

## Bioavailability of Vitamin D<sub>2</sub> and D<sub>3</sub> in Healthy Volunteers, a Randomized Placebo-Controlled Trial

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**Background:** The bioequivalence of the different forms of vitamin D, ergocalciferol (vitamin D<sub>2</sub>) and cholecalciferol (vitamin D<sub>3</sub>), has been questioned. Earlier studies have suggested that vitamin D<sub>2</sub> is less biologically active than vitamin D<sub>3</sub>.

**Objective and Design:** In a parallel study, we tested the effects of supplementation with 50- $\mu$ g/d doses of vitamin D<sub>2</sub> or D<sub>3</sub> or a placebo over a period of 8 weeks on 25(OH)D<sub>2</sub>, 25(OH)D<sub>3</sub>, their sum 25(OH)D (primary outcome variables), and PTH in healthy volunteers applying a double-blind, randomized study design. The study was conducted during the winter of 2012 in Halle (Saale), Germany, at latitude 51°47N, when UVB irradiation is virtually absent. Blood samples for the determinations of vitamin D status and PTH were collected at baseline and after 4 and 8 weeks of supplementation.

**Results:** In the placebo group (n = 19), 25(OH)D<sub>3</sub> decreased from 39.4  $\pm$  14.2 to 31.1  $\pm$  12.4 nmol/L after 8 weeks (P < .01). In the vitamin D<sub>3</sub> group (n = 42), the concentrations of 25(OH)D<sub>3</sub> increased from 41.5  $\pm$  22.8 nmol/L at baseline to 88.0  $\pm$  22.1 nmol/L after 8 weeks (P < .01). In the group receiving vitamin D<sub>2</sub> (n = 46), the 25(OH)D<sub>2</sub> concentrations increased significantly, whereas the 25(OH)D<sub>3</sub> concentration fell from 36.4  $\pm$  13.3 nmol/L at baseline to 16.6  $\pm$  6.3 nmol/L after 8 weeks (P < .01). The total 25(OH)D was not different between the groups at baseline but differed significantly between the groups after 4 and 8 weeks (P < .001).

**Conclusions:** Vitamin D<sub>3</sub> increases the total 25(OH)D concentration more than vitamin D<sub>2</sub>. Vitamin D<sub>2</sub> supplementation was associated with a decrease in 25(OH)D<sub>3</sub>, which can explain the different effect on total 25(OH)D. (*J Clin Endocrinol Metab* 98: 4339–4345, 2013)

Vitamin D exists in two different forms: ergocalciferol (vitamin D<sub>2</sub>), which occurs in plants, mainly in mushrooms; and cholecalciferol (vitamin D<sub>3</sub>), which occurs in animals and is also produced in human skin. Vitamins D<sub>2</sub> and D<sub>3</sub> differ only in their side chains. The best dietary sources of vitamin D are fatty fish and products fortified with vitamin D (1, 2). It has been estimated that most of the vitamin D<sub>3</sub> in humans is derived from endogenous synthesis in the epidermis, which contains 7-dehydrocholesterol as a precursor for vitamin D<sub>3</sub>, after irradi-

ation with UVB light at wavelengths of 290–330 nm (3). Although vitamin D<sub>2</sub> is less frequently used in Europe, it is the standard form of fortification and supplementation outside Europe.

Thus, both forms can be found in human blood, as well as the hydroxylated forms 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>.

It has been debated for many years whether the two forms are bioequivalent. A number of studies have shown that vitamin D<sub>2</sub> does not increase the serum total 25(OH)D concentrations to the same extent as vitamin D<sub>3</sub>

(4–6), but this finding has also been questioned by other investigators (7, 8). Because fortification or supplementation with vitamin D is currently the subject of much discussion owing to the widespread occurrence of vitamin D deficiency in nearly all populations investigated (9–19), it is important to know which form is more effective in supplementation and fortification. Although some studies have already shown that serum 25(OH)D<sub>3</sub> is lowered after the administration of vitamin D<sub>2</sub>, either these studies lack sufficient statistical power (5, 20) and a control group (21) and they measured only total 25(OH)D (6, 7), or they were conducted in specific population groups (eg, elderly) (21, 22). Furthermore, it seems that the route of administration (bolus vs daily) may affect the comparison of both vitamin D forms. A recent meta-analysis showed that there was no significant difference in total 25(OH)D after daily administration of either vitamin D<sub>2</sub> or vitamin D<sub>3</sub> (1). In this meta-analysis, studies using 1000–1600 IU of vitamin D<sub>2</sub> or vitamin D<sub>3</sub> were included, but it was also estimated that larger, more robust trials are required that further address this issue.

