 1000 IU daily as vitamin D2 (baseline 16.9 ± 10.5 ng/ml; 11 wk 26.8 ± 9.6 ng/ml), vitamin D3 (baseline 19.6 ± 11.1 ng/ml; 11 wk 28.9 ± 11.0 ng/ml), or a combination of 500 IU vitamin D2 and 500 IU vitamin D3 (baseline 20.2 ± 10.4 ng/ml; 11 wk 28.4 ± 7.7 ng/ml). The 25-hydroxyvitamin D3 levels did not change in the group that received 1000 IU vitamin D2 daily. The 1000 IU dose of vitamin D2 or vitamin D3 did not raise 25-hydroxyvitamin D levels in vitamin D-deficient subjects above 30 ng/ml.

Conclusion: A 1000 IU dose of vitamin D2 daily was as effective as 1000 IU vitamin D3 in maintaining serum 25-hydroxyvitamin D levels and did not negatively influence serum 25-hydroxyvitamin D3 levels. Therefore, vitamin D2 is equally as effective as vitamin D3 in maintaining 25-hydroxyvitamin D status. (J Clin Endocrinol Metab 93: 677–681, 2008)

Vitamin D2, which comes from the UV irradiation of ergosterol obtained from yeast, has been the mainstay for the prevention and treatment of vitamin D deficiency in children and adults for more than 80 yr (1, 2). As little as 100 IU vitamin D2 was found to be effective in the prevention of rickets (2–4). When humans are exposed to sunlight, 7-dehydrocholesterol in the skin absorbs UVB (290–315 nm) radiation resulting in the production of vitamin D3 (1, 3). Vitamin D3 is found naturally in cod liver oil and oily fish such as salmon (1, 3). Vitamin D3 is also made by irradiating 7-dehydrocholesterol obtained from lanolin from sheep’s wool with UVB radiation (1). Both vitamin D2 and vitamin D3 when ingested undergo metabolism in the liver to form 25-hydroxyvitamin D (25(OH)D; D represents either D2 or D3) and in the kidneys to 1,25-dihydroxyvitamin D (1, 3, 5, 6). Both vitamin D2 and vitamin D3 are available in supplements, but only vitamin D3 is available as a pharmaceutical preparation because its use predated the Food and Drug Administration and, thus, was grandfathered as a pharmaceutical drug. Vitamin D3 was commercially developed in the 1950s and has not been approved as a pharmaceutical agent in the United States but is used in food supplementation and vitamin supplements.

Since the 1930s, vitamin D2 has been considered to be equally
as effective as vitamin D₃ for bone health (3, 7). Recently, it was suggested that vitamin D₂ was less effective than vitamin D₃ in maintaining serum 25(OH)D levels when given either as 4000 IU/d for 2 wk (8) or as a single dose of 50,000 IU (9). Furthermore, it was observed that when a single dose of 50,000 IU vitamin D₂ was given to healthy adults that the serum 25(OH)D levels decreased more rapidly than the placebo group, suggesting that vitamin D₂ not only was less effective in maintaining serum 25(OH)D levels but also enhanced the degradation of 25(OH)D₃ (9).

These two observations have led to the conclusion that vitamin D₂ is approximately 30–50% as effective as vitamin D₃ in maintaining serum 25(OH)D in humans (8, 9). Our purpose was to evaluate in healthy adults what the effect of ingesting 1000 IU vitamin D₂, 1000 IU vitamin D₃, or a combination of 500 IU vitamin D₂ and 500 IU vitamin D₃ daily for 11 wk at the end of the winter had on circulating levels of total 25(OH)D as well as 25(OH)D₂ and 25(OH)D₃.

### Subjects and Methods

**Subjects**

Healthy, white, African-American, Hispanic, Asian, and Native American adults between the ages of 18 and 84 yr were enrolled in February 2007 after signing a consent form approved by our Institutional Review Board at Boston University Medical Center. We excluded those with chronic liver and kidney disease and those taking medications, including anticonvulsants, glucocorticoids, and barbiturates, that might affect vitamin D metabolism as well as subjects who were taking a vitamin D supplement. Subjects were permitted to take their multivitamin, a majority of which contained 400 IU vitamin D₃ (Table 1).

