Differential Effects of Oral Boluses of Vitamin D_2 vs Vitamin D_3 on Vitamin D Metabolism: A Randomized Controlled Trial

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Context: Vitamin D_2 and vitamin D_3 have been hypothesized to exert differential effects on vitamin D metabolism.

Objective: To compare the influence of administering vitamin D_2 vs vitamin D_3 on metabolism of vitamin D_3.

Methods: We measured baseline and 4-month serum concentrations of vitamin D_3, 25-hydroxyvitamin D_3 [25(OH)D_3], 25-hydroxyvitamin D_2, 24R,25-dihydroxyvitamin D_3 [24R,25$(OH)_2D_3$], 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], and 4β,25-dihydroxyvitamin D_3 [4β,25$(OH)_2D_3$] in 52 adults randomized to receive a total of four oral bolus doses of 2.5 mg vitamin D_2 (n = 28) or vitamin D_3 (n = 24) over four months. Metabolite-to-parent compound ratios were calculated to estimate hydroxylase activity. Pairwise before vs after comparisons were made to evaluate effects of vitamin D_2 and vitamin D_3 on metabolism of vitamin D. Mean postsupplementation metabolite-to-parent ratios were then compared between groups.

Results: Vitamin D_2 was less effective than vitamin D_3 in elevating total serum 25(OH)D concentration. Vitamin D_2 suppressed mean four-month serum concentrations of 25$(OH)D_3$, 24R,25$(OH)_2D_3$, 1α,25$(OH)_2D_3$, and 4β,25$(OH)_2D_3$ and mean ratios of 25$(OH)D_3$ to D_3 and 1α,25$(OH)_2D_3$ to 25$(OH)D_3$, while increasing the mean ratio of 24R,25$(OH)_2D_3$ to 25$(OH)D_3$. Vitamin D_3 increased mean four-month serum concentrations of 25$(OH)D_3$, 24R,25$(OH)_2D_3$, 1α,25$(OH)_2D_3$, and 4β,25$(OH)_2D_3$ and the mean ratio of 24R,25$(OH)_2D_3$ to 25$(OH)D_3$. Participants receiving vitamin D_2 had lower mean postsupplementation ratios of 25$(OH)D_3$ to vitamin D_3 and 1α,25$(OH)_2D_3$ to 25$(OH)D_3$ than those receiving vitamin D_3. Mean postsupplementation ratios of 24R,25$(OH)_2D_3$ to 25$(OH)D_3$ and 4β,25$(OH)_2D_3$ to 25$(OH)D_3$ did not differ between groups.

Conclusions: Bolus-dose vitamin D_2 is less effective than bolus-dose vitamin D_3 in elevating total serum 25(OH)D concentration. Administration of vitamin D_2 reduces 25-hydroxylation of vitamin D_3 and 1-α hydroxylation of 25$(OH)D_3$, while increasing 24R-hydroxylation of 25$(OH)D_3$. (***J Clin Endocrinol Metab*** **104: 5831–5839, 2019)**

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Abbreviations: 1α,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 4β,25$(OH)_2D_3$, 4β,25-dihydroxyvitamin D_3; 24R,25$(OH)_2D_3$, 24R,25-dihydroxyvitamin D_3; 25(OH)D, 25-hydroxyvitamin D; 25$(OH)D_3$, 25-hydroxyvitamin D_3; LLOQ, lower limit of quantitation.

Vitamin D has two forms: ergocalciferol (vitamin D_2) is synthesized *via* UV irradiation of ergosterol, a steroid found in fungi and some plants, whereas cholecalciferol (vitamin D_3) is synthesized via UV irradiation of 7-dehydrocholesterol to previtamin D_3, followed by a thermal isomerization step. In humans, the source of vitamin D_3 may be endogenous (*i.e.,* obtained via cutaneous synthesis) or exogenous (*i.e.*, ingested in foods or supplements), whereas vitamin D_2 is only available from exogenous sources. Vitamin D_2 and vitamin D_3 are structurally distinct: the side chain of vitamin D_2 contains a double bond between carbons 22 and 23 and a methyl group on carbon 24, both of which are absent from the side chain of vitamin D_3. The two forms also have differing pharmacokinetics: of 14 publications comparing effects of vitamin D_2 vs vitamin D_3 (1–14), all but three (12–14) reported that vitamin D_2 was less effective than vitamin D_3 in elevating total 25(OH)D levels. A meta-analysis of data from seven of these studies found that this effect was only statistically significant when vitamin D was administered using intermittent bolus dosing, as opposed to daily administration (15). 25-hydroxyvitamin D_3 [25(OH)D_3] subsequently undergoes a second hydroxylation step to form the active vitamin D metabolite 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2D_3$] or the inactive metabolites 24R,25-dihydroxyvitamin D_3 [24R,25(OH)$_2D_3$] and 4β,25-dihydroxyvitamin D [4β,25(OH)$_2D_3$; Fig. 1]. It also undergoes conjugation to circulating inactive sulfate and glucuronide metabolites that may be recycled back to 25(OH)D_3 rather than excreted (16, 17).