We therefore conducted a bioavailability study in healthy volunteers who received a placebo—50 μg/d of vitamin D<sub>2</sub> or 50 μg/d of vitamin D<sub>3</sub> (2000 IU/d). The aim was to investigate the effects of this high dose on the serum levels of the hydroxylated forms 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> and on their sum total 25(OH)D. In addition, we investigated PTH concentrations, which are regarded as a functional parameter of vitamin D status (23). The measurability of 25(OH)D<sub>3</sub> serum or plasma levels is superior to that of 1,25(OH)<sub>2</sub>D<sub>3</sub>, owing to the much lower concentrations of 1,25(OH)<sub>2</sub>D<sub>3</sub> and its shorter half-life compared with 25(OH)D<sub>3</sub> (24). The PTH concentrations are higher in the presence of vitamin D deficiency and decline upon supplementation with vitamin D; they can therefore be used as a functional parameter of vitamin D metabolism.

Furthermore, due to the inclusion of a placebo group, we were able to monitor the decrease of 25(OH)D<sub>3</sub> and total 25(OH)D in healthy subjects during wintertime at latitude 51°North.

## Subjects and Methods

### Design

The trial was conducted as a double-blind, randomized study in parallel groups during January, February, and March 2012, when virtually no UVB irradiation is measurable in Halle and the surrounding region. Study visits were scheduled at baseline and after 4 and 8 weeks. The subjects were randomized (stratified for body mass index [BMI] as determined during the screening visit) to receive vitamin D<sub>2</sub> (50 μg/d; n = 46), vitamin D<sub>3</sub> (50 μg/d; n = 42), or placebo (n = 19).

The supplements were manufactured commercially (Zein-Pharma) and were outwardly indistinguishable from one another. The tablets were tested for their vitamin D content after the study by a liquid chromatography, tandem mass spectrometry method in four separate runs, and the content was found to be 54 ± 12 μg for vitamin D<sub>2</sub> and 48 ± 6 μg for vitamin D<sub>3</sub> per tablet.

The participants were issued containers of tablets at baseline and after 4 weeks and were instructed to take one tablet orally per day and to return any remaining tablets at 4 and 8 weeks. The containers were numbered by an investigator with no involvement in the trial. All investigators were unaware of the order of numbering. The participants were enrolled by the physician involved in the trial but were assigned to the intervention by another investigator. Compliance, which was checked by counting the returned tablets, was 97%. During each study visit, a venous blood sample was collected for determination of 25(OH)D<sub>2</sub>, 25(OH)D<sub>3</sub>, their sum 25(OH)D, PTH, and serum calcium. The samples were frozen at –80°C until the time of analysis. The study protocol had been evaluated and approved by the Ethics Committee of the Medical Faculty at the Martin-Luther-University Halle-Wittenberg, and each participant gave his or her written, informed consent before the start of the study. The study was registered at clinicaltrials.gov (NCT01503216).

### Subjects

Participants were recruited through newspaper advertisements, personal contacts, and information in public institutions. During a screening in the autumn (about 2 mo before the start of the study), the participants answered a self-administered questionnaire on their medical history, weight, height, lifestyle (smoking, use of sun blocker-containing cosmetics), and dietary habits relating to food rich in vitamin D. The exclusion criteria were: use of vitamin D and calcium supplements, history of chronic illness and elevated serum creatinine (in females, ≥1.1 mg/dL; in males, ≥1.3 mg/dL), elevated serum calcium, pregnancy or lactation, and vacations in areas with abundant UVB irradiation in the course of the study.

