

## Long-term vitamin D<sub>3</sub> supplementation is more effective than vitamin D<sub>2</sub> in maintaining serum 25-hydroxyvitamin D status over the winter months

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### Abstract

Public health recommendations do not distinguish between vitamin D<sub>2</sub> and vitamin D<sub>3</sub>, yet disagreement exists on whether these two forms should be considered equivalent. The objective of the present study was to evaluate the effect of a daily physiological dose of vitamin D<sub>2</sub> or vitamin D<sub>3</sub> on 25-hydroxyvitamin D (25(OH)D) status over the winter months in healthy adults living in Dunedin, New Zealand (latitude 46°S). Participants aged 18–50 years were randomly assigned to 25 µg (1000 IU) vitamin D<sub>3</sub> (*n* 32), 25 µg (1000 IU) vitamin D<sub>2</sub> (*n* 31) or placebo (*n* 32) daily for 25 weeks beginning at the end of summer. A per-protocol approach, which included ≥90% supplement compliance, was used for all analyses. Serum 25-hydroxyvitamin D<sub>3</sub> (25(OH)D<sub>3</sub>), 25-hydroxyvitamin D<sub>2</sub> (25(OH)D<sub>2</sub>) and parathyroid hormone (PTH) were measured at baseline and at 4, 8, 13 and 25 weeks. Geometric mean total serum 25(OH)D concentrations (sum of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>) at baseline was 80 nmol/l. After 25 weeks, participants randomised to D<sub>2</sub> and placebo had a significant reduction in serum 25(OH)D<sub>3</sub> concentrations over the winter months compared with vitamin D<sub>3</sub>-supplemented participants (both *P*<0.001). Supplementation with vitamin D<sub>2</sub> increased serum 25(OH)D<sub>2</sub> but produced a 9 (95% CI 1, 17) nmol/l greater decline in the 25(OH)D<sub>3</sub> metabolite compared with placebo (*P*<0.036). Overall, total serum 25(OH)D concentrations were 21 (95% CI 14, 30) nmol/l lower in participants receiving vitamin D<sub>2</sub> compared with those receiving D<sub>3</sub> (*P*<0.001), among whom total serum 25(OH)D concentrations remained unchanged. No intervention-related changes in PTH were observed. Daily supplementation of vitamin D<sub>3</sub> was more effective than D<sub>2</sub>; however, the functional consequence of the differing metabolic response warrants further investigation.

**Key words:** Vitamin D<sub>2</sub>; Vitamin D<sub>3</sub>; Serum 25-hydroxyvitamin D; Parathyroid hormone

During recent years, a number of studies have been performed in human subjects to investigate the relative potencies of two commonly used forms of vitamin D, ergocalciferol (vitamin D<sub>2</sub>) and cholecalciferol (vitamin D<sub>3</sub>)<sup>(1–10)</sup>. Vitamin D<sub>3</sub>, the form produced in the skin of humans after exposure of 7-dehydrocholesterol to sunlight, is found either naturally in animal products such as fatty fish and cod-liver oil, or added as a fortificant to foods. Commercial production of vitamin D<sub>3</sub> is performed by UV irradiation of 7-dehydrocholesterol extracted from the lanolin of sheep wool. Vitamin D<sub>2</sub> is made either naturally or synthetically from the UV irradiation of ergosterol obtained from yeast, and added to foods. Structurally, vitamin D<sub>2</sub> differs from vitamin D<sub>3</sub> in that its side chain has an added methyl group on carbon 24 and an additional double bond between carbons 22 and 23. These structural differences, however, do not prevent the metabolic activation

of the two forms. Before exerting their biological effects, both vitamin D<sub>2</sub> and vitamin D<sub>3</sub> must undergo 25-hydroxylation to form 25-hydroxyvitamin D<sub>2</sub> (25(OH)D<sub>2</sub>) or 25-hydroxyvitamin D<sub>3</sub> (25(OH)D<sub>3</sub>), respectively, followed by 1 $\alpha$ -hydroxylation to produce the respective biologically active metabolites 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D).