Administration of vitamin D_2 has been reported to reduce circulating concentrations of 25(OH)D_3 in eight studies (1–7, 11); a ninth study reports a nonstatistically significant trend in the same direction (12). These observations have led investigators to speculate that vitamin D_2 may influence metabolism of vitamin D_3. In keeping with this hypothesis, a recent study reported that administration of vitamin D_2 increases the ratio of 24R,25(OH)$_2D_3$ to 25(OH)D_3 in the circulation and decreases the ratio of 1α,25[OH]$_2D_3$ to 25(OH)D_3, findings taken to indicate that vitamin D_2 induces 24R-hydroxylation and suppresses 1α-hydroxylation of 25(OH)D_3 (18). However, it is not yet known if these effects are vitamin D_2-specific, because administration of vitamin D_3 also influences the rate of conversion of parent vitamin D_3 to its hydroxylated metabolites (19). Moreover, the influence of administering vitamin D_2 on circulating concentrations of vitamin D_3 and 4β,25(OH)$_2D_3$ has yet to be determined.

Studies making a head-to-head comparison of the influence of identical doses of vitamin D_2 vs vitamin D_3 on circulating concentrations of vitamin D_3, 25(OH)D_3 and its major dihydroxylated metabolites are needed to resolve these questions. An opportunity to conduct such an investigation recently arose in the context of a randomized controlled trial that we conducted in the United Kingdom to evaluate the effect of administration of four monthly oral doses of 2.5 mg vitamin D_2 vs the same dose of vitamin D_3 on glycated hemoglobin concentration among people at risk for type 2 diabetes mellitus (11). We therefore determined concentrations of vitamin D_3, 25(OH)D_3, 24R,25(OH)$_2D_3$, 1α,25(OH)$_2D_3$, and 4β,25(OH)$_2D_3$ in serum samples taken from a subset of trial participants before and after administration of vitamin D_2 vs vitamin D_3, and calculated the change in postsupplementation metabolite-to-parent ratios to gain insight into the relative effects of vitamin D_2 vs vitamin D_3 on the activity of enzymes catalyzing 25-hydroxylation of vitamin D_3 and 1α-hydroxylation, 24R-hydroxylation, and 4β-hydroxylation of 25(OH)D_3. We also measured concentrations of vitamin D_2 and 25(OH)D_2 in the same samples and compared the influence of vitamin D_2 vs vitamin D_3 on 25-hydroxylation of vitamin D_2.

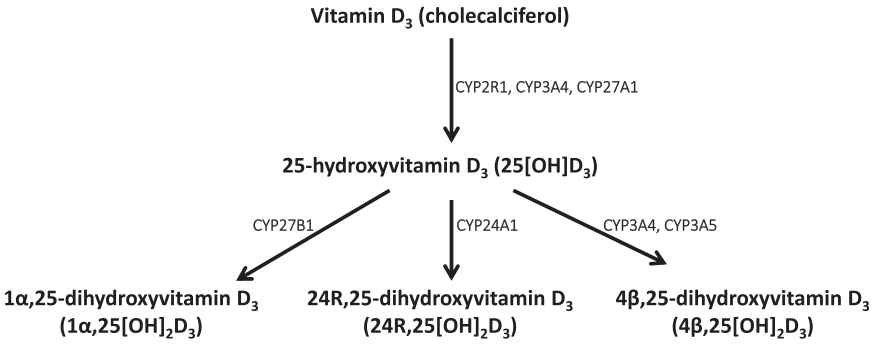

Figure 1. Vitamin D_3 oxidation pathways. The monohydroxylated and dihydroxylated metabolites investigated in the current study are shown, with the cytochrome P450 enzymes catalyzing each conversion in capitals.

Methods

Trial design and participants

As previously described, we conducted a double-blind, randomized placebo-controlled trial that enrolled a total of 340 men and women aged 30 to 75 years who had been identified as being at increased risk of developing type 2 diabetes mellitus in London and Cambridge, United Kingdom (11). Full details of inclusion and exclusion criteria are described in the published protocol (20). Eligible participants were randomly allocated to one of three groups on a 1:1:1 basis within

four strata defined by age (30 to 50 or 51 to 75 years) and sex, with a block size of six within each stratum. One group received four monthly oral bolus doses of 2.5 mg vitamin D_2: each dose was presented as 5 mL Sterogyl solution (Desma Pharma, Paris, France) containing 0.5 mg vitamin D_2 per milliliter in ethanol. The second group received four monthly oral bolus doses of 2.5 mg vitamin D_3: each dose was presented as 5 mL Vigantol oil (Merck Serono, Darmstadt, Germany) containing 0.5 mg vitamin D_3 per milliliter in Miglyol oil (Caesar & Loretz, Hilden, Germany). The third group received four monthly oral doses of placebo (Miglyol oil). The order of treatments within each block was determined by a computer-generated pseudo-random sequence, generated by the study medication manufacturer (Nova Laboratories, Leicester, UK). Neither the participants, the investigators, nor the laboratory staff knew the treatment allocation. Each participant was followed-up for a total of four months from their first visit; serum samples were collected at baseline and at the end of the study. Baseline and four-month serum samples taken from a subset of 28 participants and allocated to vitamin D_2 and 25 participants allocated to vitamin D_3 were sent for determination of concentrations of vitamin D_3 and its metabolites as detailed below. The subset of participants contributing samples to the current study were selected on the basis that they were all recruited in London; that they had each received four directly observed doses of vitamin D_2 or vitamin D_3; and that they were the 28 samples in each group having the greatest volume of serum available at both baseline and four-month follow-up to be sent for further analysis. For the vitamin D_3 group, only 25 samples had sufficient sample volume for analysis. Ethical approval for the trial was provided by the Charing Cross Medical Ethics Committee (ref 09/H0711/85) and the Cambridge Local Research Ethics Committee (ref 04/Q0108/19), and written informed consent was obtained from all participants. The trial was registered under the numbers EudraCT 2009-011264-11 and ISRCTN86515510 on 23 October 2009.