#### Design

Sixty-eight subjects were randomly assigned in a double-blinded fashion to receive daily in a capsule for 11 wk 1) placebo, 2) 1000 IU (25 µg) vitamin D₂ (ergocalciferol), 3) 1000 IU (25 µg) vitamin D₃ (cholecalciferol), or 4) 500 IU vitamin D₂ plus 500 IU vitamin D₃. All of the capsules made by Tishcon Corp. (Salisbury, MD) contained lactose (98.75%), magnesium stearate (1.0%), and silicon dioxide (1.25%). All of the products were analyzed in our laboratory by HPLC and found to contain either no vitamin D (placebo) or concentrations within 10% of their specified content. All subjects had blood samples collected at baseline and every week for a total of 11 wk. Each subject was given a dietary questionnaire at baseline to assess multivitamin and milk consumption. Pill compliance (Table 1) was determined by a pill count at each visit.

#### Analytical methods

Serum 25(OH)D₂ and 25(OH)D₃ were determined by liquid chromatography tandem mass spectroscopy at Quest Diagnostics Nichols Institute, San Juan Capistrano, CA (10). The detection limit for the assay was 4 ng/ml, and the interassay coefficient of variation was about 10%. Values for serum 25(OH)D₂ reported as less than 4 ng/ml were obtained by subtracting 25(OH)D₃ from the total 25(OH)D.

#### Statistical methods

The results are presented as means ± SD. Data were analyzed using mixed-effects regression to perform a repeated-measures analysis of 25(OH)D levels across time and groups. Pairwise comparisons were

### Table 1. Subject demographics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Placebo group (n = 14)</th>
<th>D₂+D₃ group (n = 18)</th>
<th>D₃ group (n = 20)</th>
<th>D₂ group (n = 16)</th>
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<tbody>
<tr>
<td>Mean ± SD (ng/ml)</td>
<td>40.5 ± 11.7</td>
<td>35.5 ± 14.6</td>
<td>40.0 ± 18.0</td>
<td>38.4 ± 12.0</td>
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<td>18–70</td>
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<td>18–59</td>
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<td>13 (72.2)</td>
<td>12 (65)</td>
<td>10 (62.5)</td>
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<td>Females, n (%)</td>
<td>3 (21.4)</td>
<td>5 (27.8)</td>
<td>7 (35)</td>
<td>6 (37.5)</td>
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<td>Males, n (%)</td>
<td>29.3</td>
<td>31.7</td>
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<td>Body mass index</td>
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<td>5 (27.8)</td>
<td>7 (35)</td>
<td>6 (37.5)</td>
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<td>Multivitamin, n (%)</td>
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<td>5 (27.8)</td>
<td>7 (35)</td>
<td>6 (37.5)</td>
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<td>Multivitamin-D₂, n (%)</td>
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<td>3 (16.7)</td>
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<td>Vitamin D supplement intake</td>
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<td>Mean initial 25(OH)D ± SD (ng/ml)</td>
<td>18.6 ± 8.9</td>
<td>20.2 ± 10.4</td>
<td>19.6 ± 11.1</td>
<td>16.9 ± 10.5</td>
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<tr>
<td>Mean final 25(OH)D ± SD (ng/ml)</td>
<td>18.8 ± 7.9</td>
<td>28.4 ± 7.7</td>
<td>28.9 ± 11.0</td>
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<td>Mean differences ± SD</td>
<td>0.2 ± 5.3</td>
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<td>8 (40)</td>
<td>9 (56.3)</td>
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<td>6 (42.9)</td>
<td>6 (33.3)</td>
<td>6 (30)</td>
<td>4 (25)</td>
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</tbody>
</table>

CI, Confidence interval.

a = P = 0.041 for D₂+D₃ vs. placebo.
b = P = 0.027 for D₃ vs. placebo.
c = P = 0.023 for D₂ vs. placebo.
d No statistically significant difference between D₂+D₃, D₃, and D₂ (P = 0.957).