With the use of appropriate assay systems to detect the 25(OH)D<sub>2</sub> metabolite, several randomised trials using large oral dose preparations ranging from 1250 to 7500 µg (50 000–300 000 IU) have suggested that vitamin D<sub>2</sub> is less effective in elevating or maintaining total serum 25-hydroxyvitamin D (25(OH)D) levels in healthy adults<sup>(3,6,9,10)</sup>, whereas the few studies which have directly compared daily administered low-dose preparations of vitamin D<sub>3</sub> and vitamin D<sub>2</sub> have yielded inconsistent results<sup>(1,2,5,7–9)</sup>. In addition to varying dose and dosing regimens, these latter studies have been

**Abbreviations:** 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.

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limited by short study duration (4–12 weeks), small participant numbers and have included confounding by other variables such as BMI and contribution of vitamin D<sub>3</sub> from cutaneous production, fortified foods and supplemental sources.

In an attempt to address some of the limitations of previous research, we conducted a randomised, double-blind, placebo-controlled trial to evaluate the efficacy of a daily physiological dose of vitamin D<sub>2</sub> and vitamin D<sub>3</sub> in healthy-weight adults for a 25-week period beginning at the end of summer. We specifically investigated the time course of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> serum levels and the concomitant variations in parathyroid hormone (PTH) concentration after a daily initiation of 25 µg (1000 IU) vitamin D<sub>2</sub> or D<sub>3</sub> supplementation. The study was conducted in New Zealand at a latitude of 46°S where food fortification of vitamin D is neither mandated nor common.

## Methods

### Participants

A total of ninety-five healthy, adult women and men aged 18–50 years, inclusive, were recruited from the staff and student population at the University of Otago, Dunedin, New Zealand (latitude 46°S), and from the community through advertisements in the local newspaper. This region has a temperate climate with a summer mean temperature of 14°C and a winter mean temperature of 5°C with mean sunshine hours in the winter ranging from 98 to 122 h/month<sup>(11)</sup>. The nadir in UV radiation occurs midwinter (July) after the peak 6 months earlier in summer (December). Participants were excluded if they had a BMI ≥ 25 kg/m<sup>2</sup>, had reported granulomatous conditions, gastrointestinal disease, liver or kidney disease, or diabetes, were taking medications that might affect vitamin D metabolism (e.g. anticonvulsants, steroids in any form or barbiturates), or were planning to travel during the course of the study to a location at which the latitude would be expected to result in cutaneous synthesis of vitamin D.

### Study design

The study was designed as a 24-week randomised, double-blind, placebo-controlled trial. At the baseline study visit, a non-fasting venous blood sample was collected and participants completed a brief self-administered sociodemographic and health questionnaire. They were asked to report the use of any vitamin D- and Ca-containing supplements over the past 3 months and current prescription medications (including oral contraceptives). Height was measured to the nearest 0.1 cm using a calibrated self-made stadiometer, and weight was measured to the nearest 0.1 kg using a calibrated platform digital scale (Seca). Lastly, participants were given detailed verbal and written instructions on how to collect diet records to assess dietary Ca intakes, and were asked to record all foods and beverages consumed for five weekdays and two weekend days within the next 14 d. Participants were then randomly assigned to receive one of three daily tablets labelled to contain 25 µg (1000 IU) vitamin D<sub>2</sub>,

25 µg (1000 IU) vitamin D<sub>3</sub> or a placebo. The vitamin D and placebo supplements were manufactured as hard tablets by New Zealand Nutritionals Limited, and were identical in appearance. The tablet content was independently verified on 19 August 2009 (New Zealand Laboratory Services Limited), and the actual amounts labelled to provide 25 µg (1000 IU) of vitamin D<sub>2</sub> and vitamin D<sub>3</sub> were 32 µg (1295 IU) (CV 6%) and 28 µg (1110 IU) (CV 6%), respectively. Randomisation of participants was completed with a computer-generated block randomisation scheme stratified by sex. All the participants began the study between 6 March and 13 March 2009 (early autumn) and completed the study between 27 August and 14 September 2009 (late winter).