Laboratory assays

Serum concentrations of vitamin D_3, vitamin D_2, 25(OH)D_3, 25(OH)D_2, 24R,25(OH)$_2D_3$, 1α,25(OH)$_2D_3$, 1α,25(OH)$_2D_2$, and 4β,25(OH)$_2D_3$ were determined by liquid chromatography-tandem mass spectrometry in the Thummel Laboratory, Department of Pharmaceutics, University of Washington, Seattle, Washington, as previously described (21). Lower limits of quantitation (LLOQ) were 0.23 nmol/L for vitamin D_3, 0.15 nmol/L for vitamin D_2, 0.50 nmol/L for 25(OH)D_3, 0.24 nmol/L for 25(OH)D_2, 0.14 nmol/L for 24R,25(OH)$_2D_3$, and 7.7 pmol/L for 1α,25(OH)$_2D_3$, 1α,25(OH)$_2D_2$ and 4β,25(OH)$_2D_3$. Where concentrations of a given analyte were less than the LLOQ, a value equal to the LLOQ divided by the $\sqrt{2}$ was imputed, as performed elsewhere (22). Intraday and interday coefficients of variation were <15% for all analytes, as previously reported (21).

Sample size and statistical analyses

We estimated that paired before and after serum samples from 21 participants would need to be evaluated to have 90% power to detect a 15 nmol/L difference in 25(OH)D_3 concentration preadministration vs postadministration of vitamin D_2 with $\alpha = 0.05$, based on a standard deviation for post-supplementation serum 25(OH)D_3 concentration of 20 nmol/L (11). This sample size was inflated to 28 to allow for potential assay failure. Serum samples from a similar number of participants allocated to vitamin D_3 were also evaluated.

Statistical analyses were conducted using GraphPad Prism version 6.04 (GraphPad Software Inc., La Jolla, CA) and STATA IC version 12 (StataCorp, College Station, TX). Intragroup differences in absolute concentrations of vitamin D_3 and its metabolites before vs after supplementation with vitamin D_2 or vitamin D_3 were evaluated using paired Student *t* tests. Intergroup differences in end-study values of these parameters were evaluated with linear regression, adjusting for baseline values. Mean differences are presented with 95% CI and *P* values, with statistical significance inferred where *P* values are less than 0.05.

Results

Participant enrollment and baseline characteristics

A total of 340 adults were randomly assigned to receive supplementation with vitamin D_2 (n = 112) vs vitamin D_3 (n = 114) vs placebo (n = 114) between 2010 and 2012, of whom 285 (94 randomized to vitamin D_2 vs 99 randomized to vitamin D_3 vs 92 randomized to placebo) took all four doses of study medication and completed follow-up. For the current study, baseline and four-month serum samples collected from a subset of participants recruited in London who took four doses of vitamin D_2 (n = 28) or vitamin D_3 (n = 25) were sent for determination of concentrations of vitamin D and its metabolites (Fig. 2). Effects of the intervention on the primary outcome of the main trial, and on safety, are reported elsewhere (11). The trial ended on the date of the final study visit of the last participant to be randomized. One participant selected for the substudy and randomly assigned to vitamin D_3 was found to have a high outlying baseline 25(OH)D_2 concentration (51.8 nmol/L) and was excluded from statistical analyses at a reviewer's request. Baseline characteristics of participants whose serum samples contributed to the current study are presented in Table 1. Overall, mean age was 55.6 years (SD 10.0 years) and 21 of 52 (40.4%) participants were female. Baseline demographic and clinical characteristics, serum concentrations of vitamin D_3, vitamin D_2 and their metabolites and metabolite-to-parent ratios were similar for those randomized to receive vitamin D_2 vs vitamin D_3 where measurable; concentrations of 1α,25(OH)$_2D_2$ were undetectable (<7.7 pmol/L) in all samples.

Influence of vitamin D_2 vs vitamin D_3 on total 25(OH)D concentrations

Both vitamin D_2 and vitamin D_3 elevated total 25(OH)D concentrations at follow-up: the mean increase in total 25(OH)D concentrations after administration of vitamin D_2 was 31.4 nmol/L (95% CI 21.5 to 41.2 nmol/L,

