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Impact of a single oral dose of 100,000 IU vitamin D3 on profiles of serum 25(OH)D3 and its metabolites 24,25(OH)$_2$D3, 3-epi-25(OH)D3, and 1,25(OH)$_2$D3 in adults with vitamin D insufficiency

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Abstract

Background: We investigate the effect of a high dose of vitamin D3 on circulating concentrations of 25(OH)D3 and its metabolites 24,25(OH)$_2$D3, 3-epi-25(OH)D3, and 1,25(OH)$_2$D3 in healthy individuals with self-perceived fatigue and vitamin D insufficiency [25(OH)D3 <50 nmol/L].

Methods: One hundred and seven study participants (age 20–50 years) were randomized to receive a single 100,000 IU dose of vitamin D3 (n = 52) or placebo (n = 55). Vitamin D metabolite concentrations in serum were measured before, and 4 weeks after, supplementation.

Results: Overall, 52% of participants receiving vitamin D3 attained a serum 25(OH)D3 level >75 nmol/L. Among individuals who received vitamin D3, there were significant increases in serum concentrations of 25(OH)D3 and its metabolites 24,25(OH)$_2$D3, 3-epi-25(OH)D3, and 1,25(OH)$_2$D3 at 4 weeks; however, inter-individual variability in these changes was substantial. Positive correlations between serum 25(OH)D3 and 24,25(OH)$_2$D3 and 3-epi-25(OH)D3, and a significant negative correlation between serum 1,25(OH)$_2$D3 and 3-epi-25(OH)D3, were found 4 weeks after supplementation. The 24,25(OH)$_2$D3/25(OH)D3 and 24,25(OH)$_2$D3/1,25(OH)$_2$D3 ratios were significantly increased, compared with baseline, in participants receiving vitamin D3. Baseline 25(OH)D3 concentration was the only factor predictive of the change in 25(OH)D3 after supplementation.

Conclusions: Administration of a single high dose of vitamin D3 leads to a significant increase in concentrations of 25(OH)D3, 24,25(OH)$_2$D3, 3-epi-25(OH)D3 and 1,25(OH)$_2$D3; induction of the catabolic pathway predominates over the production of 1,25(OH)$_2$D3. Due to the high inter-individual variation in the 25(OH)D3 response to supplementation, any given dose of vitamin D is unlikely to achieve optimal vitamin D status in all treated individuals

Keywords: 1,25(OH)$_2$D3; 24,25(OH)$_2$D3; 25(OH)D3; 3-epi-25(OH)D3; supplementation; vitamin D.

Introduction

Vitamin D plays a key role in the regulation of calcium and phosphate homeostasis, and deficiency of this vitamin is associated with secondary hyperparathyroidism, an increase in bone turnover and bone loss [1]. Vitamin D synthesized in the skin [vitamin D3 (cholecalciferol)] or orally ingested [either vitamin D3 or vitamin D2 (ergocalciferol)] is metabolized in the liver by the enzyme 25-hydroxylase (CYP2R1) to form 25-hydroxy vitamin D3 [25(OH)D3], which is then further metabolized primarily in the kidney by 1α-hydroxylase (CYP27B1) to form the active vitamin D metabolite, 1,25-dihydroxy vitamin D3 [1,25(OH)$_2$D3]. Both 25(OH)D3 and 1,25(OH)$_2$D3 undergo further metabolism, predominantly by renal 24-hydroxylase (CYP24A1), to generate 24,25-dihydroxy vitamin D3 [24,25(OH)$_2$ D3] and 1,24,25-trihydroxyvitamin D3 [1α,24,25(OH)$_3$D3], respectively [2–4]. Mutations in the CYP24A1 gene are associated with partial or total loss of 24-hydroxylase activity, which in turn leads to hypercalcaemic conditions [5–7]. The production of 24,25(OH)$_2$D3 has been shown to be 25(OH)D3-dependent, and is moderately affected by

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vitamin D supplementation [5, 6]; the physiological role of this metabolite remains to be established, although it is known to be involved in embryogenesis, cartilage development and fracture repair [8–10].

Measurement of total 25(OH)D [comprising both 25(OH)D3 and 25(OH)D2] in serum is widely accepted as a marker of vitamin D status; however, the optimum threshold concentration of 25(OH)D continues to be debated. The Institute of Medicine (IOM) recommends a threshold of 50 nmol/L for bone health [11], whereas the Endocrine Society recommend a threshold of 75 nmol/L for optimal reductions in fall or fracture risk [1].

The 25(OH)D3 response to vitamin D supplementation varies markedly between individuals, and a significant proportion of patients may have persistent suboptimal levels despite supplementation [12–17]. Furthermore, the relationship between circulating 25(OH)D3 concentrations and clinical outcomes such as osteoporosis and fracture risk may differ between racial groups, raising the question of whether 25(OH)D3 provides a reliable estimate of vitamin D status in all populations [18, 19]. For these reasons, increasing attention is being paid to the measurement of 24,25$(OH)_2$D3 (the major circulating catabolite of vitamin D), and the ratio of 24,25$(OH)_2$D3 to 25(OH)D3, as potential markers of vitamin D catabolism and predictors of the serum 25(OH)D response to vitamin D supplementation [5, 6, 12, 18, 20].