A total of 119 subjects had been recruited for the intervention study (age range, 19–67 y), were finally included in the study, and were randomized by a computer-generated randomization list to the intervention groups with the BMI as the stratification criterion. Participants were randomized into three groups according to their BMI: normal weight (BMI below 25 kg/m<sup>2</sup>), overweight (25–30 kg/m<sup>2</sup>), and obese (above 30 kg/m<sup>2</sup>). Before the start of the intervention, seven subjects (placebo group, n = 1; vitamin D<sub>2</sub> group, n = 3; vitamin D<sub>3</sub> group, n = 3) dropped out. During the study period, five subjects (vitamin D<sub>2</sub> group, n = 1; vitamin D<sub>3</sub> group, n = 4) dropped out for personal reasons. During each visit, the participants were interviewed about any adverse effect. In addition, the calcium concentration in serum was measured in serum obtained at each visit.

After completion of the study, all subjects, including those in the control group, were informed about their vitamin D status and offered vitamin D supplements.

### Analytical methods

Serum concentrations of total 25(OH)D, 25(OH)D<sub>3</sub>, and 25(OH)D<sub>2</sub> were determined by liquid chromatography coupled with mass spectrometry (MassChrom 25-OH Vitamin D<sub>3</sub>/D<sub>2</sub> reagent kit for liquid chromatography, tandem mass spectrom-

**Table 1.** Characteristics of Study Participants at Baseline

	Vitamin D <sub>2</sub> Group	Vitamin D <sub>3</sub> Group	Placebo Group	P (ANOVA)
n	46	42	19	
Age, y	33.2 ± 12.4	35.6 ± 13.5	31.6 ± 9.3	.445
No. of males/females	15/31	16/26	8/11	.745
BMI, kg/m <sup>2</sup>	23.7 ± 3.8	24.0 ± 4.2	23.7 ± 4.9	.928
Systolic blood pressure, mm Hg	121 ± 14	120 ± 15	115 ± 8	.201
Diastolic blood pressure, mm Hg	76 ± 8	76 ± 10	75 ± 6	.894
Creatinine at screening, mg/dL	0.80 ± 0.22	0.86 ± 0.23	0.88 ± 0.24	.298

Data are expressed as mean ± SD.

etry analysis; Chromsystems Instruments and Chemicals GmbH) on an API 2000 system (Applied Biosystems). The coefficient of variation for the determination of 25(OH)D<sub>2</sub> was 3.1% at a concentration of 44.8 nmol/L; for 25(OH)D<sub>3</sub>, it was 5.3% at a concentration of 42.8 nmol/L. Total 25(OH)D was calculated as the sum of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>. The detection limit for both 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> was 2.5 nmol/L, and the limit of quantification was 7.5 nmol/L. However, the measured levels were used for the calculation of total 25(OH)D as the sum of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>, even in subjects with 25(OH)D<sub>2</sub> levels below the limit of quantification.

Intact PTH was measured in the serum by an ELISA (Biomerica Inc). Serum creatinine was determined spectrophotometrically (DiaSys Diagnostic Systems GmbH).

### Statistical analyses

Statistical analyses were performed using PASW version 18.0 (SPSS Inc). All data are expressed in the form of means ± SD, with  $P < .05$  as the significance threshold. The primary outcome variables were the 25(OH)D<sub>2</sub>, 25(OH)D<sub>3</sub>, and total 25(OH)D concentrations. These variables and PTH concentrations are presented in Table 2. Because changes in total 25(OH)D and PTH tend to depend on the baseline level, we used repeated measure analysis to analyze changes upon supplementation. We used the generalized linear models repeated measures procedure in PASW for this analysis. Total 25(OH)D and 25(OH)D<sub>3</sub> at baseline and at 4 and 8 weeks were used as the within-subjects factor, and the supplementation group was used as the between-subjects factor. In addition, post hoc analyses by Scheffé were used to detect differences between single groups. PTH was highly skewed and was therefore analyzed by the nonparametric Kruskal-Wallis test.

In addition, we calculated the absolute change and the percentage change in total 25(OH)D, 25(OH)D<sub>3</sub>, and PTH (8 wk – baseline) and compared these changes among groups by ANOVA (Table 3).