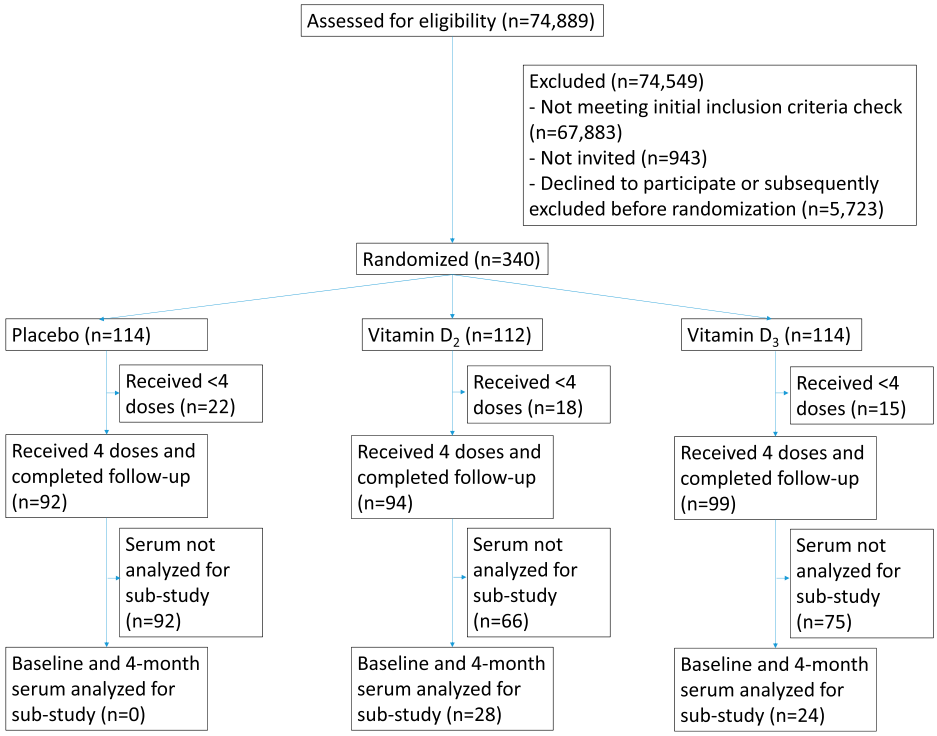

Figure 2. Trial profile.

$P < 0.001$), and the corresponding increase in total 25(OH)D concentrations after administration of vitamin D_3 was 46.4 nmol/L (95% CI 33.7 to 59.0 nmol/L, $P < 0.001$). The difference in the mean change in total 25(OH)D concentration at follow-up for participants randomized to vitamin D_3 vs vitamin D_2 was 13.0 nmol/L (95% CI -0.5 to 26.6 nmol/L, $P = 0.06$; Table 2; Fig. 3). No difference in the ratio of total 25(OH)D to total parent vitamin D at follow-up was seen between participants randomized to vitamin D_2 vs vitamin D_3 ($P = 0.32$).

Influence of vitamin D_2 on metabolism of vitamin D_3

To characterize effects of vitamin D_2 on metabolism of D_3, we conducted pairwise statistical analyses comparing circulating concentrations of vitamin D_3 and its metabolites in 28 individuals before vs after oral administration of four monthly doses of 2.5 mg vitamin D_2. Results are presented in Table 2. Administration of vitamin D_2 had no statistically significant effect on serum concentrations of vitamin D_3 ($P = 0.07$), but it did reduce mean serum concentrations of 25(OH)D_3 (43.2% decrease, $P < 0.001$; Fig. 3), 24R,25(OH)$_2D_3$ (37.5% decrease, $P = 0.007$), 1α,25(OH)$_2D_3$ (53.4% decrease, $P < 0.001$; Fig. 3) and 4β,25(OH)$_2D_3$ (42.0% decrease, $P = 0.03$). Administration of vitamin D_2 reduced molar ratios of 25(OH)D_3 to vitamin D_3 (76.2% decrease, $P = 0.003$) and 1α,25(OH)$_2D_3$ to 25(OH)D_3 (52.2% decrease, $P = 0.02$), but increased the molar ratio of 24R,25(OH)$_2D_3$ to 25(OH)D_3 (24.5% increase, $P = 0.04$). No statistically significant effect of vitamin D_2 on the molar ratio of 4β,25(OH)$_2D_3$ to 25(OH)D_3 was seen ($P = 0.97$).

Influence of vitamin D_3 on its own metabolism

Having characterized the effects of vitamin D_2 on metabolism of vitamin D_3, we proceeded to conduct a second set of pairwise before and after statistical analyses to evaluate the effects of vitamin D_3 on the same biochemical parameters in a separate group of 24 individuals who received four monthly doses of 2.5 mg vitamin D_3. Results of these analyses (Table 2) show that administration of vitamin D_3 elevated serum concentrations of vitamin D_3 (80.0% increase, $P = 0.03$), 25(OH)D_3 (110.0% increase, $P < 0.001$; Fig. 3), 24R,25(OH)$_2D_3$ (165.5% increase, $P < 0.001$), 1α,25(OH)$_2D_3$ (66.6% increase, $P = 0.005$), and 4β,25(OH)$_2D_3$ (214.5% increase, $P = 0.02$). Administration of vitamin D_3 also increased the ratio of 24R,25(OH)$_2D_3$ to 25(OH)D_3 (32.6% increase, $P = 0.006$) but had no statistically significant effect on ratios of 25(OH)D_3 to vitamin D_3 ($P = 0.09$), 1α,25(OH)$_2D_3$ to 25(OH)D_3 ($P = 0.06$), or 4β,25(OH)$_2D_3$ to 25(OH)D_3 ($P = 0.06$).