Participants were to return to the clinic at weeks 4, 8, 12 and 24 to provide non-fasting blood samples at a standard time in the day between 08.00 and 11.00 hours. The average number of days from baseline for each targeted study week was as follows: 4 weeks (28.3 d); 8 weeks (56.0 d); 12 weeks (92.3 d, equivalent to 13 weeks); 24 weeks (175 d, equivalent to 25 weeks). At each subsequent visit, participants were asked questions relating to the use of prescription medications and travel outside of the surrounding area since the last study visit. Measurements of height and weight were taken once again at the completion of the study. Compliance was assessed using cumulative pill counts at the end of the study. The blinding of the arms of the study was maintained for all researchers, including the statistician, until the final data analyses were completed. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human participants were approved by the Human Ethics Committee at the University of Otago, Dunedin, New Zealand. Written informed consent was obtained from all participants. The study was registered at [www.actr.org.au](http://www.actr.org.au) as ACTRN12609000273280.

### Biochemical analyses

Serum 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> concentrations were determined by isotope dilution liquid chromatography–tandem MS on a API 3200 instrument (Applied Biosystems) according to the method of Maunsell *et al.*<sup>(12)</sup>. The limit of quantification for the assay was <5 nmol/l for both metabolites. Values for serum 25(OH)D reported as less than 5 nmol/l were considered to be zero. To assess accuracy and inter-assay variability, we prepared an internal quality control by adding 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> to a pooled serum sample and ran an external quality control serum material (UTAK Laboratories) with a verified 25(OH)D<sub>2</sub> value of 24.2 nmol/l (mean 23.3 (SD 1.2) nmol/l; CV 5.2%) and a 25(OH)D<sub>3</sub> value of 27.5 nmol/l (mean 25.9 (SD 2.4) nmol/l; CV 9.3%). For the internal quality control, the inter-assay CV for 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> were 3.8% at 69.8 nmol/l and 4.5% at 83.6 nmol/l, respectively. Intact PTH was measured by an automated electrochemiluminescence immunoassay (Elecsys 1010<sup>®</sup>; Roche Diagnostics). Manufacturer-provided controls (Elecsys Preci-Control Bone 1, 2 and 3) were analysed with each reagent kit. The means and CV (%) for the three controls were 45.2

(SD 2.8) pg/ml and 6.1%, 155.7 (SD 10.3) pg/ml and 6.6%, and 650.7 (SD 33.5) pg/ml and 5.2%, respectively, and were within the range of the results provided by the manufacturer. Serum samples from each subject (for all clinic visits) were analysed together in the same batch.

**Statistical analysis**

A per-protocol approach, which included all participants  $\geq 90\%$  compliant with the study supplement, was used for all analyses. Additionally, an intent-to-treat analysis that included all randomly assigned subjects irrespective of

compliance was performed. The intent-to-treat results were only slightly different from the per-protocol results and the differences were not clinically significant (see Table S1, available online). Therefore, only the per-protocol results are presented, unless noted otherwise.

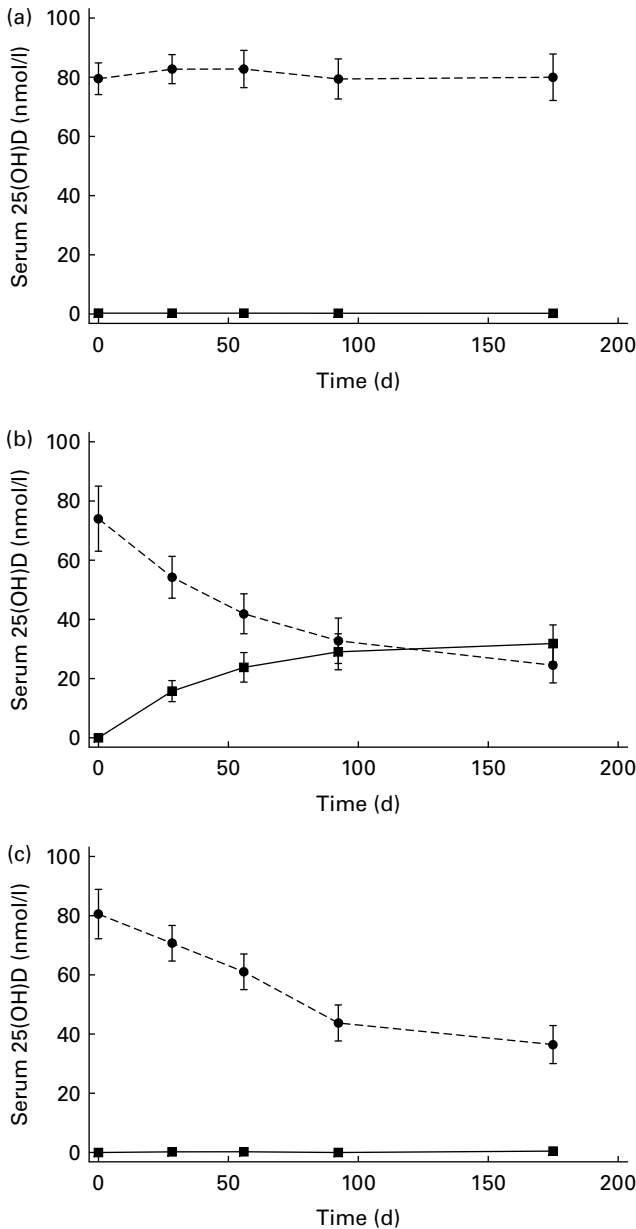
The original study design included a sample size calculation based on a planned intention-to-treat analysis before the decision to examine the efficacy aspects of supplementation in the primary analysis reported herein. As such, there is no power analysis reported for the per-protocol study, and the power to detect practically important effect sizes is reflected in the widths of the CI included in the results.