According to a power calculation, 50 subjects per group would be required to show a difference of 10 nmol/L in the mean total 25(OH)D concentration after 8 weeks of supplementation between the vitamin D<sub>2</sub> and D<sub>3</sub> groups (at an assumed standard variation of 15 nmol/L for each group, at a power of 80%, and a significance level of 0.05). Because it was the main aim to compare vitamin D<sub>2</sub> with D<sub>3</sub>, the size of the placebo group was only about half that of the vitamin D groups. Only subjects who finished the study according to protocol were included into the analyses.

### Results

The characteristics of the subjects are presented in Table 1. The average total 25(OH)D concentration at baseline in January was 40.2 ± 18.0 nmol/L, indicating a high degree of suboptimal vitamin D status in these healthy, young volunteers, with no significant differences between the groups. The total 25(OH)D concentration increased significantly throughout the study in the groups supplemented with vitamin D<sub>2</sub> or vitamin D<sub>3</sub> and decreased significantly to 33.1 ± 13.9 nmol/L after 4 weeks and to 32.1 ± 12.8 nmol/L after 8 weeks in the placebo group. After 4 and 8 weeks, the 25(OH)D concentrations differed significantly between the groups (Table 2).

At baseline, the 25(OH)D<sub>2</sub> concentration was below the limit of quantification (7.5 nmol/L) in all but two participants. In neither the vitamin D<sub>3</sub> group nor the placebo group did the average 25(OH)D<sub>2</sub> rise above the limit of quantification in the course of the study. In the vitamin D<sub>2</sub> group, 25(OH)D<sub>2</sub> increased significantly to 39.6 ± 11.7 nmol/L at 4 weeks and to 51.2 ± 18.5 nmol/L at 8 weeks (Table 2).

At baseline, there was no difference in the 25(OH)D<sub>3</sub> concentration between the groups. Although in the vitamin D<sub>3</sub> group 25(OH)D<sub>3</sub> increased significantly after 4 and 8 weeks, it decreased significantly in the vitamin D<sub>2</sub> and placebo groups. The decrease was more pronounced in the vitamin D<sub>2</sub> group, and the difference from the placebo group was significant at both 4 and 8 weeks (Table 2).

The increases (4-wk baseline, 8-wk baseline) in the specific hydroxylated forms of vitamin D [either 25(OH)D<sub>2</sub> or 25(OH)D<sub>3</sub>] were as follows: in the case of 25(OH)D<sub>2</sub> in the vitamin D<sub>2</sub> group, 38.4 ± 11.0 nmol/L after 4 weeks and 50.0 ± 18.0 nmol/L after 8 weeks; in the case of 25(OH)D<sub>3</sub> in the vitamin D<sub>3</sub> group, 34.2 ± 17.2 nmol/L after 4 weeks and 46.7 ± 21 nmol/L after 8 weeks. The increase was calculated from the baseline value in this group, without taking the decrease in 25(OH)D<sub>3</sub> in the placebo group into account. The increase was not significantly different at either 4 or 8 weeks.

**Table 2.** Vitamin D Metabolites in Healthy Volunteers Receiving Supplementation With Vitamin D<sub>2</sub>, Vitamin D<sub>3</sub>, or Placebo for 8 Weeks

	Vitamin D <sub>2</sub> Group	Vitamin D <sub>3</sub> Group	Placebo Group	P (ANOVA)
n	46	42	19	
Total 25(OH)D				
Baseline, nmol/L	37.6 ± 13.3	43.7 ± 23.3	40.7 ± 14.5	.292
4 wk, nmol/L	59.9 ± 15.2 <sup>a</sup>	77.1 ± 23.5 <sup>b</sup>	33.1 ± 13.9	.001
8 wk, nmol/L	67.8 ± 20.1 <sup>a</sup>	89.2 ± 22.1 <sup>b</sup>	32.1 ± 12.8	.001
Repeated measure analysis				<.001
25(OH)D <sub>3</sub>				
Baseline, nmol/L	36.4 ± 13.3	41.5 ± 22.8	39.4 ± 14.2	.409
4 wk, nmol/L	20.3 ± 8.1 <sup>a</sup>	75.7 ± 23.2 <sup>b</sup>	31.1 ± 13.9	.001
8 wk, nmol/L	16.6 ± 6.3 <sup>a</sup>	88.0 ± 22.1 <sup>b</sup>	31.1 ± 12.4	.001
Repeated measure analysis				.001
25(OH)D <sub>2</sub>				
Baseline, nmol/L	<7.5 <sup>c</sup>	<7.5	<7.5	.110
4 wk, nmol/L	39.6 ± 11.7 <sup>a</sup>	<7.5	<7.5	.001
8 wk, nmol/L	51.2 ± 18.5 <sup>a</sup>	<7.5	<7.5	.001
Repeated measure analysis				.001
PTH				
Baseline, ng/mL	69.8 ± 45.2	59.3 ± 22.6	79.4 ± 49.2	.334
4 wk, ng/mL	63.0 ± 33.2	49.1 ± 19.5	65.0 ± 40.0	.086
8 wk, ng/mL	56.8 ± 26.5	40.3 ± 19.5	60.8 ± 38.1	.007
Repeated measure analysis				.651