Comparing effects of vitamin D_2 vs vitamin D_3 on metabolism of vitamin D_3

To determine whether the two forms of vitamin D exerted different effects on metabolism of vitamin D_3, we

Table 1. Participants' Baseline Characteristics by Allocation

	Characteristics	Vitamin D_2 (n=28)	Vitamin D_3 (n = 24)
Sex	Female, n (%)	10 (35.7)	11 (45.8)
	Male, n (%)	18 (64.3)	13 (54.2)
Mean age, y (SD)		56.0 (10.8)	55.0 (9.1)
Ethnic origin	White, n (%)	18 (64.3)	18 (75.0)
	Other, n (%)	10[a] (35.7)	6 (25.0)[b]
Total serum concentration of vitamin D and its metabolites[c]	Mean total vitamin D, nmol/L (SD)[d]	6.5 (6.7)	8.6 (8.5)
	Mean total 25(OH)D, nmol/L (SD)[e]	49.7 (32.3)	45.5 (25.0)
	Mean total 25(OH)D-to-total vitamin D molar ratio (SD)	19.3 (27.5)	18.6 (22.4)
Serum concentration of vitamin D_3 and its metabolites	Mean vitamin D_3, nmol/L (SD)	2.9 (3.3)	3.5 (4.2)
	Mean 25(OH)D_3, nmol/L (SD)	46.1 (32.4)	42.0 (24.7)
	Mean 1α,25(OH)$_2D_3$, pmol/L (SD)	77.2 (46.0)	96.9 (55.1)
	Mean 24R,25(OH)$_2D_3$, nmol/L (SD)	3.2 (2.7)	2.9 (1.8)
	Mean 4β,25(OH)$_2D_3$, pmol/L (SD)	126.4 (133.8)	97.1 (104.9)
	Mean 25(OH)D_3-to-vitamin D_3 molar ratio (SD)	39.0 (47.2)	34.1 (44.2)
	Mean 24R,25(OH)$_2D_3$-to-25(OH)D_3 molar ratio (SD)	0.07 (0.03)	0.07 (0.04)
	Mean 1α,25(OH)$_2D_3$-to-25(OH)D_3 molar ratio (SD)	0.0023 (0.0018)	0.0029 (0.0022)
	Mean 4β,25(OH)$_2D_3$-to-25(OH)D_3 molar ratio (SD)	0.0024 (0.0016)	0.0020 (0.0014)
Serum concentration of vitamin D_2 and its metabolites	Mean vitamin D_2, nmol/L (SD)	3.6 (5.3)	5.1 (7.7)
	Mean 25(OH)D_2, nmol/L (SD)	3.5 (1.3)	3.5 (1.5)
	Mean 1α,25(OH)$_2D_2$, pmol/L, (SD)	<7.7[f]	<7.7[f]
	Mean 25(OH)D_2-to-vitamin D_2 molar ratio (SD)	18.5 (17.2)	16.2 (19.5)

[a]Of whom 6 were of black or black British ethnic origin and 4 were of Asian or Asian British ethnic origin.

[b]Of whom 5 were of Asian or Asian British ethnic origin and 1 was of black or black British ethnic origin.

[c]Not calculated for 1,25-dihydroxyvitamin D (1,25-dihydroxyvitamin D_2 was undetectable in all) or 24,R,25-dihydroxyvitamin D/4β-dihydroxyvitamin D (neither 24,R,25-dihydroxyvitamin D_2 nor 4β-dihydroxyvitamin D_2 were measured).

[d]Calculated by summing values for vitamin D_2 and vitamin D_3.

[e]Calculated by summing values for 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3.

[f]1α,25(OH)$_2D_2$ undetectable in all; lower limit of quantification for this metabolite was 7.7 pmol/L.

undertook unpaired statistical analyses comparing postsupplementation values of vitamin D_3 metabolite-to-parent ratios between individuals supplemented with vitamin D_2 vs vitamin D_3, using linear regression with adjustment for baseline values. Results of these analyses (Table 2) reveal that participants receiving vitamin D_2 had lower mean postsupplementation ratios of 25(OH)D_3 to vitamin D_3 ($P = 0.002$) and 1α,25(OH)$2D_3$ to 25(OH)D_3 ($P = 0.03$) than those who received vitamin D_3. No statistically significant difference in mean postsupplementation ratios of 24R,25(OH)$_2D_3$ to 25(OH)D_3 ($P = 0.42$) or 4β,25(OH)$_2D_3$ to 25(OH)D_3 ($P = 0.25$) was seen between participants receiving vitamin D_2 vs vitamin D_3.

Influence of vitamin D_3 vs vitamin D_2 on metabolism of vitamin D_2

Although the primary focus of our study was to compare the effects of vitamin D_2 vs vitamin D_3 on metabolism of vitamin D_3, limited data were also available to evaluate relative effects of the two forms of vitamin D on metabolism of vitamin D_2 (Table 2). Pairwise analyses revealed that administration of vitamin D_2 increased mean serum 25(OH)D_2 concentration ($P < 0.001$; Fig. 3) and mean 25(OH)D_2-to-D_2 molar ratio ($P = 0.01$), but had no statistically significant effect on mean serum concentration of vitamin D_2 itself ($P = 0.09$). By contrast, administration of vitamin D_3 reduced vitamin D_2 concentrations over time ($P = 0.008$) but did not influence 25(OH)D_2 concentrations ($P = 0.64$; Fig. 3) or 25(OH)D_2-to-D_2 ratio ($P = 0.07$). Unpaired analysis comparing post-supplementation 25(OH)D_2-to-D_2 ratios between individuals supplemented with vitamin D_2 vs vitamin D_3, with adjustment for baseline values, showed that the mean postsupplementation ratio of 25(OH)D_2 to vitamin D_2 among participants receiving vitamin D_2 was higher than that of participants who received vitamin D_3 ($P = 0.03$).