The outcome variables were total serum 25(OH)D, 25(OH)D<sub>2</sub> and PTH concentration. Mixed-effects linear regression models were used to evaluate the fixed effect of the intervention on total serum 25(OH)D, serum 25(OH)D<sub>2</sub> and PTH including a random subject effect to account for the repeated measures within subjects and controlled for baseline levels. Models with PTH as an outcome variable also included dietary Ca. Natural log transformations were used where this improved residual normality and/or homoscedasticity. The difference in changes between the groups was assessed using combinations of treatment group effects and group  $\times$  time interactions. Stata (version 11.0; Stata Corporation) was used for all analyses and a two-sided  $P < 0.05$  was considered statistically significant in all cases.

**Results**

Of the ninety-five participants recruited and enrolled in the study, eighty-five completed the intervention trial (vitamin D<sub>3</sub>, thirty out of the thirty-two enrolled; vitamin D<sub>2</sub>, twenty-five out of thirty-one; placebo, thirty out of thirty-two). Reasons for discontinuing the intervention were as follows: lack of time ( $n$  3); personal reason ( $n$  1); unspecified health reasons ( $n$  4); exacerbation of eczema ( $n$  2). An additional twenty-four participants were excluded from the per-protocol analysis for compliance  $< 90\%$  (vitamin D<sub>3</sub>,  $n$  6; vitamin D<sub>2</sub>,  $n$  12; placebo,  $n$  5) and compliance unknown (did not return pill bottle) (vitamin D<sub>3</sub>,  $n$  1), resulting in a total of sixty-one participants who completed the study with known supplement compliance  $\geq 90\%$ : 25  $\mu$ g (1000 IU) vitamin D<sub>3</sub>/d,  $n$  24, 25  $\mu$ g (1000 IU) vitamin D<sub>2</sub>/d,  $n$  13; placebo,  $n$  25. At baseline, the mean age of participants included in the analysis was 29 years (range 18–50 years) and the majority of the participants were New Zealand Europeans (84%), well educated with at least some tertiary education (89%), and female (79%). All of the participants were classified as normal weight (BMI 18–24.9 kg/m<sup>2</sup>) with a mean BMI of 22.7 (SD 2.3) kg/m<sup>2</sup>. Median dietary Ca intake (fifty-three out of sixty-one participants who completed baseline diet records) was 869 (25th and 75th percentile 696, 1029) mg/d. There were eight (14%) participants who regularly took a Ca-containing supplement. There was no evidence of a significant difference in dietary Ca among the three groups ( $P = 0.98$ ).

Total serum 25(OH)D concentrations (i.e. the sum of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>) at baseline ranged from 40 to 136 nmol/l with the geometric mean serum total 25(OH)D of



**Fig. 1.** Mean serum 25-hydroxyvitamin D<sub>3</sub> (25(OH)D<sub>3</sub>, ---) and 25-hydroxyvitamin D<sub>2</sub> (25(OH)D<sub>2</sub>, —) concentrations over time in healthy adult participants treated with (a) 25  $\mu$ g/d vitamin D<sub>3</sub>, (b) 25  $\mu$ g/d vitamin D<sub>2</sub> or (c) placebo over a 6-month intervention ( $n$  61). Values are means, with 95% CI represented by vertical bars.