Measurement of vitamin D metabolites as biomarkers of vitamin D status has been further complicated in recent years by the identification of C3 epimeric forms of 25(OH)D3 and 1,25$(OH)_2$D3 [21]. These epimers were originally identified in infants and neonates, in whom they account for approximately 21% of total 25(OH)D3 concentrations [21], but were subsequently shown to be present in lower concentrations in adults, in whom they account for approximately 6% of total 25(OH)D3 [21–23]. The 3-epi-25(OH)D3 metabolite is produced endogenously, and circulating concentrations increase following vitamin D supplementation [22]; however, the physiological significance of these epimers remains to be established [20, 21].

In view of the continuing uncertainty surrounding the clinical utility of different vitamin D metabolites as markers of vitamin D status, and to better understand the vitamin D metabolism pathway in response to supplementation, the present study was performed to investigate the effect of a single high dose (100,000 IU) of vitamin D3 on profiles of circulating 25(OH)D3 and its metabolites 24,25$(OH)_2$D3, 3-epi25(OH) D3, and 1,25$(OH)_2$D3 in healthy individuals with self-perceived fatigue and vitamin D insufficiency [25(OH)D3 <50 nmol/L], and to assess the inter-individual variability in the response to vitamin D supplementation. A further objective was to investigate the hypothesis that the baseline 24,25$(OH)_2$D3/25(OH)D3 ratio is a predictor of the response to supplementation.

Materials and methods

Clinical samples

Frozen serum samples (n = 214) were obtained from a prospective randomized, double-blind, placebo-controlled clinical trial conducted at the University Hospital of Zurich, Switzerland (latitude 47°22′ N) (ClinicalTrials.gov Registry number NCT02022475). The trial was conducted in accordance with the declaration of Helsinki and Good Clinical Practice guidelines; the study protocol and its amendment were approved by the Zurich Cantonal Ethical Committee and Swissmedic, and informed consent was obtained from all participants prior to enrolment. The primary aim of the trial was to determine the effects of a single high dose of vitamin D3, compared with placebo, on serum 25(OH)D3 concentrations and clinical outcomes such as fatigue at 4 weeks after treatment. Full details of this trial has been described elsewhere [23].

The trial involved 107 participants [age 20–50 years, body mass index (BMI) 18–25 kg/m^2] who had serum 25(OH)D3 concentrations below 50 nmol/L. The 50 nmol/L threshold for vitamin D insufficiency was used in accordance with the recommendation of the Institute of Medicine (IOM) [11]. Participants were randomized to receive either a single 100,000 IU dose of vitamin D3 (n = 52) or placebo (n = 55).

Blood samples were obtained at a screening visit immediately before treatment and at a second visit 4 weeks after supplementation. Serum was separated by centrifugation at 2000 *g* for 10 min, and aliquots were stored at −80 °C prior to analysis. Serum concentrations of 25(OH)D3, 3-epi 25(OH)D3, 24,25$(OH)_2$D3 and 25-hydroxy vitamin D2 [25(OH)D2] were measured by a validated NIST traceable LC-MS/MS assay using a Micromass Quattro Ultima Pt mass spectrometer (Waters Corp., Milford, MA, USA) at Bioanalytical Facility, University of East Anglia, Norwich, UK; details of the assay are provided in the online supplementary material. For all analytes, the assay showed good linearity ($r^2 \geq 0.98$) and low intra-assay and inter-assay variability (see Supplementary Table S1).

Measurements of total 1,25$(OH)_2$D3 were performed using a commercial immunoextraction enzyme immunoassay kit (IDS, Bolden, UK). The inter- and intra-assay imprecision, as expressed by the coefficient of variation (CV), was less than 12.5%. Serum concentrations of calcium, phosphate, parathyroid hormone (PTH), C-reactive protein (CRP), and creatinine were measured using a Cobas 8000 analyzer (Roche Diagnostics, Mannheim, Germany) at the Institute of Clinical Chemistry, University Hospital of Zurich. All analyses were carried out according to the manufacturer's instructions. For all analytes, intra-assay and inter-assay variability, as expressed by the coefficient of variation (CV), were ≤1.7% and 3.1%, respectively.

Statistical analyses

Demographic data and serum concentrations of vitamin D metabolites at baseline and follow-up were summarized using descriptive

statistics (means, SDs, medians and interquartile ranges). Differences between baseline and post-supplementation values were analysed by means of paired t tests for vitamin D metabolites, unpaired t-tests for normally distributed demographic variables, Mann-Whitney rank tests for non-normally distributed variables, and χ^2 tests for categorical variables. All comparisons were two-sided. Associations between vitamin D3 metabolites, and other clinical variables (age, BMI, serum calcium, serum phosphate and serum PTH), at baseline and at 4 weeks after supplementation were investigated using Spearman rank correlation analysis.

Simple and multiple regression analyses were used to build prediction models for the 25(OH)D3 response to vitamin D3 supplementation. Four different models were used: model 1 included only baseline 25(OH)D3 concentrations as covariate; model 2 included baseline 25(OH)D3, 24,25$(OH)_2$D3 and 3-epi-25(OH)D3 concentrations as covariates; model 3 included the same covariates as model 2 in addition to age, gender and body mass index (BMI), while model 4 included baseline 1,25$(OH)_2$D3 concentrations in addition to the same covariates as model 2. All analyses were performed using IBM SPSS Statistics 22 software (SPSS Inc., Chicago, IL, USA), and p-values below 0.05 were considered significant.