Discussion

To our knowledge, this is the first investigation to evaluate the influence of vitamin D_2 on circulating concentrations of parent vitamin D_3 and its dihydroxylated metabolite 4β,25(OH)$_2D_3$ in addition to serum concentrations of 25(OH)D_2 and 25(OH)D_3. We

Table 2. Serum Concentrations of Vitamin D_3, Vitamin D_2 and Their Metabolites After Administration of Vitamin D_2 vs Vitamin D_3

			Paired Analyses				Unpaired Analysis	
	Post-Vitamin D_2 (n = 28)	Post-Vitamin D_3 (n = 24)	Mean Difference, Post-Vitamin vs Pre-Vitamin D_2 (95% CI)	*P*	Mean Difference, Post-Vitamin vs Pre-Vitamin D_3 (95% CI)	*P*	Mean Difference, Post-Vitamin D_3 vs Post-Vitamin D_2 (95% CI)[a]	*P*
Total vitamin D and its metabolites[b]								
Mean total vitamin D, nmol/L (SD)[c]	7.4 (7.8)	6.8 (5.7)	0.9 (−3.1 to 5.0)	0.65	−1.7 (−5.8 to 2.3)	0.39	−0.6 (−4.6 to 3.3)	0.75
Mean total 25(OH)D, nmol/L (SD) [d]	81.0 (21.9)	91.9 (34.5)	31.4 (21.5 to 41.2)	<0.001	46.4 (33.7 to 59.0)	<0.001	13.0 (−0.5 to 26.6)	0.06
Mean total 25(OH)D-to-total vitamin D molar ratio (SD)	27.8 (49.1)	17.6 (7.9)	8.5 (−13.9 to 31.0)	0.44	−1.1 (−10.2 to 8.1)	0.81	−10.3 (−30.9 to 10.3)	0.32
Vitamin D_3 and its metabolites								
Mean vitamin D_3, nmol/L (SD)	5.6 (7.3)	6.3 (5.5)	2.7 (−0.3 to 5.8)	0.07	2.8 (0.3 to 5.3)	0.03	0.5 (−3.2 to 4.1)	0.80
Mean 25(OH)D_3, nmol/L (SD)	26.2 (19.1)	88.2 (34.1)	−19.9 (−29.3 to −10.5)	<0.001	46.2 (33.3 to 59.2)	<0.001	64.0 (51.0 to 77.1)	<0.001
Mean 24R,25$(OH)_2D_3$, nmol/L	2.0 (1.5)	7.7 (3.0)	−1.2 (−2.0 to −0.3)	0.007	4.8 (3.7 to 6.0)	<0.001	5.8 (4.7 to 7.0)	<0.001
Mean 1α,25$(OH)_2D_3$, pmol/L	36.0 (50.5)	161.4 (95.4)	−41.1 (−63.8 to 18.5)	<0.001	64.5 (21.4 to 107.6)	0.005	119.7 (77.6 to 161.8)	<0.001
Mean 4β,25$(OH)_2D_3$, pmol/L	73.2 (81.9)	305.4 (438.6)	−53.2 (−99.2 to −7.2)	0.03	208.3 (40.7 to 375.9)	0.02	258.8 (98.0 to 419.7)	0.002
Mean 25(OH)D_3-to-vitamin D_3 molar ratio (SD)	9.3 (11.4)	18.2 (7.7)	−29.7 (−48.5 to −10.9)	0.003	−15.9 (−34.5 to 2.7)	0.09	9.0 (3.4 to 14.6)	0.002
Mean 24R,25$(OH)_2D_3$-to-25(OH)D_3 molar ratio (SD)	0.0822 (0.0435)	0.0928 (0.0297)	0.0163 (0.0010 to 0.0315)	0.04	0.0214 (0.0069 to 0.0360)	0.006	0.0078 (−0.0114 to 0.0270)	0.42
Mean 1α,25$(OH)_2D_3$-to-25(OH)D_3 molar ratio (SD)	0.0011 (0.0015)	0.0020 (0.0011)	−0.0012 (−0.0022 to −0.0002)	0.02	−0.0009 (−0.0020 to 0.0001)	0.06	0.0009 (0.0001 to 0.0017)	0.03
Mean 4β,25$(OH)_2D_3$-to-25(OH)D_3 molar ratio (SD)	0.0024 (0.0022)	0.0030 (0.0027)	0.0000 (−0.0009 to 0.0009)	0.97	0.0010 (−0.0001 to 0.0021)	0.06	0.0008 (−0.0006 to 0.0021)	0.25
Vitamin D_2 and its metabolites								
Mean vitamin D_2, nmol/L (SD)	1.8 (1.1)	0.6 (0.8)	−1.8 (−4.0 to 0.3)	0.09	−4.5 (−7.7 to −1.3)	0.008	−1.2 (−1.8 to −0.6)	<0.001
Mean 25(OH)D_2, nmol/L (SD)	54.8 (12.9)	3.6 (1.5)	51.2 (46.2 to 56.3)	<0.001	0.1 (−0.5–0.7)	0.64	−51.1 (-56.5 to −45.7)	<0.001
Mean 1α,25$(OH)_2D_2$, pmol/L	<7.7[e]	<7.7[e]	—	—		—	—	—
Mean 25(OH)D_2-to-vitamin D_2 molar ratio, (SD)	111.0 (185.2)	24.6 (18.5)	92.5 (21.8 to 163.3)	0.01	8.4 (−0.8 to 17.7)	0.07	−83.7 (−160.0 to −7.4)	0.03

[a]Adjusted for baseline value.