**Table 1.** Per-protocol analysis of total serum 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone concentrations at baseline and over the 25-week intervention period (Geometric means and 95% confidence intervals)

Measurement and treatment groups	Baseline		Week 4		Week 8		Week 13		Week 25		Estimated change after 25 weeks adjusting for baseline
	Geometric mean	95% CI	Geometric mean	95% CI	Geometric mean	95% CI	Geometric mean	95% CI	Geometric mean	95% CI	
<b>Total serum 25(OH)D (nmol/l)</b>											
Vitamin D <sub>3</sub> (25 µg/d), n 23	80	75, 85	83	78, 88	83	77, 89	80	73, 86	80	72, 88	Reference
Vitamin D <sub>2</sub> (25 µg/d), n 13	74	63, 85	70	63, 78	66	59, 72	62	54, 69	56	50, 63	-21 (-13, -29) nmol/l*
Placebo, n 25	81	72, 89	71	65, 77	61	55, 67	44	38, 50	37	30, 43	-44 (-37, -50) nmol/l†
<b>Parathyroid hormone (pg/ml)</b>											
Vitamin D <sub>3</sub> (25 µg/d), n 23	36.9	32.1, 41.7	34.0	29.2, 38.8	29.2	25.0, 33.3	32.2	27.2, 37.2	40.1	33.7, 46.4	Reference
Vitamin D <sub>2</sub> (25 µg/d), n 13	38.4	29.6, 47.2	32.7	26.2, 39.2	34.5	27.4, 41.6	33.1	27.2, 39.1	43.3	35.0, 51.5	8.4 (-13.7, 36.0) %
Placebo, n 25	37.6	31.2, 44.0	32.3	28.0, 36.6	31.8	25.8, 37.8	32.0	28.6, 35.5	43.4	37.3, 49.5	10.2 (-8.8, 33.1) %

\* Estimates were significantly different from the vitamin D<sub>3</sub> group after adjustment for baseline concentration ( $P < 0.001$ ).

† Estimates were significantly different from the vitamin D<sub>2</sub> group after adjustment for baseline concentration ( $P < 0.001$ ).

80 (SD 18) nmol/l. There were three (5%) participants who had 25(OH)D concentrations below 50 nmol/l. The concentration of 25(OH)D<sub>2</sub> at baseline was below detectable limits (<5 nmol/l) for most participants. Mean serum PTH was 37.5 (SD 14.2) pg/ml. Secondary hyperparathyroidism, defined as a PTH concentration > 65 pg/ml, was observed in four (7%) of the sixty-one participants at baseline.

The time course of serum 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> is shown in Fig. 1, which presents the mean values at each sampling point. Supplementation with vitamin D<sub>2</sub> increased serum 25(OH)D<sub>2</sub>, whereas participants who received vitamin D<sub>3</sub> or placebo showed no significant change in serum 25(OH)D<sub>2</sub> throughout the study. The mean absolute increase in serum 25(OH)D<sub>2</sub> levels achieved per 25 µg vitamin D<sub>2</sub> daily was 32 nmol/l. Serum 25(OH)D<sub>3</sub> concentration significantly declined in the D<sub>2</sub>-supplemented group (53 (95% CI 45, 61) nmol/l;  $P < 0.001$ ) and placebo (44 (95% CI 37, 51) nmol/l;  $P < 0.001$ ) relative to D<sub>3</sub>. After adjustment for baseline, D<sub>2</sub>-supplemented participants experienced a 9 (95% CI 1, 17) nmol/l greater decline in the 25(OH)D<sub>3</sub> metabolite compared with placebo ( $P < 0.036$ ).