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performed between all treatment groups as well as each treatment group vs. placebo. Interactions between treatment group and time compared the linear change in 25(OH)D over time between the groups. A repeated-measures mixed-effect model also compared the 25(OH)D$_2$ and 25(OH)D$_3$ across visits for each of the treatment groups. Statistical analysis was performed using SAS (SAS Institute, Inc., Cary, NC).

**Results**

Sixty percent of our healthy adult subjects were vitamin D deficient [25(OH)D < 20 ng/ml], and 87% were insufficient [25(OH)D < 30 ng/ml], even though about 29% took a multivitamin daily that contained 400 IU vitamin D and about 47% drank about 1.2 glasses of milk per day. Adults who received the placebo capsule daily for 3 months demonstrated no significant change in their total 25(OH)D levels during the winter and early spring (Fig. 1). Adults who ingested 1000 IU vitamin D$_2$/d gradually increased their total 25(OH)D levels from 16.9 ± 10.5 ng/ml to 25.8 ± 6.6 ng/ml during the first 6 wk and then remained stable (Fig. 1). Adults who ingested 1000 IU vitamin D$_3$ had a baseline 25(OH)D of 19.6 ± 11.1 ng/ml that was statistically no different from the baselines of either the placebo group or the groups that took 1000 IU vitamin D$_2$/d or 500 IU vitamin D$_2$ plus 500 IU vitamin D$_3$/d (P = 0.79). The vitamin D$_3$ group increased their serum 25(OH)D$_3$ levels similar to that of the group that ingested vitamin D$_2$. The 25(OH)D$_2$ levels in the vitamin D$_3$ group began to plateau by wk 6 and was 28.9 ± 11.0 ng/ml at the end of the study, which was not statistically different from the vitamin D$_2$ group (26.8 ± 9.6 ng/ml) (Fig. 1).

To determine whether vitamin D$_2$ ingestion had any effect on circulating levels of 25(OH)D$_3$, we determined 25(OH)D$_2$ and 25(OH)D$_3$ in the samples. The 25(OH)D$_2$ levels increased from undetectable (<4 ng/ml) to 14 ± 5.3 ng/ml by wk 6 and remained at approximately 14 ng/ml for the ensuing 5 wk in the group that received 1000 IU vitamin D$_2$ (Fig. 2A). The baseline 25(OH)D$_3$ level in the same subjects was 15.1 ± 9.8 ng/ml and did not significantly change during the entire study and was 13.6 ± 10.2 ng/ml at the end of the study (P = 0.14) (Fig. 2A). Similarly, the group that received vitamin D$_3$ showed no significant change in the serum 25(OH)D$_2$ throughout the study (P = 0.33) (Fig. 2B).

To determine further whether vitamin D$_2$ interfered with vitamin D$_3$ metabolism, we gave one group of subjects 500 IU vitamin D$_2$ mixed with 500 IU vitamin D$_3$. The rise in the total 25(OH)D was identical to that observed for the groups who received either 1000 IU vitamin D$_2$ or 1000 IU vitamin D$_3$. 

![FIG. 1. Mean (± SEM) serum 25(OH)D levels after oral administration of vitamin D$_2$ and/or vitamin D$_3$. Healthy adults recruited at the end of the winter received placebo (•), 1000 IU vitamin D$_2$ (D$_2$, n = 20), 500 IU vitamin D$_2$ plus 500 IU vitamin D$_3$ (D$_2$ + D$_3$, n = 18) daily for 11 wk. The total 25(OH)D levels are demonstrated over time. *, P = 0.027 comparing 25(OH)D$_2$ over time between vitamin D$_2$ and placebo; **, P = 0.014 comparing 25(OH)D$_3$ over time between vitamin D$_2$ and placebo; ***, P = 0.023 comparing 25(OH)D$_2$ over time between vitamin D$_2$ and placebo.](image1)