[b]Not calculated for 1,25-dihydroxyvitamin D (1,25-dihydroxyvitamin D_2 was undetectable in all) or 24,R,25-dihydroxyvitamin D/4β-dihydroxyvitamin D (neither 24,R,25-dihydroxyvitamin D_2 nor 4β-dihydroxyvitamin D_2 were measured).

[c]Calculated by summing values for vitamin D_2 and vitamin D_3.

[d]Calculated by summing values for 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3.

[e]1α,25$(OH)_2D_2$ undetectable in all; LLOQ for this metabolite was 7.7 pmol/L.

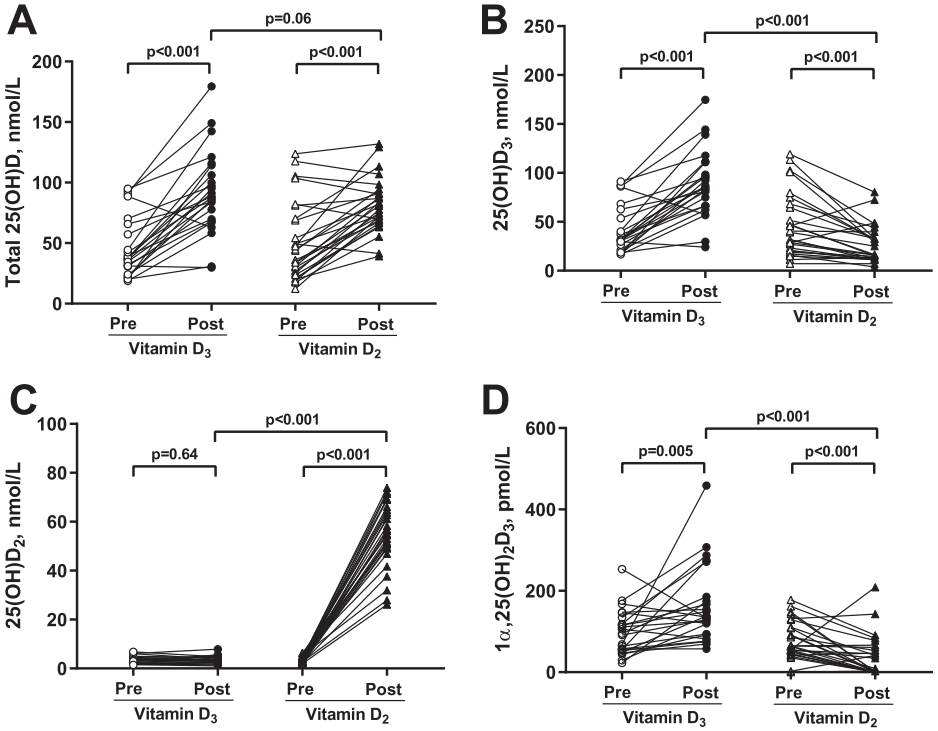

Figure 3. Influence of oral administration of vitamin D_3 and vitamin D_2 on serum concentrations of (A) total 25(OH)D, (B) 25(OH)D_3, (C) 25(OH)D_2, and (D) 1α,25(OH)$_2D_3$. Baseline and 4-mo data are presented for 24 adults receiving four bolus doses of 2.5 mg vitamin D_3 at 0, 1, 2, and 3 mo postrandomization and 28 adults receiving an equivalent regimen of vitamin D_2. Lines link data points from the same individual; *P* values for within-group comparisons before vs after supplementation are from paired Student *t* tests. *P* values for intergroup comparisons of postsupplementation values in participants randomized to vitamin D_3 vs vitamin D_2 are from linear regression with adjustment for baseline values.

found that administration of vitamin D_2 exerted a greater inhibitory effect than administration of vitamin D_3 on mean ratios of 25(OH)D_3 to D_3 and 1α,25(OH)$_2D_3$ to 25(OH)D_3 in the circulation. We also observed that vitamin D_2 and vitamin D_3 increased the mean 24R,25(OH)$_2D_3$-to-25(OH)D_3 ratio to a similar extent, and that neither form of vitamin D had a statistically significant effect on the mean serum 4β,25(OH)$_2D_3$-to-25(OH)D_3 ratio. By contrast with findings of a recently published study (6), we found that administration of vitamin D_3 did not suppress serum concentrations of 25(OH)D_2, nor did it influence mean serum 25(OH)D_2-to-vitamin D_2 ratio. Administration of vitamin D_2 resulted in an increase in the mean 25(OH)D_2-to-D_2 ratio.