Results

Baseline demographic and clinical characteristics of study participants are summarized in Table 1. No statistically significant differences between the vitamin D supplemented and placebo groups were observed. At baseline, 3-epi-25(OH)D3 was present in 88% of study participants, at a mean concentration equivalent to 3.9% of serum 25(OH)D3 concentrations (Table 1).

Changes in vitamin D metabolites following vitamin D supplementation

Serum concentrations of vitamin D metabolites at baseline are summarized in Table 1, and changes in these concentrations 4 weeks after a single oral dose of 100,000 IU vitamin D3 are presented in Figure 1. At 4 weeks, participants receiving vitamin D3 showed significant absolute increases in serum 25(OH)D3, 24,25$(OH)_2$D3, 3-epi-25(OH)D3 and 1,25$(OH)_2$D3 concentrations (all $p < 0.001$ vs. baseline), whereas no such changes were seen in placebo-treated participants.

Interestingly, the ratios of 24,25$(OH)_2$D3 to 25(OH)D3 and 24,25(OH)2D3 to 1,25(OH)3D3 were significantly increased, compared with baseline, in study participants receiving vitamin D3 supplementation (Figure 1). The mean 24,25(OH)D3/25(OH)D3 ratio at baseline was 0.076 ± 0.02, and this had increased to 0.086 ± 0.02 ($p = 0.006$) at 4 weeks after supplementation. Similarly, the ratio of 24,25$(OH)_2$D3 to 1,25$(OH)_2$D3 increased 2.4-fold after vitamin D3 supplementation, from 0.023 ± 0.01 at baseline to 0.056 ± 0.025 ($p < 0.0001$) at 4 weeks. In participants receiving placebo, both ratios remained unchanged following supplementation ($p = 0.36$ and $p = 0.92$, respectively, vs. baseline), as shown in Figure 1E and F.

At 4 weeks after dosing, all participants in the vitamin D3 group had attained a serum 25(OH)D3 concentration ≥50 nmol/L, except for one patient in whom the 25(OH)D3 concentration increased from a baseline value of 17.5 nmol/L to 35.6 nmol/L. Overall, 52% of participants

Table 1: Baseline demographic and clinical characteristics.

	Therapy (n = 52)	Placebo (n = 55)	p-Value
Age, years	29 (6)	28 (6)	0.30
Gender, females/males	27/25 (52%/48%)	26/29 (47%/53%)	0.15[a]
BMI, kg/m^2	22 (2)	22 (2)	0.54
Arterial blood pressure, mmHg			
Systolic	123 (11)	126 (11)	0.16
Diastolic	78 (9)	77 (8)	0.44
Parathyroid hormone, ng/L	44 (16)	46 (18)	0.59
Calcium, mmol/L[b]	2.23 (0.07)	2.22 (0.07)	0.97
Phosphate, mmol/L	0.99 (0.18)	1.00 (0.15)	0.69
Creatinine, µmol/L	71 (14)	75 (13)	0.13
C-reactive protein, mg/L[c]	0.5 (0.0–1.2)	0.6 (0.3–1.8)	0.27
24,25$(OH)_2$D3, nmol/L	2.2 (0.9)	2.5 (1.0)	0.08
25(OH)D3, nmol/L	28 (9)	32 (11)	0.06
1,25$(OH)_2$D3, pmol/L	100 (29)	94 (25)	0.23
3-epi-25(OH)D3, nmol/L	1.0 (0.9)	1.3 (0.93)	0.08
25(OH)D2, nmol/L[c]	1.8 (1.1–2.2)	2 (1.4–2.6)	0.06

Data are shown as mean (SD), and groups were compared using unpaired two-sided t tests, unless indicated otherwise. [a]χ^2-test; [b]adjusted for serum albumin concentrations; [c]median (interquartile range).

