

Vitamin D supplementation increases calcium absorption without a threshold effect^{1–3}

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ABSTRACT

Background: The maximal calcium absorption in response to vitamin D has been proposed as a biomarker for vitamin D sufficiency.

Objective: The objective was to determine whether there is a threshold beyond which increasing doses of vitamin D, or concentrations of serum 25-hydroxyvitamin D [25(OH)D], no longer increase calcium absorption.

Design: This was a placebo-controlled, dose-response, randomized, double-blind study of the effect of vitamin D on calcium absorption in healthy postmenopausal women. Seventy-six healthy postmenopausal women were randomly assigned to placebo or 800 IU (20 μ g), 2000 IU (50 μ g), or 4000 IU (100 μ g) vitamin D₃ for 8 wk. The technique of dual isotopes of stable calcium was used with a calcium carrier to measure calcium absorption at baseline and after 8 wk.

Results: Seventy-one women with a mean \pm SD age of 58.8 \pm 4.9 y completed the study. The mean calcium intake was 1142 \pm 509 mg/d and serum 25(OH)D was 63 \pm 14 nmol/L at baseline. A statistically significant linear trend of an increase in calcium absorption adjusted for age and body mass index with increasing vitamin D₃ dose or serum 25(OH)D concentration was observed. A 6.7% absolute increase in calcium absorption was found in the highest vitamin D₃ group (100 μ g). No evidence of nonlinearity was observed in the dose-response curve.

Conclusions: No evidence of a threshold of calcium absorption was found with a serum 25(OH)D range from 40 to 130 nmol/L. Calcium absorption in this range is not a useful biomarker to determine nutritional recommendations for vitamin D. This trial was registered at clinicaltrials.gov as NCT01119378. *Am J Clin Nutr* 2014;99:624–31.

INTRODUCTION

It has been suggested that there is a vitamin D intake or a serum 25-hydroxyvitamin D [25(OH)D]⁴ concentration (the measure of vitamin D status) above which there is no further influence on calcium absorption. This purported “threshold” could be used as an indicator of vitamin D sufficiency; ie, once calcium absorption is maximized, there would be no rationale for increasing the vitamin D intake above the threshold for skeletal health (proposed to be as high as 80–90 nmol/L). Other investigators propose that a decreased calcium absorption occurs only when there is a substrate deficiency of 25(OH)D [<10 ng/mL (25 nmol/L)], resulting in a lack of calcitriol synthesis (1).

Hansen et al (2) carried out a study of 18 subjects, given 50,000 IU vitamin D₂/d for 15 d. Calcium absorption was measured in the same subjects by using dual calcium isotopes. Calcium absorption efficiency (mean \pm SD) increased only 3% (from 24 \pm 7%

to 27 \pm 6%; $P = 0.04$). There was no evidence of a threshold. A single megadose of vitamin D₂ was used in this uncontrolled study. Shapses et al (3), using a dual-isotope study with a single dose of vitamin D₃ of 375 μ g/wk and 10 μ g/d, noted a 3.7% increase in calcium absorption. Gallagher et al (4) reported on a prolonged (1 y) dose-response, placebo-controlled study of 163 postmenopausal women with vitamin D insufficiency. They concluded that the increase in calcium absorption observed with supplementation of 4800 IU vitamin D₃/d was only the equivalent of drinking a small glass of milk. Calcium absorption was measured by using a single-isotope technique and a low calcium carrier (100 mg). Other studies in children and elderly women have also not found an increase in calcium absorption with vitamin D supplementation (5, 6).

A double-isotope technique is preferable for measurement of calcium absorption because it corrects for calcium recycling (7, 8). We performed a dose-response, placebo-controlled, randomized double-blind study of the effect of vitamin D₃ supplementation on intestinal calcium absorption. We used the dual-isotope technique with a calcium intake of 300 mg to answer the question: Is there a serum 25(OH)D concentration or intake of vitamin D₃ above which calcium absorption no longer increases?