The effect of vitamin D supplementation on total serum 25(OH)D concentrations (sum of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>) over the 25-week intervention period is reported in Table 1. As expected, participants randomised to the placebo had a significant reduction in total serum 25(OH)D concentrations (sum of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>) over the winter months compared with participants who received vitamin D<sub>3</sub> and vitamin D<sub>2</sub> (both  $P < 0.001$ ) (Table 1). Total serum 25(OH)D concentrations were also significantly lower in participants receiving vitamin D<sub>2</sub> compared with those receiving D<sub>3</sub> ( $P < 0.001$ ), among whom there was no change in total serum 25(OH)D (paired  $t$  test;  $P = 0.90$ ) (Table 1). At the end of the study, 84% (twenty-one out of twenty-five) and 15% (two out of thirteen) of participants in the placebo and D<sub>2</sub> groups, respectively, had total 25(OH)D concentrations < 50 nmol/l compared with only 9% (two out of twenty-three) of participants in the D<sub>3</sub> group. Despite the significant decrease in total 25(OH)D concentrations from baseline in the vitamin D<sub>2</sub> and placebo groups, there was no evidence of significant intervention-related changes in serum PTH concentrations among the three groups ( $P = 0.81$ ; Table 1). This model was repeated adjusting for dietary Ca with the same conclusion being reached ( $P = 0.77$ ).

## Discussion

The present results show that a daily intake of 25 µg (1000 IU) vitamin D<sub>3</sub> is more effective than 25 µg (1000 IU) vitamin D<sub>2</sub> in maintaining serum 25(OH)D concentration during the autumn and winter months. In New Zealand (latitude ranging from 35°S to 47°S), very few foods are fortified with vitamin D, and the relative contribution of dermal production of vitamin D<sub>3</sub> is markedly diminished during the winter months. Of the unsupplemented (placebo) participants, 84% had low vitamin D status (< 50 nmol/l). To our knowledge, this is the first study comparing vitamin D<sub>3</sub> and D<sub>2</sub> by mapping the time course of serum 25(OH)D from the summertime peak through to the



wintertime nadir. As expected, total serum 25(OH)D concentrations decreased substantially among the present study participants assigned to the placebo group over the course of the study, whereas a daily intake of 25 µg (1000 IU) vitamin D<sub>3</sub> was efficacious in maintaining summertime serum 25(OH)D levels. In contrast, while participants assigned to the vitamin D<sub>2</sub> group exhibited significantly higher total serum 25(OH)D levels than the placebo group, concentrations were 21 nmol/l lower (equivalent to 28% of the baseline mean) at the end of the study relative to the vitamin D<sub>3</sub> group. Furthermore, the greater fall in 25(OH)D<sub>3</sub> levels observed in the D<sub>2</sub>-supplemented participants compared with placebo suggests a more rapid metabolism or clearance of circulating 25(OH)D<sub>3</sub> following D<sub>2</sub> supplementation, which may partly explain the inability of this form to maintain the total serum 25(OH)D levels.

Previous studies employing higher-dosage regimens ranging from 100 µg/d (4000 IU/d) to a single dose of 7500 µg (300 000 IU) have consistently reported a substantial discrimination in favour of vitamin D<sub>3</sub><sup>(1–3,6,7,9,10)</sup>, while limited evidence generated from studies using lower physiological daily doses have argued that D<sub>2</sub> and D<sub>3</sub> are essentially equivalent<sup>(5,8)</sup>. Holick *et al.*<sup>(5)</sup> provided the first published evidence of the effective equivalence of the two forms in human subjects in a randomised, placebo-controlled trial demonstrating that serum 25(OH)D levels increased to the same extent in participants receiving 25 µg (1000 IU) daily as vitamin D<sub>2</sub>, vitamin D<sub>3</sub> or a combination of 12.5 µg (500 IU) vitamin D<sub>2</sub> and 12.5 µg (500 IU) vitamin D<sub>3</sub>. These findings suggested that the pharmacokinetic parameters of vitamin D<sub>2</sub> and vitamin D<sub>3</sub> change with increasing dose such that low doses of D<sub>2</sub> and D<sub>3</sub> appear equivalent while higher doses of D<sub>2</sub> are less effective than D<sub>3</sub>. However, Heaney *et al.*<sup>(10)</sup> have argued that at lower doses, the increase in serum 25(OH)D would be relatively small and thus not sufficient to allow detection of differences between the two forms due to the combined effects of analytical and biological variability of serum 25(OH)D. Participants in Holick's study<sup>(5)</sup> were generally obese (mean BMI 30 kg/m<sup>2</sup>), which may have an effect on the outcome measure of total serum 25(OH)D, and nearly 40% (six out of sixteen) of the participants assigned to the D<sub>2</sub> group were taking a 10 µg (400 IU) vitamin D<sub>3</sub>-containing supplement during the time period of the study. We sought to avoid the effects of potential predictors in the present study by excluding participants with a BMI greater than or equal to 25 kg/m<sup>2</sup> and prohibiting the use of vitamin D-containing supplements during the study. Although we did not measure background dietary vitamin D intakes from food sources, the absence of widespread fortification of food with vitamin D in New Zealand makes it unlikely that the present results were confounded by dietary intake from fortified food sources. Moreover, the serum 25(OH)D assay detection limit of 10 nmol/l in Holick's study was high, and values for serum 25(OH)D<sub>2</sub> less than 10 nmol/l were obtained by subtracting 25(OH)D<sub>3</sub> from the total 25(OH)D. This method employed to quantify serum 25(OH)D<sub>2</sub> levels may have led to an overestimation of actual concentrations. In contrast, our assay was more sensitive with a non-detectable serum