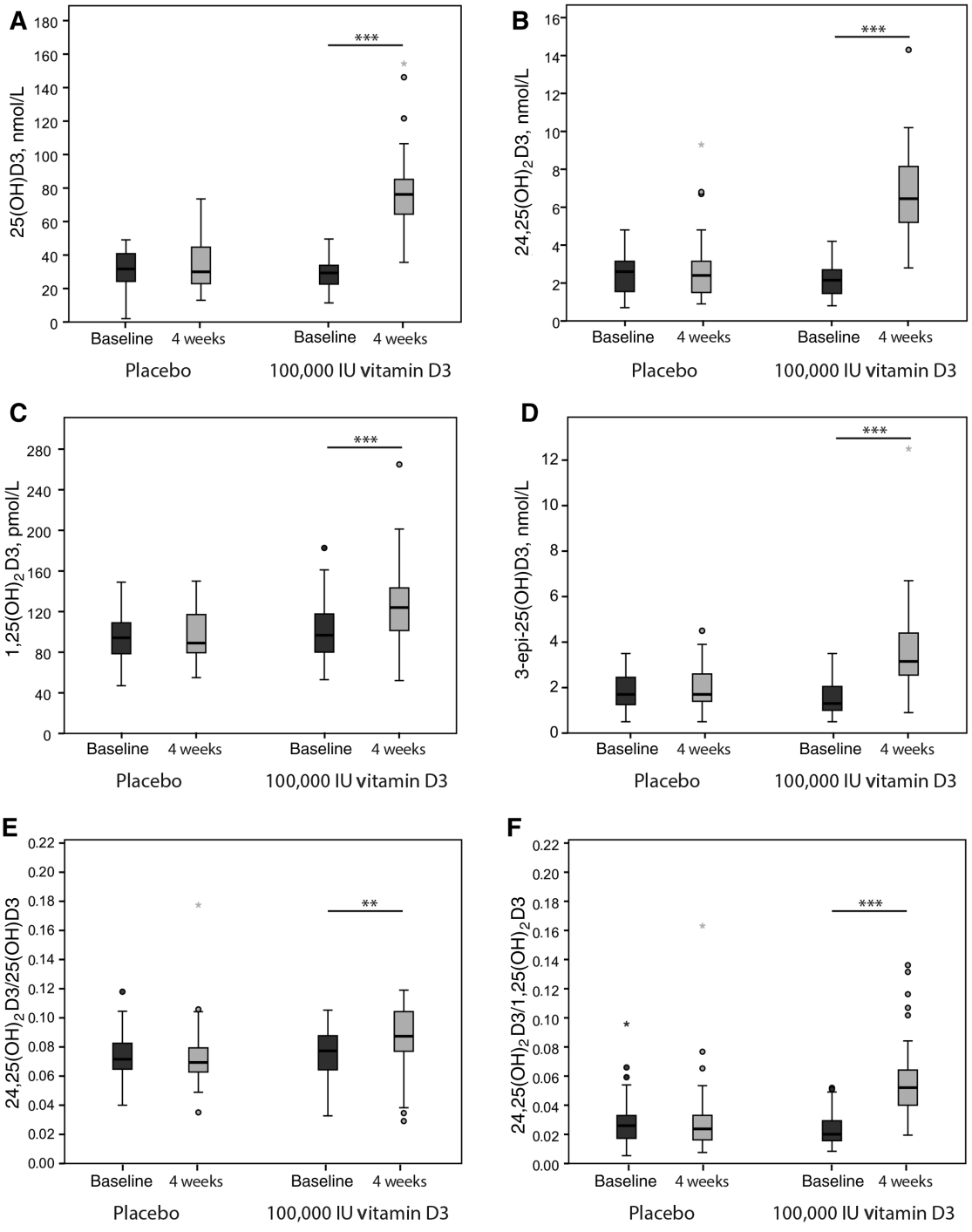

Figure 1: Absolute changes in vitamin D metabolites from baseline (dark shading) to 4 weeks after (light shading) a single 100,000 IU oral dose of vitamin D3.
(A) 25(OH)D3; (B) 24,25(OH)D3; (C) 1,25$(OH)_2$ D3; (D) 3-epi-25(OH)D3; (E) 24,25$(OH)_2$D3/(25(OH)D3; (F) 24,25$(OH)_2$D3/1.25$(OH)_2$D3.

receiving vitamin D3 supplementation attained a serum 25(OH)D3 concentration of >75 nmol/L, while 46% attained a serum 25(OH)D3 concentration between 50 and 75 nmol/L. No significant differences were observed in vitamin D metabolite concentration changes from baseline in study subjects who attained 25(OH)D3 concentration between 50 and 75 nmol/L, as compared to those who attained a serum 25(OH)D3 concentration >75 nmol/L (Table 2).

Substantial inter-individual variability in changes in serum 25(OH)D3, 3-epi-25(OH)D3, 24,25$(OH)_2$D3 and 1,25$(OH)_2$D3 was observed following administration of 100,000 IU vitamin D3. This variability was not dependent

Table 2: Mean (±SD) vitamin D metabolite concentration changes from baseline in supplemented subjects who attained serum 25(OH)D3 concentrations between 50 and 75 nmol/L vs. those who attained a serum 25(OH)D3 concentration >75 nmol/L, 4 weeks after a single oral dose of 100,000 IU vitamin D3 administration.

Vitamin D metabolites	50–75 nmol/L (n=24)	>75 nmol/L (n=27)	p-Value
25(OH)D3, nmol/L	39.2±10.3	59.6±18.9	<0.001
$24,25(OH)_2D3$, nmol/L	3.9±1.2	4.9±2.8	0.13
$1,25(OH)_2D3$, pmol/L	20.9 (−29.4–78.0)[a]	32.3 (−45.8–83.5)[a]	0.20[a]
3-epi-25(OH)D3, nmol/L	1.7±1.6	2.4±1.9	0.17
$24,25(OH)_2D3/25(OH)D3$	0.091±0.016	0.081±0.026	0.10
$24,25(OH)_2D3/1,25(OH)_2D3$	0.053±0.017	0.060±0.031	0.36

[a]Median (5th–95th percentile), Mann-Whitney test.

on baseline serum levels of the respective analytes, as shown in Figure 2.

Overall, 25(OH)D3 accounted for approximately 89%–90% of circulating vitamin D metabolites at baseline, $24,25(OH)_2D3$ accounted for 7%, and 3-epi-25(OH)D3 for approximately 3%–4%. These proportions did not change after vitamin D3 supplementation (Figure 3).