SUBJECTS AND METHODS

Subjects

Recruitment was carried out during the winter months in consecutive years. Recruitment started in November 2010, and the study ended in March 2012. Letters were sent to our existing research patients who had expressed the desire to enroll their family members and friends into a clinical trial. Ads were placed in the local newspapers, and a direct mailing was used. Healthy postmenopausal women between the ages of 50 and 70 y were eligible for enrollment. Exclusion criteria included the following:

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⁴ Abbreviations used: CTX, C-terminal telopeptides of type I collagen; IOM, Institute of Medicine; PTH, parathyroid hormone; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

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1) any chronic medical illness; 2) subjects with a BMI (in kg/m²) >35; 3) use of medication that influences vitamin D and bone metabolism; 4) significant deviation from normal in medical history, physical examination, or laboratory tests as evaluated by the primary investigator; 5) hypercalciuria (urine calcium:creatinine ratio >0.37, hypercalcemia (serum calcium >10.6, nephrolithiasis, and active sarcoidosis; 6) unexplained weight loss >15% during the previous year or history of anorexia nervosa; 7) participation in another investigational trial in the past 30 d before the screening evaluation; 8) alcohol intake reported by patient of >2 drinks/d; 9) baseline 25(OH)D concentration >70 nmol/L; 10) smoking of more than one pack per day; 11) dietary calcium intake >2000 mg; and 12) unwillingness to forego multivitamin and vitamin D supplements during the study. The study was approved by the Winthrop University Hospital Institutional Review Board. Subjects gave written informed consent.

Protocol

Subjects had a baseline visit for screening. If eligible, they returned for a randomization visit at week 2, at which time calcium absorption was measured and medication was dispensed. The subjects made a revisit after 8 wk of supplementation (week 10), at which time calcium absorption and the baseline laboratory studies were repeated. Total daily calcium intake was estimated by using a dietary recall (Short Calcium Questionnaire 2002; NIH Clinical Center) at the baseline and final visits. The subjects were randomly assigned to 1 of 4 groups with each receiving placebo, 800 IU (20 µg), 2000 IU (50 µg), or 4000 IU (100 µg) vitamin D₃/d, respectively. A computer-generated block randomization was used (20 blocks of 4). The random allocation sequence was generated by the statistician, and the research pharmacist assigned participants. Investigators and participants were blinded to group assignment. Supplements and placebo appeared identical in size, shape, color, and weight.

Calcium absorption

Calcium absorption was performed at baseline and again after 8 wk of supplementation. A dual-tracer-isotope method was used to measure calcium absorption efficiency (7, 9–11). Subjects were given a breakfast that was a fixed meal providing 300 mg Ca. Toward the end of breakfast, the subjects were given a stable isotope of calcium, ~24.75 µg ⁴⁶Ca that had been mixed with

240 mL Ca-fortified orange juice (7). After breakfast, a different calcium stable isotope (~1.75 mg ⁴²Ca) was infused intravenously within 5 min, flushing the line with saline. The syringes were weighed before and after the infusion to determine the precision of the intravenous isotope doses. After administration of the calcium isotopes, a complete 24-h urine sample was collected by the patient and was handed over in person to the research team the next day.

The relative fraction of the oral, compared with the intravenous, dose in this 24-h urine pool was determined and represented the fraction of the oral tracer dose that was absorbed (7). Urine samples were prepared for mass spectrometric analysis by using the oxalate precipitation technique. Samples were analyzed for isotopic enrichment by using thermal ionization mass spectrometry as previously described (7). The analyses were performed in the laboratory of the USDA/Agricultural Research Service Children's Nutrition Research Center.