25(OH)D<sub>2</sub> level of less than 5 nmol/l. We also conservatively assumed a value of zero for any 25(OH)D reported as <5 nmol/l.

More recently, Binkley *et al.*<sup>(9)</sup> evaluated daily administration of 40 µg (1600 IU) of vitamin D<sub>2</sub> and D<sub>3</sub> and concluded that vitamin D<sub>3</sub> was significantly more effective than D<sub>2</sub> on the basis of a greater absolute increase in serum 25(OH)D levels for those participants treated with vitamin D<sub>3</sub> (23 nmol/l) compared with D<sub>2</sub> (15 nmol/l). However, mean baseline 25(OH)D levels in the D<sub>2</sub> participants in the present study were higher than the D<sub>3</sub> participants, and there was no evidence of a significant difference in mean 25(OH)D levels after 12 months of intervention (97.5 nmol/l in the D<sub>3</sub> group when compared with 95.3 nmol/l in the D<sub>2</sub> group). The present study is therefore the first intervention, to our knowledge, to clearly demonstrate that long-term administration of a daily physiological dose of D<sub>3</sub> produces a substantially larger effect than D<sub>2</sub> in a healthy adult population. The more rapid metabolism of vitamin D<sub>2</sub> than D<sub>3</sub> could reflect the lower affinity of serum 25(OH)D<sub>2</sub> for vitamin D-binding protein than 25(OH)D<sub>3</sub> (i.e. shorter circulating half-life) and/or the increased affinity of 25(OH)D<sub>2</sub> for the 24-hydroxylase enzyme (i.e. greater rate of catabolism)<sup>(13,14)</sup>. Furthermore, the present findings of a greater decline in serum 25(OH)D<sub>3</sub> levels in the D<sub>2</sub>-treated participants than in the placebo group have also been previously reported in other studies ranging from 25 µg (1000 IU)/d to 1250 µg (50 000 IU) single and weekly dose regimens<sup>(3,7,10)</sup>. It has been proposed that an up-regulation in mechanisms required to metabolise vitamin D<sub>2</sub> and its metabolites may increase the metabolic degradation of circulating 25(OH)D<sub>3</sub> levels<sup>(3)</sup>.