Correlations between vitamin D3 metabolites before and after vitamin D3 supplementation

In the overall study population (n=107), there were significant correlations at baseline between serum concentrations of 25(OH)D3 and $1,25(OH)_2D3$, $24,25(OH)_2D3$ or 3-epi-25(OH)D3 (ρ=0.39, 0.86 and 0.36, respectively;

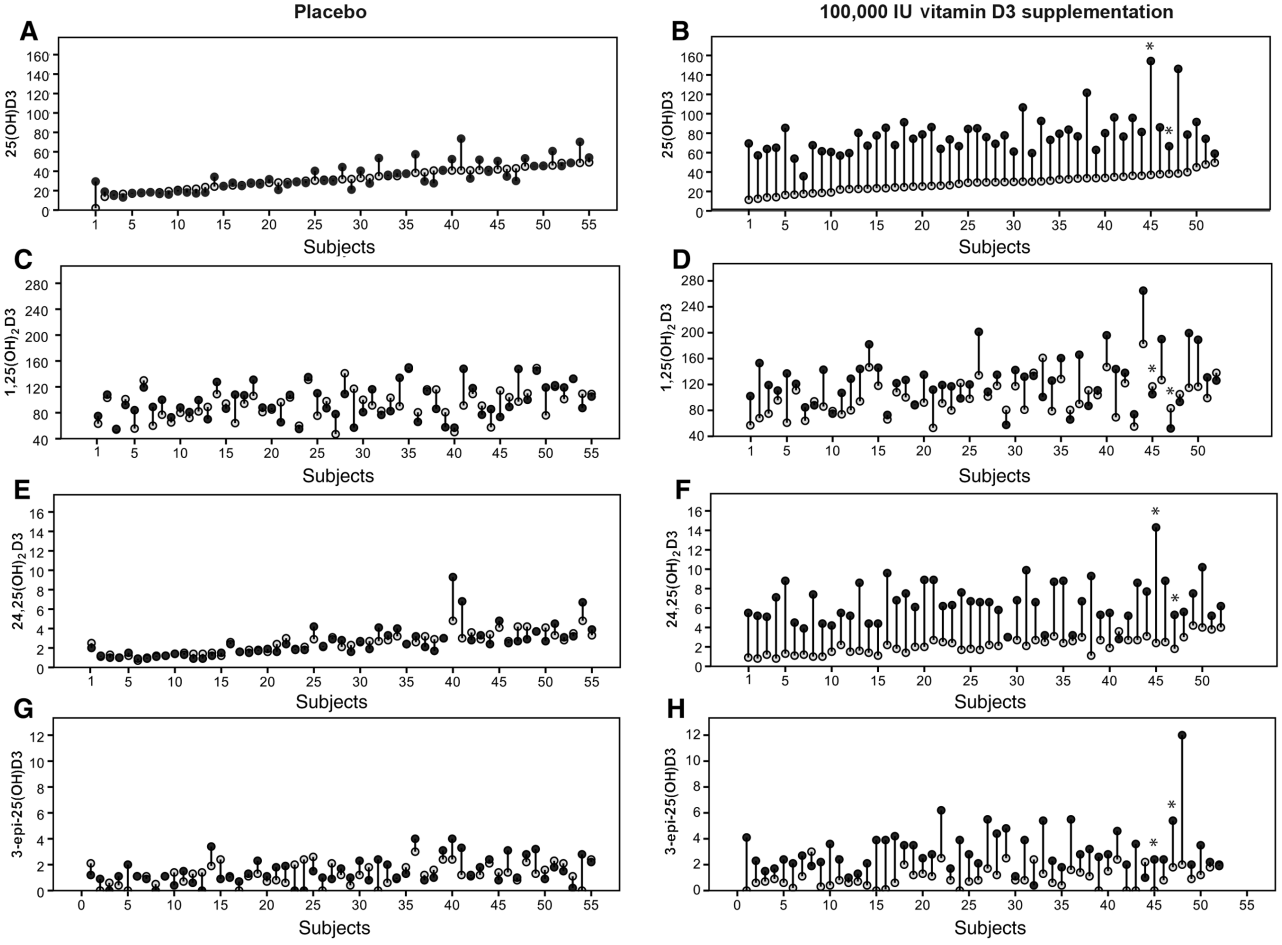

Figure 2: Changes in serum 25(OH)D3 (nmol/L) (A, B), 1,25(OH)2D3 (pmol/L) (C, D), 24,25(OH)2D3 (nmol/L) (E, F), 3-epi-25(OH)D3 (nmol/L) (G, H) concentrations from baseline to 4 weeks after vitamin D supplementation in individual participants.
Open circles: baseline, black-filled circles: post-supplementation; asterisks indicate participants specifically referred to in the discussion.

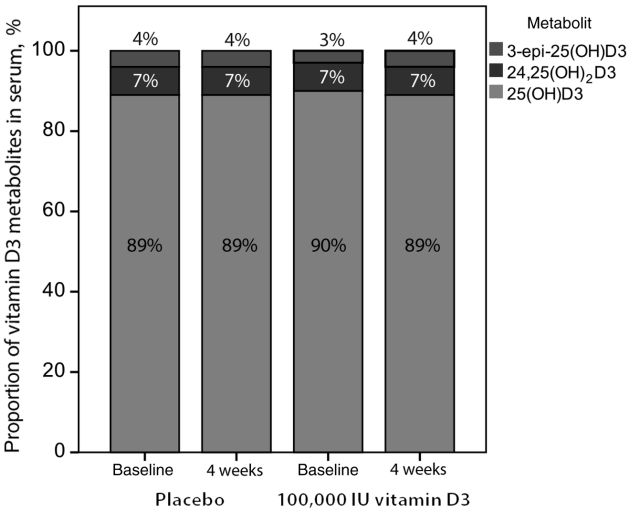

Figure 3: Relative proportions of vitamin D3 metabolites in serum at baseline (visit A) and 4 weeks after a 100,000 IU single oral dose of vitamin D3 or placebo (visit B).