Data are shown as mean ± SD. Differences between the groups at the various time points of the study were tested by one-way ANOVA with post hoc Scheffé comparison. The overall effect of supplementation was tested by an ANOVA with repeated measurement (PASW procedure GLM repeated measure). Due to the high degree of skewness, the Kruskal-Wallis test was used for testing differences in PTH between groups.

<sup>a</sup> Significantly different at  $P < .01$  from vitamin D<sub>3</sub> group and placebo.

<sup>b</sup> Significantly different at  $P < .01$  from vitamin D<sub>2</sub> group and placebo.

<sup>c</sup> Values for 25(OH)D<sub>2</sub> at baseline and in the vitamin D<sub>3</sub> and placebo groups in the course of the study are only provided for those levels exceeding the limit of detection (>2.5 nmol/L).

The PTH concentrations were not significantly different between the groups at baseline or after 4 and 8 weeks (Table 2). PTH concentrations decreased significantly during the course of the study in all groups.

The absolute and percentage differences in total 25(OH)D, 25(OH)D<sub>3</sub>, and 25(OH)D<sub>2</sub> between baseline and 8 weeks were significant among the supplementation groups. Absolute or percentage differences in PTH

concentrations were not significant among the groups (Table 3).

No adverse effects were reported by the participants. Serum calcium did not exceed the normal range in any of the participants (data not shown). The analysis for total 25(OH)D, the primary outcome variable, was repeated with all randomized subjects included (intention-to-treat analysis). This did not change the results (data not shown).

**Table 3.** Absolute and Percentage Changes in Total 25(OH)D, 25(OH)D<sub>3</sub>, 25(OH)D<sub>2</sub> (Absolute Change Only), and PTH at 8 Weeks Compared to Baseline

	Vitamin D <sub>2</sub> Group	Vitamin D <sub>3</sub> Group	Placebo Group	P (ANOVA)
n	46	42	19	
Δ Total 25(OH)D at 8 wk (to baseline), nmol/L	+30.2 ± 20.1 <sup>c</sup>	+45.5 ± 21.7 <sup>a</sup>	-8.6 ± 7.3	.001
% Total 25(OH)D at 8 wk (of baseline)	200 ± 97% <sup>a</sup>	259 ± 149% <sup>a</sup>	79 ± 16%	.001
Δ 25(OH)D <sub>3</sub> at 8 wk (to baseline), nmol/L	-19.8 ± 9.6 <sup>c</sup>	+46.5 ± 21.3 <sup>b</sup>	-8.3 ± 6.1	.001
% 25(OH)D <sub>3</sub> at 8 wk (of baseline)	47 ± 14%	280 ± 183% <sup>b</sup>	79 ± 15%	.001
Δ 25(OH)D <sub>2</sub> at 8 wk (to baseline), nmol/L	+43.7 ± 18.5 <sup>d</sup>	<7.5	<7.5	.001
Δ PTH at 8 wk (to baseline), ng/mL	-13.0 ± 35.4	-19.0 ± 29.4	-18.6 ± 35.1	.658
% PTH at 8 wk (of baseline)	95 ± 47%	80 ± 58%	82 ± 38%	.354

Data are shown as mean ± SD. Significance was tested by ANOVA, followed by a post hoc Scheffé comparison.

<sup>a</sup> Significantly different from placebo group.

<sup>b</sup> Significantly different from vitamin D<sub>2</sub> and placebo groups.