Despite the differential response of serum 25(OH)D to vitamin D<sub>2</sub> and D<sub>3</sub>, there was no evidence of a difference in PTH concentrations between the treatment groups, which raises the more important question of whether the ingestion of D<sub>2</sub> *v.* D<sub>3</sub> makes any functional difference. We did not measure the active form of vitamin D, 1,25(OH)<sub>2</sub>D, and thus it is not known whether the lower level of circulating 25(OH)D observed in our D<sub>2</sub>-treated participants would result in lower production of the dihydroxylated form. The kidney is the major site of production of 1,25(OH)<sub>2</sub>D, and adequate production of this metabolite is dependent on the level of the serum 25(OH)D precursor and the 25(OH)D-1-α-hydroxylase or cytochrome P27B1 (CYP27B1) enzyme, which converts 25(OH)D to 1,25(OH)<sub>2</sub>D<sup>(15,16)</sup>. The expression of renal CYP27B1 is tightly regulated and plays an essential role in maintaining Ca and phosphate homeostasis. When serum 25(OH)D levels fall, there is a rise in PTH, which stimulates CYP27B1 enzyme activity<sup>(15)</sup>. Given the strong interdependence of vitamin D and Ca, it is likely that the relatively high dietary Ca intakes in the present study population may have suppressed the rise in serum PTH that typically accompanies declining 25(OH)D concentrations<sup>(17–19)</sup>. As a result, renal synthesis of 1,25(OH)<sub>2</sub>D may not be increased appropriately. Although knowledge of the regulation of CYP27B1 expression in extra-renal tissues such as skeletal muscle, liver and lung is limited, the tissue-specific synthesis of 1,25(OH)<sub>2</sub>D<sub>2</sub> appears to be directly related to the availability



of the 25(OH)D precursor for CYP27B1, and thus low circulating 25(OH)D may lead to an earlier decline in local *v.* circulating levels of 1,25(OH)<sub>2</sub>D<sup>(16)</sup>. We did not specifically address these outcomes in the present study, but further investigation in this area is warranted.

The relatively higher attrition rate and failure to sufficiently achieve 90% or greater self-reported compliance in participants assigned to the vitamin D<sub>2</sub> group compared with those assigned to the D<sub>3</sub> or placebo group ( $\chi^2$  test,  $P=0.011$ ) is a potential limitation. However, there appears to be no reasonable explanation for the significant difference in loss across the groups. The study was double-blinded (investigators and participants), allocation was concealed, all tablets were indistinguishable and there were no reports of adverse events. Given that the per-protocol analyses conveyed similar results to the intention-to-treat analyses, we assume that the potential bias from non-random dropout of participants or exclusion for poor compliance had no major impact on the results.

The Food and Nutrition Board of the Institute of Medicine has recommended 10 µg (400 IU) vitamin D daily to meet the needs of half of adults up to the age of 70 years, and 15 µg (600 IU) daily to meet the needs of 97.5% of these adults<sup>(20)</sup>. In the present study, a daily intake of 25 µg (1000 IU) vitamin D<sub>3</sub> maintained the summer 25(OH)D levels. Using data from controlled trials, a regression analysis of the relationship between the serum 25(OH)D level and the total intake of vitamin D predicts that a daily intake of 25 µg (1000 IU) would be associated with a mean serum 25(OH)D level of 68 nmol/l ( $y=9.9 \ln(\text{total vitamin D intake in IU/d})^{(20)}$ , where 40 IU vitamin D is equal to 1 µg vitamin D). The predicted level is noticeably less than the present study's mean total serum 25(OH)D level of 80 nmol/l achieved after 25 weeks supplementation in the D<sub>3</sub>-treated participants; however, it should be noted that the simulated intake–response relationship has been determined under conditions of minimal sun exposure, which may not be fully met at latitudes below 49°<sup>(20)</sup>. In contrast, the predicted value is substantially higher than the mean total serum 25(OH)D level of 56 nmol/l observed in our D<sub>2</sub> participants at the end of the study. Neither the simulated intake–response relationship nor the newly revised Dietary Reference Intake (DRI) distinguish between vitamin D<sub>2</sub> and vitamin D<sub>3</sub>.

In conclusion, daily supplementation of 25 µg (1000 IU) vitamin D<sub>3</sub> over a 25-week intervention period was more effective than vitamin D<sub>2</sub> in maintaining serum 25(OH)D levels. These findings contribute to the accumulating evidence that vitamin D<sub>3</sub> and vitamin D<sub>2</sub> have different pharmacokinetic profiles for serum 25(OH)D. As a result, care should be taken to distinguish the form of vitamin D used for both clinical studies and therapeutic use, particularly given that the dose employed in the present study is commonly used in over-the-counter dietary supplements. Nonetheless, conclusions about the biological significance of the different functional effects of the two forms cannot be drawn. Additional studies are needed to determine whether even lower doses would also suggest differences in pharmacokinetic parameters between vitamin D<sub>2</sub> and vitamin D<sub>3</sub>.

## Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S0007114512002851>

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