$p < 0.001$ for all) as shown in Figure 4. Serum concentrations of $24,25(OH)_2D3$ at baseline correlated significantly with 3-epi-25(OH)D3 ($\rho = 0.37$, $p < 0.001$), but there were no other significant correlations between the other metabolites. There were also weak but significant correlations at baseline between serum 25(OH)D3 and calcium concentrations ($\rho = 0.24$, $p = 0.013$), and between serum $24,25(OH)_2D3$ and PTH concentrations ($\rho = 0.20$, $p = 0.043$).

Among participants who received vitamin D3 supplementation ($n = 52$), there were significant positive correlations at 4 weeks between serum 25(OH)D3 concentrations and $24,25(OH)_2D3$ ($\rho = 0.47$, $p < 0.001$) and 3-epi-25(OH)D3 ($\rho = 0.35$, $p = 0.011$), and a significant negative correlation between serum $1,25(OH)_2D3$ and 3-epi-25(OH)D3 ($\rho = -0.46$, $p < 0.001$). The change in serum 25(OH)D3 concentrations from baseline to 4 weeks after supplementation was significantly correlated with the change in $24,25(OH)_2D3$ concentrations ($\rho = 0.49$, $p < 0.0001$), but not with changes in $1,25(OH)D_2D3$ concentrations ($\rho = 0.05$, $p = 0.71$).

Predictors of 25(OH)D3 response to vitamin D3 supplementation

Multiple regression analyses were performed to identify predictors of the 25(OH)D3 response to vitamin D3 supplementation. The results of these analyses are summarized in Table 3. The variance in the 25(OH)D3 level after supplementation explained by a simple regression model that included only 25(OH)D3 at baseline was 15% ($R^2 = 0.17$, $F(1,50) = 10.2$, $p = 0.002$) Adjustment for other vitamin D3 metabolites [$1,25(OH)_2D3$, $24,25(OH)_2D3$ or 3-epi-25(OH)D3], age, sex or BMI did not further improve the prediction of 25(OH)D3 levels after supplementation. Similarly, other putative markers of vitamin D3 status, including the $24,25(OH)_2D3$/25(OH)D3 ratio alone or in combination with age, sex, and BMI were not predictive of 25(OH)D3 concentrations after vitamin D3 supplementation. None of the regression models could predict the variance in the 25(OH)D3 change after supplementation (Table 3).

Changes in other circulating biomarkers of calcium homeostasis

Participants receiving vitamin D supplementation showed a significant decrease in PTH concentrations at 4 weeks, whereas PTH concentrations were increased in placebo-treated participants (mean change -2.6 ± 13 vs. 3.9 ± 18 ng/L, respectively; $p = 0.03$). Calcium and phosphate concentrations remained unchanged in both groups.

Discussion

This study has shown that serum concentrations of 25(OH)D3, $24,25(OH)_2D3$, 3-epi-25(OH)D3 and $1,25(OH)_2D3$ all increase significantly 4 weeks after a single high oral dose of 100,000 IU vitamin D3, whereas no such changes are seen in placebo-treated participants. The increase in 25(OH)D3 concentrations after supplementation was significantly associated with the increase in $24,25(OH)_2D3$ concentrations after supplementation.

Taking the $24,25(OH)_2D$ values and the ratio of $24,25(OH)_2D$/25(OH)D3 and $24,25(OH)_2D3/1,25(OH)_2D3$ as markers of vitamin D catabolism, we found significant increases in these variables following supplementation with a high dose of vitamin D3, which indicates induction of the vitamin D catabolic pathway. This suggests that, when adequate amounts of biologically active vitamin D are available, the production of the vitamin D catabolite 24,25(OH)D is favoured over the active metabolite 1,25(OH)D3, due to increased activity of 24-hydroxylase (CYP24A1), thereby avoiding excessive production of 1,25(OH)D3 and associated toxicity. Interestingly, a previous study from our group, which analysed vitamin D metabolite profiles in three supplementation studies, showed that the production of $24,25(OH)_2D3$ is favoured over $1,25(OH)_2D3$ following administration of high doses of vitamin D3, compared with lower doses [20].

