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**VITAMIN D₂ IS AS EFFECTIVE AS VITAMIN D₃ IN MAINTAINING
CIRCULATING CONCENTRATIONS OF 25-HYDROXYVITAMIN D**

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Disclosure:

Michael F. Holick is on the Speaker's Bureau for Merck, Proctor and Gamble, Eli Lilly, and a consultant for Amgen, Novartis, Quest Diagnostics, P&G and Merck.

Rachael M. Biancuzzo, Tai C. Chen, Ellen K. Klein, Azzie Young, Douglass Bibuld, Wael Salameh, Allen Ameri, and Andrew D. Tannenbaum have nothing to declare.

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ABSTRACT

Context

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

Objective

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 years who received either placebo, 1,000 IU of vitamin D₃, 1,000 IU of vitamin D₂, or 500 IU of vitamin D₂ plus 500 IU of vitamin D₃ daily for 11 weeks 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 \pm SD) increased to the same extent in the groups that received 1,000 IU daily as vitamin D₂ (baseline 16.9 ± 10.5 ng/ml; 11 weeks 26.8 ± 9.6 ng/ml); vitamin D₃ (baseline 19.6 ± 11.1 ng/ml; 11 weeks 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 weeks 28.4 ± 7.7 ng/ml). The 25-hydroxyvitamin D₃ levels did not change in the group that received 1,000 IU vitamin D₂ daily. 1,000 IU of

vitamin D₂ or vitamin D₃ did not raise 25-hydroxyvitamin D levels in vitamin D deficient subjects above 30 ng/ml.

Conclusion

1,000 IU of vitamin D₂ daily was as effective as 1,000 IU of 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.

Introduction

Vitamin D₂, which comes from the ultraviolet irradiation of ergosterol obtained from yeast, has been the main stay for the prevention and treatment of vitamin D deficiency in children and adults for more than 80 years (1, 2). As little as 100 IU of 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 ultraviolet B (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 D₂ 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 1950's, and has not been approved as a pharmaceutical agent in the United States, but is used in food supplementation and vitamin supplements.

Since the 1930's, 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 4,000 IU/d for two weeks (8) or as a single dose of 50,000 IU (9). Furthermore, it was observed that when a single dose of 50,000 IU of 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 was not only 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 1,000 IU of vitamin D₂, 1,000 IU vitamin D₃, or a combination of 500 IU vitamin D₂ and 500 IU vitamin D₃ daily for 11 weeks 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 18-84 years 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 weeks 1) placebo, 2) 1,000 IU (25 micrograms) vitamin D₂ (ergocalciferol), 3) 1,000 IU (25 micrograms) vitamin D₃ (cholecalciferol), or 4) 500 IU of 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 high performance liquid chromatography 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 eleven weeks. 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 ~10%. Values for serum 25(OH)D₂ reported as < 4 ng/ml were obtained by subtracting 25(OH)D₃ from the total 25(OH)D.

Statistical Methods

The results are presented as means \pm SDs. Data was analyzed using mixed effects regression to perform a repeated measures analysis of 25(OH)D levels across time and

groups. Pairwise comparisons were performed between all treatment groups as well as each treatment group versus 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)D₂ and 25(OH)D₃ across visits for each of the treatment groups. Statistical analysis was performed using SAS (SAS Institute, Inc.).

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 ~29% took a multivitamin/d that contained 400 IU vitamin D and ~47% drank ~1.2 glasses of milk/d. Adults who received the placebo capsule daily for three months demonstrated no significant change in their total 25(OH)D levels during the winter and early spring (Fig. 1). Adults who ingested 1,000 IU vitamin D₂/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 six weeks then remained stable (Fig. 1). Adults who ingested 1,000 IU of vitamin D₃ 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 1,000 IU of vitamin D₂/d or 500 IU vitamin D₂ plus 500 IU vitamin D₃/d (P=0.79). The vitamin D₃ group increased their serum 25(OH)D levels similar to that of the group that ingested vitamin D₂. The 25(OH)D levels in the vitamin D₃ group began to plateau by week 6 and was 28.9 ± 11.0 ng/ml at the end of the study which was not statistically different from the vitamin D₂ group (26.8 ± 9.6 ng/ml) (Fig. 1).

To determine whether vitamin D₂ ingestion had any effect on circulating levels of 25(OH)D₃, we determined 25(OH)D₂ and 25(OH)D₃ in the samples. The 25(OH)D₂ levels increased from undetectable (<4 ng/ml) to 14 ± 5.3 ng/ml by week 6 and remained at approximately 14 ng/ml for the ensuing five weeks in the group that received 1,000 IU vitamin D₂ (Fig. 2A). The baseline 25(OH)D₃ 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 D₃ showed no significant change in the serum 25(OH)D₂ throughout the study (P = 0.33) (Fig. 2B).