![FIG. 2. Effect of vitamin D$_2$ or vitamin D$_3$ on serum 25(OH)D$_2$ and 25(OH)D$_3$ levels. Serum levels of 25(OH)D$_2$ (•) and serum 25(OH)D$_3$ (■) were measured in healthy subjects receiving 1000 IU vitamin D$_2$ (A), 1000 IU vitamin D$_3$ (B), or 500 IU vitamin D$_2$ plus 500 IU vitamin D$_3$ (C) daily for 11 wk. Results are presented as means ± SEM over time. *, P < 0.0001 comparing 25(OH)D$_2$ between baseline and 11 wk (A); *, P < 0.0001 comparing 25(OH)D$_3$ between baseline and 11 wk (B); *, P = 0.0014 comparing between 25(OH)D$_3$ and placebo group (C); **, P = 0.0031 comparing 25(OH)D$_2$ and placebo group (C). Note serum 25(OH)D$_2$ levels less than 4 ng/ml were obtained by subtracting the total 25(OH)D$_3$ from the total 25(OH)D levels.](image2)
daily, and the total 25(OH)D levels at the end of the study were no different in all three groups \( (P = 0.957) \) (Fig. 1). An analysis of the 25(OH)D\(_2\) and 25(OH)D\(_3\) also demonstrated a comparable increase in both levels in the group that received the combination of 500 IU vitamin D\(_2\) (5.7 ± 4.5 ng/ml) and 500 IU vitamin D\(_3\) (6.1 ± 4.3 ng/ml) (Fig. 2C).

**Discussion**

Many multivitamin preparations and some foods are fortified with vitamin D\(_2\). Two recent observations have raised questions as to whether vitamin D\(_2\) should be used either as a pharmaceutical agent or as a supplement because it appeared that vitamin D\(_2\) not only was less effective than vitamin D\(_3\) in maintaining 25(OH)D levels (8, 9) but that it also had a negative effect on 25(OH)D status (9). There has also been concern that vitamin D\(_2\) may not be bioequivalent to vitamin D\(_3\) in maintaining bone health (11).

The Food and Nutrition Board has recommended that adults up to the age of 50 yr require 200 IU vitamin D/d, whereas adults 51–70 yr and 71 yr and older require 400 and 600 IU/d, respectively (12). However, many experts now agree that in the absence of adequate sun exposure, at least 1000 IU vitamin D/d is required to maintain 25(OH)D in the sufficient range (1, 13).

Because the placebo group did not demonstrate any change in their circulating levels of 25(OH)D, there was little influence of environmental sun exposure or dietary or supplemental vitamin D intake on their vitamin D status. Subjects who received 1000 IU vitamin D\(_2\) or 1000 IU vitamin D\(_3\) daily gradually increased blood levels of 25(OH)D to the same levels throughout the study. The increase from baseline in the total 25(OH)D levels at the end of the study was 9.3 ng/ml for the vitamin D\(_3\) group, 9.9 ng/ml for the vitamin D\(_2\) group, and 8.2 ng/ml for the vitamin D\(_2\) plus vitamin D\(_3\) group, which is consistent with the observation that serum 25(OH)D levels increased by 1 ng/ml for every 100 IU vitamin D\(_3\) (14). However, the 25(OH)D levels did not rise above 30 ng/ml, which is now considered to be the vitamin D-sufficient range, suggesting that more than 1000 IU vitamin D\(_2\) or vitamin D\(_3\) is necessary to maintain serum 25(OH)D levels above 30 ng/ml when the sun provides no vitamin D\(_3\).