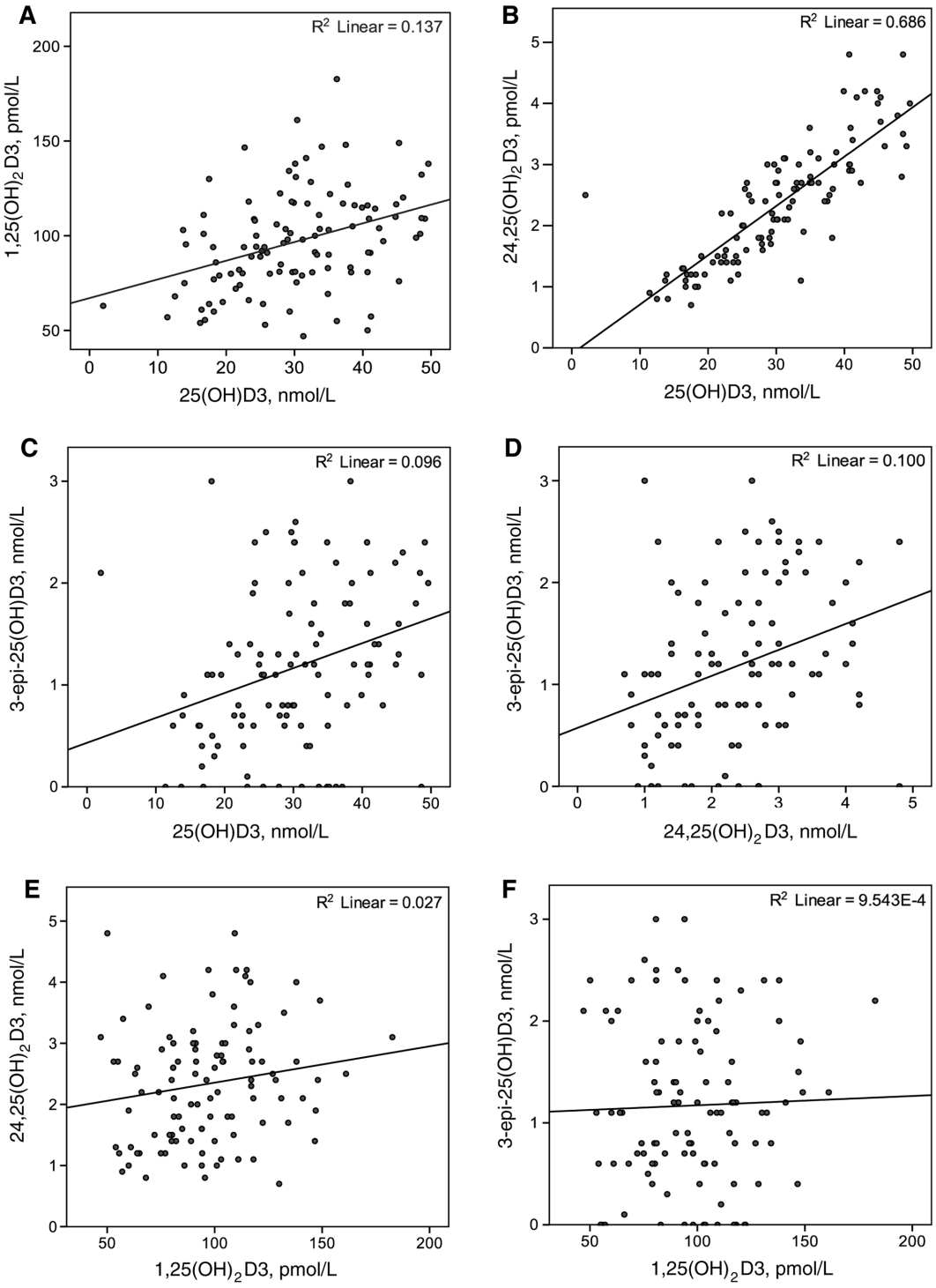

Figure 4: Correlations between baseline concentrations of vitamin D metabolites.

The majority of participants receiving vitamin D3 attained serum 25(OH)D3 concentrations above 50 nmol/L, a widely accepted threshold for vitamin D insufficiency [11], but only 52% of subjects attained serum 25(OH)D3 concentrations above 75 nmol/L. This indicates that the use of a single high dose of vitamin D is not sufficient to ensure that adequate vitamin D levels are attained in all study participants. This would be consistent with the finding by Binkley et al. [14] that suboptimal 25(OH)D3 levels persisted in approximately 20% of individuals despite dosing with

Table 3: Regression models for the 25(OH)D3 response to vitamin D3 supplementation.

Model	Covariate	β coefficient (95% CI)	p-Value
Model 1	25(OH)D3	0.41 (0.36 to 1.58)	0.002
Model 2	25(OH)D3	0.71 (0.58 to 2.79)	0.004
	24,25(OH)2D3	−0.34 (−19.17 to 3.17)	0.156
	3-epi-(OH)2D3	−0.06 (−8.86 to 5.56)	0.648
Model 3	25(OH)D3	0.74 (0.57 to 2.93)	0.005
	24,25(OH)2D3	−0.38 (−20.61 to 2.60)	0.125
	3-epi-(OH)2D3	−0.01 (−7.78 to 7.21)	0.939
	Age	−0.18 (−1.58 to 0.34)	0.203
	Sex	0.005 (−11.02 to 11.40)	0.973
	BMI	−0.11 (−4.05 to 1.79)	0.440
Model 4	25(OH)D3	0.84 (0.73 to 3.21)	0.002
	24,25(OH)2D3	−0.41 (−21.3 to 1.96)	0.101
	3-epi-(OH)2D3	−0.05 (−8.61 to 5.84)	0.702
	1,25(OH)2D3	−0.15 (−0.33 to 0.11)	0.308

Model summaries: Model 1: $R^2 = 0.17$, adjusted $R^2 = 0.15$, $F(1,50) = 10.2$, $p = 0.002$; Model 2: $R^2 = 0.21$, adjusted $R^2 = 0.16$, $F(3,48) = 4.3$, $p = 0.009$; Model 3: $R^2 = 0.27$, adjusted $R^2 = 0.17$, $F(6,45) = 4.3$, $p = 0.023$; Model 4: $R^2 = 0.23$, adjusted $R^2 = 0.16$, $F(3,47) = 4.3$, $p = 0.014$.