To further determine whether vitamin D₂ interfered with vitamin D₃ metabolism, we gave one group of subjects 500 IU of vitamin D₂ mixed with 500 IU of vitamin D₃. The rise in the total 25(OH)D was identical to that observed for the groups who received either 1,000 IU of vitamin D₂ or 1,000 IU of vitamin D₃ 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)D₂ and 25(OH)D₃ also demonstrated a comparable increase in both levels in the group that received the combination of 500 IU vitamin D₂ (5.7 ± 4.5 ng/ml) and 500 IU vitamin D₃ (6.1 ± 4.3 ng/ml) (Fig. 2C).

Discussion

Many multivitamin preparations and some foods are fortified with vitamin D₂. Two recent observations have raised questions as to whether vitamin D₂ should be used either as a pharmaceutical agent or as a supplement since it appeared that vitamin D₂ was not only less effective than vitamin D₃ 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 D₂ may not be bioequivalent to vitamin D₃ in maintaining bone health (13).

The Food and Nutrition Board has recommended that adults up to the age of 50 require 200 IU of vitamin D/d whereas adults 51-70 years and 71+ years require 400 and 600 IU/d respectively (11). However, many experts now agree that in the absence of adequate sun exposure at least 1,000 IU of vitamin D/d is required to maintain 25(OH)D in the sufficient range (1, 12).

Since the placebo group did not demonstrate any change in their circulating levels of 25(OH)D, there was little influence of environmental sun exposure, dietary or supplemental vitamin D intake on their vitamin D status. Subjects who received 1,000 IU of vitamin D₂ or 1,000 IU vitamin D₃ 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 D₃, 9.9 ng/ml for the vitamin D₂ group and 8.2 ng/ml for the vitamin D₂ plus vitamin D₃ which is consistent with the observation that serum 25(OH)D levels increased by 1 ng/ml for every 100 IU of vitamin D₃ (14). However, the 25(OH)D levels did not rise above 30 ng/ml which is now considered to be vitamin D sufficient range suggesting that more than 1,000 IU of vitamin D₂ or vitamin D₃ is necessary to maintain serum 25(OH)D levels above 30 ng/ml when the sun provides no vitamin D₃.

Armas et al (9) reported that a single dose of 50,000 IU of vitamin D₂ was less effective than 50,000 IU of vitamin D₃ in maintaining serum 25(OH)D levels over the ensuing 30 days in the summer. Furthermore, when compared to the group that received placebo, the group that received 50,000 IU of vitamin D₂ had a significant reduction in

serum 25(OH)D at the end of the study. We did not observe any negative influence of vitamin D₂ on either total 25(OH)D or 25(OH)D₃ levels (Figs. 1,2). The maintenance of the serum 25(OH)D₃ levels observed in this report (Fig. 1) was most likely due to the release of vitamin D₃ stored in the body fat since skin synthesis of vitamin D₃ does not occur during the winter in Boston (1). It is possible that a single pharmacologic dose of vitamin D₂ enhanced the destruction of both vitamin D₂ and vitamin D₃ and their 25-hydroxy derivatives. However, when 50,000 IU of vitamin D₂ was given weekly for 8 weeks (15) or twice a week for 5 weeks (16), there was on 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 D₂ when given in pharmacologic 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 4,000 IU of vitamin D₃ for two weeks was 1.7 times more effective in raising blood levels of 25(OH)D (increased 9.0 ± 2 ng/ml) than 4,000 IU of vitamin D₂/d (increased 4.2 ± 2 ng/ml) is unclear at this time. The rise in serum 25(OH)D₃ was only about 20% of what would have been expected for a 4,000 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 since we observed that 25(OH)D levels did not begin to plateau until six weeks. When vitamin D₂ was combined with vitamin D₃, there was no significant difference in the rise in 25(OH)D (Fig. 1). Furthermore, the group that received 1,000 IU of vitamin D₂ had no significant change in the level of 25(OH)D₃ suggesting that vitamin D₂ at least at 1,000 IU/d had no influence on the catabolism of vitamin D₃ or 25(OH)D₃. Thus, 1,000 IU of vitamin D₂/d is as effective as vitamin D₃ in maintaining 25(OH)D status. These observations are consistent with those of Markestad

et al (17) and Rapuri et al (18) who observed that vitamin D₂ and vitamin D₃ contributed equally to serum 25(OH)D levels in mothers and their neonates and elderly women, respectively. Furthermore, the concentrations of 1,25-dihydroxyvitamin D₂ and 1,25-dihydroxyvitamin D₃ were reported to be proportional to the distribution of 25(OH)D₂ and 25(OH)D₃ (19, 20) implying that the 25(OH)D-1-hydroxylase (CYP27B-1) recognized 25(OH)D₂ equally as well as 25(OH)D₃. Therefore, collectively these data and our results suggest that vitamin D₂ is as effective as vitamin D₃ in sustaining both 25(OH)D and 1,25(OH)₂D levels (19, 20) and improving bone health (16). More studies are needed to determine whether the media (i.e., ethanol vs oil vs lactose) that vitamin D₂ and vitamin D₃ are dissolved in influence either their bioavailability or catabolism. Our observations also suggest that 1,000 IU of vitamin D₂ or vitamin D₃ 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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FIGURE LEGENDS