<sup>c</sup> Significantly different from vitamin D<sub>3</sub> and placebo groups.

<sup>d</sup> Significantly different from vitamin D<sub>2</sub> and vitamin D<sub>3</sub> groups.

## Discussion

Our major finding is that vitamin D<sub>3</sub> increased 25(OH)D more effectively than vitamin D<sub>2</sub>. By measuring the specific hydroxylated forms, we have been able to show that the underlying reason for this difference is a substantial decrease in 25(OH)D<sub>3</sub> in subjects receiving vitamin D<sub>2</sub>. This had not been demonstrated earlier with sufficient statistical power. We have also been able to show that hydroxylation of vitamin D<sub>2</sub> was similar to hydroxylation of vitamin D<sub>3</sub> because the increase in the specific hydroxylated forms [25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>] was similar in the two groups (compare the absolute differences in Table 3).

Vitamins D<sub>2</sub> and D<sub>3</sub> have been compared earlier in a number of studies that differed in their design, supplement dosage, frequency of supplementation, use of the delivery method, and selection of participants and also in their conclusion regarding the bioequivalence of the two forms of the vitamin (4–8, 20–22, 25, 26). A recent meta-analysis that included seven of these studies (4–8, 21, 22) concluded that the change in 25(OH)D was significantly greater after supplementation with vitamin D<sub>3</sub> than after one with vitamin D<sub>2</sub>, although the effect was largely due to the studies that used a bolus dose; it was not significant in studies with daily supplementation (1). However, for the latter analysis, only six studies (6–8, 21, 22) with a total number of 248 participants were available. Our study with 42 and 46 participants in the vitamin D<sub>3</sub> and D<sub>2</sub> groups, respectively, would have changed the result of this analysis, yielding a significant effect in favor of vitamin D<sub>3</sub> compared to vitamin D<sub>2</sub> also with daily supplementation (the analysis using present data in addition to those of Tripkovic et al [1] was made using Review Manager 5.2; data not shown).

The most interesting result of our study, however, is the decrease in 25(OH)D<sub>3</sub> after supplementation with vitamin D<sub>2</sub>. This was already evident after 4 weeks, and the decrease was significantly different from the seasonal decrease observed in the placebo group. A decrease in 25(OH)D<sub>3</sub> after supplementation with vitamin D<sub>2</sub> was reported earlier by Glendenning et al (22) in elderly hip fracture patients receiving 1000 IU/d for a period of 3 months, and also by Armas et al (26), who studied single doses of 50 000 IU of D<sub>2</sub> and D<sub>3</sub> in healthy men with a follow-up period of 28 days. Interestingly, both groups of authors did not discuss these findings specifically. This was also observed by Binkley et al (21) after administration of 1600 IU daily for a period of 12 months. It is surprising that this effect was observed in only a few studies, although it should be pointed out that only studies using methods capable of distinguish-

ing between 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> would be able to show this effect. The use of immunoassays will therefore not make it possible to observe the effect. The biological reason behind this finding remains to be elucidated.

It has been suggested that an increased catabolism of 25(OH)D takes place due to supplementation with vitamin D<sub>2</sub> (5). Heaney et al (5) studied, groups of 16 and 17 subjects who received 50 000 IU once weekly for 12 weeks, and a significantly higher AUC<sub>25(OH)D</sub> was observed after 84 days for vitamin D<sub>3</sub>. Interestingly, vitamins D<sub>3</sub> and D<sub>2</sub> were also measured in the fat tissue of two participants, and a decrease in vitamin D<sub>3</sub> in fat tissue after supplementation with vitamin D<sub>2</sub> was observed. Because the authors measured vitamin D<sub>2</sub> in fat biopsies from only two participants, however, this finding did not reach statistical significance.

It has also been suggested that one reason for the lower increase in 25(OH)D after vitamin D<sub>2</sub> in comparison with supplementation with D<sub>3</sub> was due to impaired hydroxylation at C25 (atom of the vitamin D molecule) (27). We have shown that at least the increases in the specific hydroxylation products [either 25(OH)D<sub>2</sub> or 25(OH)D<sub>3</sub>] were similar. However, we cannot exclude the possibility that vitamin D<sub>2</sub> impairs hydroxylation of vitamin D<sub>3</sub>, which is also present in the circulation. Because the decrease in 25(OH)D<sub>3</sub> exceeded the observed decrease in the placebo group, this is a likely explanation. The problem should be investigated further.