vitamin D3, 50,000 IU monthly, for 1 year. Furthermore, our results demonstrate large inter-individual variations in the increase in 25(OH)D3 and 24,25$(OH)_2$D concentrations following administration of 100,000 IU vitamin D3. In addition, we provide the first evidence that the increase in the 3-epimer 25(OH)D metabolite following vitamin D supplementation also shows large inter-individual variation in adults, probably due to modifying factors, as has previously been described for 25(OH)D3 and 24,25$(OH)_2$D3 [5, 12, 14, 16]. This inter-individual variability in both 24,25(OH)D3 and 3-epi-25(OH)D3 contributes to the observed inter-individual variation in the response to vitamin D3 supplementation. For example, looking at Figure 2, it can be seen that participants 45 and 47 in the vitamin D supplementation group had similar baseline concentrations of 25(OH)D3, but the increases in 24,25$(OH)_2$D3 and 3-epi25(OH)D3 following supplementation differed markedly between the two participants. These large individual variations in the response to supplementation should be taken into account when giving recommendations for vitamin D supplementation. Clearly, a single fixed dose of vitamin D will not suffice to ensure adequate 25(OH)D levels in all patients unless the dose is very large, thereby increasing the risk of toxicity [16]. It is therefore desirable to tailor the dose of vitamin D in order to achieve pre-specified 25(OH)D3 targets in individual patients [16].

Several factors may contribute to the inter-individual variability in the response to vitamin D supplementation, including BMI, baseline 25(OH)D3 concentrations and genetic factors. Single nucleotide polymorphisms (SNPs) involved in the synthesis (DHCR7 and CYP2R1), binding and transportation (DBP/GC) and degradation (CYP24A1) of vitamin D and its metabolites have been shown to contribute to differences in the vitamin D response to supplementation [15, 24–26]. In contrast to findings from other studies [12], the change in 25(OH)D3 concentrations after therapy in our study was not dependent on the age and BMI of the study participants at baseline. This could be due to the narrow age and BMI ranges of the participants in our study (age: 29 ± 6 years; BMI: 22 ± 2 kg/m^2).

The well accepted negative correlation between baseline levels of 25(OH)D3, and the increase in this metabolite following supplementation [12, 27], was not seen in this study. Similar negative findings have been reported by Binkley et al. [16]. This lack of correlation in our study may be due to the short time period over which concentrations were measured, and the fact that only a single dose was used. In our regression model including only 25(OH)D3 at baseline, the baseline value explained 15% of the variance in the 25(OH)D3 concentration after supplementation. The inclusion of other vitamin D3 metabolites in the regression models did not improve the predictive power of baseline 25(OH)D3, and the 24,25$(OH)D_2$D3/25(OH)D3 ratio was not predictive of the 25(OH)D3 response.

The epimeric metabolite 3-epi-25(OH)D3 was present in 88% of participants at baseline in this study, at a mean concentration equivalent to 3.5% of serum 25(OH)D3 concentrations. This finding is consistent with previous studies that found vitamin D3 epimers to be present in adults, albeit in lower concentrations than in infants [17, 21, 27, 28]. However, the physiological significance of these metabolites is unknown [21, 22]. Due to the low concentrations of vitamin D epimers in adults, the inclusion of 3-epi-25(OH)D3 has only a marginal effect on the classification of vitamin D status [29]. In the present study, 3-epi-25(OH)D3 concentrations were not predictive of the increase in 25(OH)D3 following supplementation.

To our knowledge, this is the first study to report the concentrations of key vitamin D metabolites following the administration of a high oral dose of vitamin D3 in young healthy adults with vitamin D deficiency/insufficiency. It is possible that changes in vitamin D metabolites after vitamin D administration might be different in the elderly as compared to young adults. Further studies are required to address the impact of vitamin D supplementation on key vitamin D metabolite concentration changes in elderly as vitamin D deficiency/insufficiency is more common in elderly subjects. Limitations of the study include the small sample size, the narrow age and BMI ranges of the

participants and the short and non-comprehensive follow-up after supplementation. As described by Binkely et al. [14], following administration of 50,000 IU vitamin D3, 25(OH)D3 concentrations rise rapidly and reach a peak after 3 days, whereas in our study blood collection was only performed 4 weeks after dosing. An analysis of the kinetics of vitamin D catabolism by measuring changes in $24,25(OH)_2D$ concentrations over time following supplementation would be of great interest. We did not analyse the activities of enzymes involved in the enzymatic conversion of vitamin D metabolites (CYP27B1, CYP2R1, and CYP24A1), or polymorphisms of these enzymes. Moreover, we did not assess the genetic variants of vitamin D binding protein, which is well known to affect the response to vitamin D3 supplementation [30].

In conclusion, this study has shown that administration of a single high oral dose of vitamin D3 leads to a significant increase in concentrations of 25(OH)D3 and its metabolites 24,25(OH)2D3, 3-epi (OH)D3 and 1,25(OH)2D3, with induction of the catabolic pathway predominating over the production of the active metabolite 1,25(OH)D3. The study has also highlighted the substantial heterogeneity in the 25(OH)D response to supplementation, which means that any given dose of vitamin D is unlikely to achieve optimal vitamin D status in all treated individuals. New cost-effective screening strategies are urgently needed to avoid the current trend toward universal supplementation on sight, and to help identify individuals requiring lower- or higher-dose vitamin D supplements: it should be emphasised that high doses of vitamin D are often counter-productive as they may not achieve an adequate increase in 25(OH)D.

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