Armas et al. (9) reported that a single dose of 50,000 IU vitamin D\(_3\) was less effective than 50,000 IU vitamin D\(_3\) in maintaining serum 25(OH)D levels over the ensuing 30 d in the summer. Furthermore, when compared with the group that received placebo, the group that received 50,000 IU vitamin D\(_3\) had a significant reduction in serum 25(OH)D at the end of the study. We did not observe any negative influence of vitamin D\(_2\) on either total 25(OH)D or 25(OH)D\(_3\) levels (Figs. 1 and 2). The maintenance of the serum 25(OH)D\(_3\) levels observed in this report (Fig. 1) was most likely due to the release of vitamin D\(_3\) stored in the body fat because skin synthesis of vitamin D\(_3\) does not occur during the winter in Boston (1). It is possible that a single pharmacological dose of vitamin D\(_2\) enhanced the destruction of both vitamin D\(_2\) and vitamin D\(_3\) and their 25-hydroxy derivatives. However, when 50,000 IU vitamin D\(_2\) was given weekly for 8 wk (15) or twice a week for 5 wk (16), there was an average a 100% increase in serum 25(OH)D levels (15) and a significant increase in bone mineral density in both the hip and spine (16). Thus, vitamin D\(_2\) when given in pharmacological doses is effective in maintaining serum 25(OH)D levels and is beneficial for skeletal health (16). Why Trang et al. (8) observed that the daily dosing of 4000 IU vitamin D\(_2\) for 2 wk was 1.7 times more effective in raising blood levels of 25(OH)D (increased 9.0 ± 2 ng/ml) than 4000 IU vitamin D\(_2\)/d (increased 4.2 ± 2 ng/ml) is unclear at this time. The rise in serum 25(OH)D\(_3\) was only about 20% of what would have been expected for a 4000 IU dose, i.e. 40 ng/ml. This may be due to their ethanol formulation. This could also be due to the short time course because we observed that 25(OH)D levels did not begin to plateau until 6 wk. When vitamin D\(_2\) was combined with vitamin D\(_3\), there was no significant difference in the rise in 25(OH)D (Fig. 1). Furthermore, the group that received 1000 IU vitamin D\(_3\) had no significant change in the level of 25(OH)D\(_3\), suggesting that vitamin D\(_2\) at least at 1000 IU/d had no influence on the catabolism of vitamin D\(_3\) or 25(OH)D\(_3\). Thus, 1000 IU vitamin D\(_2\)/d is as effective as vitamin D\(_3\) in maintaining 25(OH)D status. These observations are consistent with those of Markesstedt et al. (17) and Rapuri et al. (18) who observed that vitamin D\(_2\) and vitamin D\(_3\) contributed equally to serum 25(OH)D\(_3\) levels in mothers and their neonates and elderly women, respectively. Furthermore, the concentrations of 1,25-dihydroxyvitamin D\(_2\) and 1,25-dihydroxyvitamin D\(_3\) were reported to be proportional to the distribution of 25(OH)D\(_2\) and 25(OH)D\(_3\) (19, 20), implying that the 25(OH)D-1-hydroxylase (CYP27B-1) recognized 25(OH)D\(_2\) equally as well as 25(OH)D\(_3\). Therefore, collectively, these data and our results suggest that vitamin D\(_2\) is as effective as vitamin D\(_3\) in sustaining both 25(OH)D and 1,25(OH)\(_2\)D\(_3\) levels (19, 20) and improving bone health (16). More studies are needed to determine whether the carrier (i.e. ethanol vs. oil vs. lactose) that vitamin D\(_2\) and vitamin D\(_3\) are dissolved in influence either their bioavailability or catabolism. Our observations also suggest that 1000 IU vitamin D\(_2\) or vitamin D\(_3\) is required to sustain blood levels of 25(OH)D above a mean of 20 ng/ml but was insufficient in raising the levels above a mean of 30 ng/ml.

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Address all correspondence and requests for reprints to: Michael F. Holick, Boston University School of Medicine, 715 Albany Street, M-1013, Boston, Massachusetts 02118. E-mail: mfholick@bu.edu.

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References