Other explanations include an increased catabolism of the 25(OH)D<sub>2</sub> molecule due to a lower degree of binding to the vitamin D binding protein (28). Our data do not support an increased catabolism of 25(OH)D<sub>2</sub>, although they cannot exclude it.

Because we did not measure any other metabolite [24,25(OH)<sub>2</sub>D metabolites, 1,24,25(OH)<sub>3</sub>D metabolites], we can only speculate about differences in the 24-hydroxylation step between 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>. Further studies should include these metabolites to obtain a deeper insight into the competitive nature of the two forms of vitamin D.

Our study has several strengths and also some limitations. The strengths of the present study include its large sample size, which allowed us to detect small differences between vitamin D<sub>2</sub> and D<sub>3</sub> treatments that earlier studies had been unable to show. Another important strength is the measurement of both 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> in this study. Measurements of the specific hydroxylated forms of vitamin D enabled us to show the effect of vitamin D<sub>2</sub> on the 25(OH)D<sub>3</sub> levels. In addition, due to the inclusion of the placebo group, we were able to monitor the decrease in total 25(OH)D concentrations within healthy subjects living at the approximate latitude 51°North. We

observed a strong decrease from January to February and no further decrease from February to March.

One limitation of our study was that we did not measure the active forms, 1,25(OH)<sub>2</sub>D<sub>2</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub>, or other metabolites. In addition, we did not obtain a dose-response curve after a single dose, and we did not determine the catabolic products 24,25(OH)<sub>2</sub>D, 24,25(OH)<sub>2</sub>D<sub>3</sub>, or 24,25(OH)<sub>2</sub>D<sub>2</sub>. Measurement of these metabolites would provide valuable insights into the metabolism of vitamin D<sub>3</sub> in the presence of vitamin D<sub>2</sub>. We also studied only one dose, and the level of 50 μg/d is beyond current recommendations and fortification levels.

In future studies, the effect of lower doses of vitamin D that are closer to the recommended daily amounts should be investigated. In light of the decrease in 25(OH)D<sub>3</sub> by vitamin D<sub>2</sub>, the effect of vitamin D<sub>2</sub> supplementation on disease outcomes, eg, bone health and fractures, should be carefully analyzed. Indeed, the effect of vitamin D<sub>2</sub> on falls was found to be lower than that of vitamin D<sub>3</sub> in recent meta-analyses (29, 30).

PTH and vitamin D are both involved in bone metabolism (31) and show an inverse correlation. PTH secretion is directly modulated (23) and suppressed by 25(OH)D concentrations (31). Leventis and Kiely (32) demonstrated that vitamin D<sub>3</sub> affected PTH concentration more than vitamin D<sub>2</sub>, a finding that is not supported by our data. However, our study was not designed to demonstrate an effect of vitamin D supplementation on PTH concentrations as the primary outcome. To demonstrate such an effect, we had to include even more subjects due to the large variation in PTH concentrations. Therefore, we may have missed an effect of vitamin D supplementation on PTH concentrations. This is in line with a number of other studies (8, 21, 22).

In conclusion, we have shown that vitamin D<sub>3</sub> is more effective in raising the vitamin D status than vitamin D<sub>2</sub> and that vitamin D<sub>2</sub> supplementation causes a decrease in 25(OH)D<sub>3</sub>. These findings question the usefulness of vitamin D<sub>2</sub> supplements. Instead, vitamin D<sub>3</sub> should be used for supplementation and fortification purposes.

## Acknowledgments

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This work was supported by Grant 0315668A from the German Ministry of Education and Research.

Author contributions: J.D. and G.I.S. designed the research. U.L., K.H., S.W., and F.H. conducted the study. J.D. and U.L. analyzed the data and wrote the paper. J.D. has primary responsibility for the final content. All authors read and approved the final manuscript. None of the authors declared any financial or

personal relationships with other persons or organizations that could have an inappropriate influence on this work. The funding agency had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Registered at clinicaltrials.gov with identifier NCT01503216.

Disclosure Summary: The authors have nothing to disclose.

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