Our findings are consistent with reports that administration of vitamin D_2 reduces serum concentrations of 25(OH)D_3 (1–4), and that this phenomenon is associated with an increase in the ratio of 24R,25(OH)$_2D_3$ to 25(OH)D_3 and a decrease in the ratio of 1α,25(OH)2D_3 to 25(OH)D_3 in the circulation. Interestingly, serum 25(OH)D_2 concentrations were markedly elevated in all study participants receiving vitamin D_2, consistent with induction of the vitamin D_2 25-hydroxylation pathway, although there was an opposite effect on 25(OH)D_3 and rate of formation in the same treated individuals (Fig. 3), raising the possibility that different enzymes might catalyze 25-hydroxylation of the two forms of vitamin D. Alternatively, the decline of 25(OH)D_3 following administration of vitamin D_2 may reflect competition of vitamin D_2 for the same 25-hydroxylation pathway as vitamin D_3. Although changes in metabolite-to-parent ratios may reflect alteration in rates of conversion of one metabolite to another, they could also be explained by removal of vitamin D and its metabolites from the circulation (*e.g.*, via direct excretion or disposition into depots such as adipose tissue and muscle). Further investigations to compare the effects of different forms of vitamin D on expression and activity of the enzymes responsible for metabolizing vitamin D_3 are needed to resolve the question of whether changes in metabolite-to-parent ratios truly reflect changes in activity of cytochrome P450 enzymes. Such studies would potentially require liver and renal biopsies: both are invasive procedures and their inclusion in a study protocol could raise issues relating to ethics and acceptability to participants.

One aspect in which our findings differ from those of other investigators relates to the lack of detectable

1,25-dihydroxyvitamin D_2 at follow-up among participants receiving vitamin D_2 in our study. By contrast, Biancuzzo *et al.* reported that daily administration of 1,000 IU vitamin D_2 for 11 weeks induced a mean increase in serum $1,25(OH)_2D_2$ concentration of 5.2 pg/mL (13.5 pmol/L) (14). This difference may reflect use of intermittent bolus dosing in the current study, which contrasts with the daily dosing regimen used by Biancuzzo *et al.*

Our findings provide insights into the differential effects of ergocalciferol and cholecalciferol on vitamin D metabolism, however, from the clinician's perspective, a key question relates to relative effects of the two forms of vitamin D on total 25(OH)D levels, which reflect vitamin D status. Among substudy participants, the mean increase in total 25(OH)D for participants randomized to vitamin D_2 (n = 28) vs vitamin D_3 (n = 25) was 31.4 nmol/L vs 46.4 nmol/L, respectively (P for intergroup comparison = 0.06). This trend is in keeping with findings from the main trial, in which the difference in increase in total 25(OH)D for participants randomized to vitamin D_2 (n = 112) vs D_3 (n= 114; 31.2 vs 38.3 nmol/L increase, respectively) attained statistical significance (P = 0.03).

Our study has several strengths. Participants randomized to vitamin D_2 vs D_3 were well matched with regard to baseline characteristics, and directly observed administration of vitamin D_2 and vitamin D_3 at identical doses via the same route allowed for a head-to-head comparison of their effects. Determination of concentrations of parent vitamin D_2 and D_3, $25(OH)D_2$, $25(OH)D_3$, and its major dihydroxylated metabolites allowed us to compare effects of vitamin D_2 vs vitamin D_3 on both the synthesis and the catabolism of $25(OH)D_3$. Moreover, we utilized the gold standard method (liquid chromatography-tandem mass spectrometry) to measure concentrations of vitamin D and its metabolites with high degrees of accuracy and sensitivity, avoiding issues of cross-reactivity between metabolites of vitamin D_2 and vitamin D_3 that may arise with immunoassays (23).

Our study also has some limitations. We measured concentrations of vitamin D metabolites at a single time point, one month after the fourth bolus dose was given; thus, we do not capture the pharmacokinetics of vitamin D metabolism at multiple points over the period of the dosing interval. In particular, conclusions relating to concentrations of parent vitamin D_2 and vitamin D_3 in the circulation should be guarded, because of their short half-life. Participants all had an elevated risk of type 2 diabetes mellitus: thus, our findings cannot necessarily be generalized to other groups. However, we have no specific reason to believe that effects of vitamin D_2 are likely to be different in this group compared with the general population. We did not measure concentrations of 1,24,25-trihydroxyvitamin D_3 or $24R,25(OH)_2D_2$: this could have provided insights into the effects of vitamin D_2 vs vitamin D_3 on 24-hydroxylation of $1\alpha,25(OH)_2D_3$ and $25(OH)D_2$, respectively. Preparations of vitamin D_2 and vitamin D_3 were presented in different vehicles (alcohol vs oily solution, respectively), which could theoretically have impacted differently on absorption and/or metabolism. However, a study in schoolchildren comparing ethanol vs oil as a vehicle for a weekly oral dose of 14,000 IU vitamin D_3 showed no difference in the 25(OH)D response to supplementation between groups over eight weeks (24), rendering this explanation for the findings in the current study unlikely.

In conclusion, the current study confirms reports that vitamin D_2 is less effective than vitamin D_3 in elevating total 25(OH)D levels, and extends prior findings by showing that administration of vitamin D_2 reduces 25-hydroxylation of vitamin D_3 and 1-α hydroxylation of $25(OH)D_3$ and increases 24R-hydroxylation of $25(OH)D_3$.

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