Fig. 1. Mean (\pm SEM) serum 25-hydroxyvitamin D levels after oral administration of vitamin D₂ and/or vitamin D₃. Healthy adults recruited at the end of the winter received either placebo [(n = 14; (···●···)], 1,000 IU of vitamin D₃ [D₃, n = 20; (-■-)], 1,000 IU of vitamin D₂ [D₂, n = 16; (-▲-)] or 500 IU of vitamin D₂ and 500 IU of vitamin D₃ [D₂ and D₃, n = 18; (-◆-)] daily for 11 weeks. The total 25-hydroxyvitamin D levels are demonstrated over time. *P = 0.027 comparing 25(OH)D over time between vitamin D₃ and placebo. **P=0.041 comparing 25(OH)D over time between 500 IU vitamin D₃ + 500 IU vitamin D₂ and placebo. ***P=0.023 comparing 25(OH)D over time between vitamin D₂ and placebo.

Fig. 2. Effect of vitamin D₂ or vitamin D₃ on serum 25-hydroxyvitamin D₂ [25(OH)D₂] and 25-hydroxyvitamin D₃ [25(OH)D₃] levels. Serum levels of 25(OH)D₂ (-■-) and serum 25(OH)D₃ (-◆-) were measured in healthy subjects receiving 1,000 IU of vitamin D₂ (Panel A), 1,000 IU of vitamin D₃ (Panel B), or 500 IU of vitamin D₂ + 500 IU of vitamin D₃ (Panel C) daily for 11 weeks. Results are presented as means \pm SEM over time. In Panel A, *P<0.0001 comparing 25(OH)D₂ between baseline and 11 weeks. In Panel B, *P<0.0001 comparing 25(OH)D₃ between baseline and 11 weeks. In Panel C, *P=0.0014 comparing between 25(OH)D₃ and placebo group, **P=0.0031 comparing 25(OH)D₂ and placebo group. Note serum 25(OH)D₂ levels <4 ng/ml were obtained by subtracting the total 25(OH)D₃ from the total 25(OH)D levels.

TABLE 1. Subject Demographics

Characteristics	Placebo Group (N=14)	D ₂ + D ₃ Group (N=18)	D ₃ Group (N=20)	D ₂ Group (N=16)
Age - yr				
Mean ± SD	40.5 ± 11.7	35.5 ± 14.6	40.0 ± 18.0	38.4 ± 12.0
Range	22 - 59	18 - 70	20 - 81	18 - 59
Female sex-no. of subjects (%)	11 (78.6)	13 (72.2)	13 (65)	10 (62.5)
Male sex-no. of subjects (%)	3 (21.4)	5 (27.8)	7 (35)	6 (37.5)
Body-mass index	29.3	31.7	30.0	31.0
Multivitamin-no. of subjects	6 (42.9)	4 (22.2)	5 (25)	6 (37.5)
Multivitamin-D ₃ -no. of subjects (%)	4 (28.6)	3 (16.7)	5 (25)	6 (37.5)
Vitamin D supplement intake	0	0	0	0
Dropout-no. of subjects (%)	4 (20)	2 (10)	0 (0)	4 (20)
Menopausal Status-no. of subjects (%)	3 (21.4)	2 (11.1)	5 (25)	3 (18.8)
Oral Contraceptive Pill Use-no. of subjects (%)	0 (0)	2 (11.1)	1 (5)	0 (0)
Compliance (%)	96.6	95.0	95.3	93.6
Mean Initial 25(OH)D levels (ng/ml) ± SD	18.6 ± 8.9	20.2 ± 10.4	19.6 ± 11.1	16.9 ± 10.5
Mean Final 25(OH)D levels (ng/ml) ± SD	18.8 ± 7.9	28.4 ± 7.7	28.9 ± 11.0	26.8 ± 9.6
Mean Differences ± SD	0.2 ± 5.3	8.2 ± 7.8 ^{†*}	9.3 ± 7.1 ^{††*}	9.9 ± 3.2 ^{†††*}
95% CI	-2.6 - 3.0	4.6 - 11.8	6.2 - 12.7	5.2 - 14.6
Demographics-no. of subjects (%)				
Asian	2 (14.3)	1 (5.6)	4 (20)	1 (6.3)
American Indian	0 (0)	0 (0)	0 (0)	1 (6.3)
Black	6 (42.9)	9 (50)	8 (40)	9 (56.3)
Hispanic	0 (0)	2 (11.1)	2 (10)	1 (6.3)
White	6 (42.9)	6 (33.3)	6 (30)	4 (25)

† indicates a p-value=0.041 for D₃+D₂ vs placebo

†† indicates a p-value=0.027 for D₃ vs placebo

††† indicates a p-value=0.023 for D₂ vs placebo

* indicates no statistically significant difference between D₃+D₂, D₃ and D₂ (p=0.957)