Laboratory analyses

Serum 25(OH)D was measured by using a radioimmunoassay from DiaSorin Inc. The intraassay variability in our laboratory was 4.1%, and the interassay variability was 7.0%. Our laboratory participates in the Vitamin D External Quality Assessment Scheme—an external quality-control program—and uses the National Institute of Standards and Technology standard (12). Serum 1,25-dihydroxyvitamin D [1,25(OH)₂D] was measured by using an enzyme immunoassay manufactured by Immuno Diagnostic System Ltd. The intraassay variability was 10.2%, and the interassay variability was 18.1%. Serum and urinary calcium were measured with *O*-cresolphthalein complex by using automated equipment (Dimension-RXL). Urinary creatinine was measured by Jaffe reaction with automated instrumentation (Dimension-RXL). The vitamin D tablets were assayed by HPLC [Waters Symmetry; C18, 3.9 × 150-mm column, mobile phase, acetonitrile:methanol (75:25)]. Serum parathyroid hormone (PTH) was measured with the Immulite 2000 Analyzer for the quantitative measurement of intact PTH (Diagnostic Products Corporation). Serum C-terminal telopeptides of type I collagen (CTX) was measured with a Serum Crosslaps ELISA kit made by Nordic Bioscience Diagnostics. Serum procollagen type 1 *N*-terminal propeptide was measured by using a UniQ P1NP RIA kit from Orion Diagnostica.

TABLE 1
Baseline characteristics by vitamin D dose¹

Measurement	Control (n = 19)	800 IU (n = 19)	2000 IU (n = 20)	4000 IU (n = 18)	P value ²
Age (y)	60 ± 4.5	57 ± 4.5	59 ± 5.8	60 ± 4.7	0.4
BMI (kg/m ²)	26.2 ± 3.8	26.4 ± 3.6	27.6 ± 4.9	26 ± 4	0.6
Calcium intake (FFQ – dietary + supplement)	1156 ± 580	1027 ± 469	1160 ± 499	1196 ± 518	0.8
Calcium absorption	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.4
Serum calcium (mg/dL)	9.6 ± 0.3	9.6 ± 0.3	9.5 ± 0.4	9.5 ± 0.4	0.95
Serum creatinine (mg/dL)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.89
25(OH)D (nmol/L)	61.7 ± 15.3	64 ± 13.8	64.8 ± 15.1	62.1 ± 14.2	0.90
1,25(OH) ₂ D (pmol/L)	97.9 ± 27.7	104.6 ± 26.3	111.7 ± 38.1	117.3 ± 64.9	0.52

¹All values are means ± SDs. FFQ, food-frequency questionnaire; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

²P values are based on an overall F test from an ANOVA comparing differences between dose groups.

Adverse events

An adverse event was defined as any undesired change in the subject as indicated by signs, symptoms, or laboratory data that occurred in association with taking the study drug whether or not it is considered to be related to the medication.

Statistical analysis

Our main outcome variable was the comparison of groups with respect to changes in calcium absorption from baseline. We determined our sample size based on a previous study (13). As per that study's design, a sample size of 25 patients per group achieved 90% power to detect a difference between groups of 7.2% (in absolute terms) in changes from baseline. Alpha was equal to 0.05.

Descriptive statistics (mean, median, and SD) of continuous clinical covariates and laboratory markers were generated to describe the sample of patients both overall and within each treatment arm. Differences in the mean levels of each covariate across treatment arm were examined via ANOVA. Similarly, categorical covariates such as race were summarized by using frequencies and percentages with differences between treatment arms assessed with chi-square tests. Relations between continuous

covariates were examined via Pearson correlation coefficients. Scatterplots of continuous variables with nonparametric smoothed curves imposed were generated to examine the degree and nature of the specific relations. For the primary outcome of calcium absorption at follow-up, further exploration of the relation between absorption and treatment weight, BMI, baseline calcium absorption, 25(OH)D, and other laboratory markers was performed by using linear regression models. Multivariable models for calcium absorption were examined to adjust for potential confounders such as age, weight, and BMI. To arrive at the final multivariable model presented, an exhaustive search of the model space was conducted, and models were ranked on the basis of their adjusted R^2 values. Because of likely collinearity between different variables, the final models presented were considered representative of other equally informative models. Model assumptions were checked via residual analysis and graphic summaries, and removal of outlying or influential observations was performed to observe the robustness of the main results.

The relation between vitamin D intake and calcium absorption at baseline and follow-up, and the change (Δ) between follow-up and baseline, were each assessed via ANOVA, and statistical significance of the overall treatment effect was determined via the global F test. To examine the hypothesized piecewise linear or

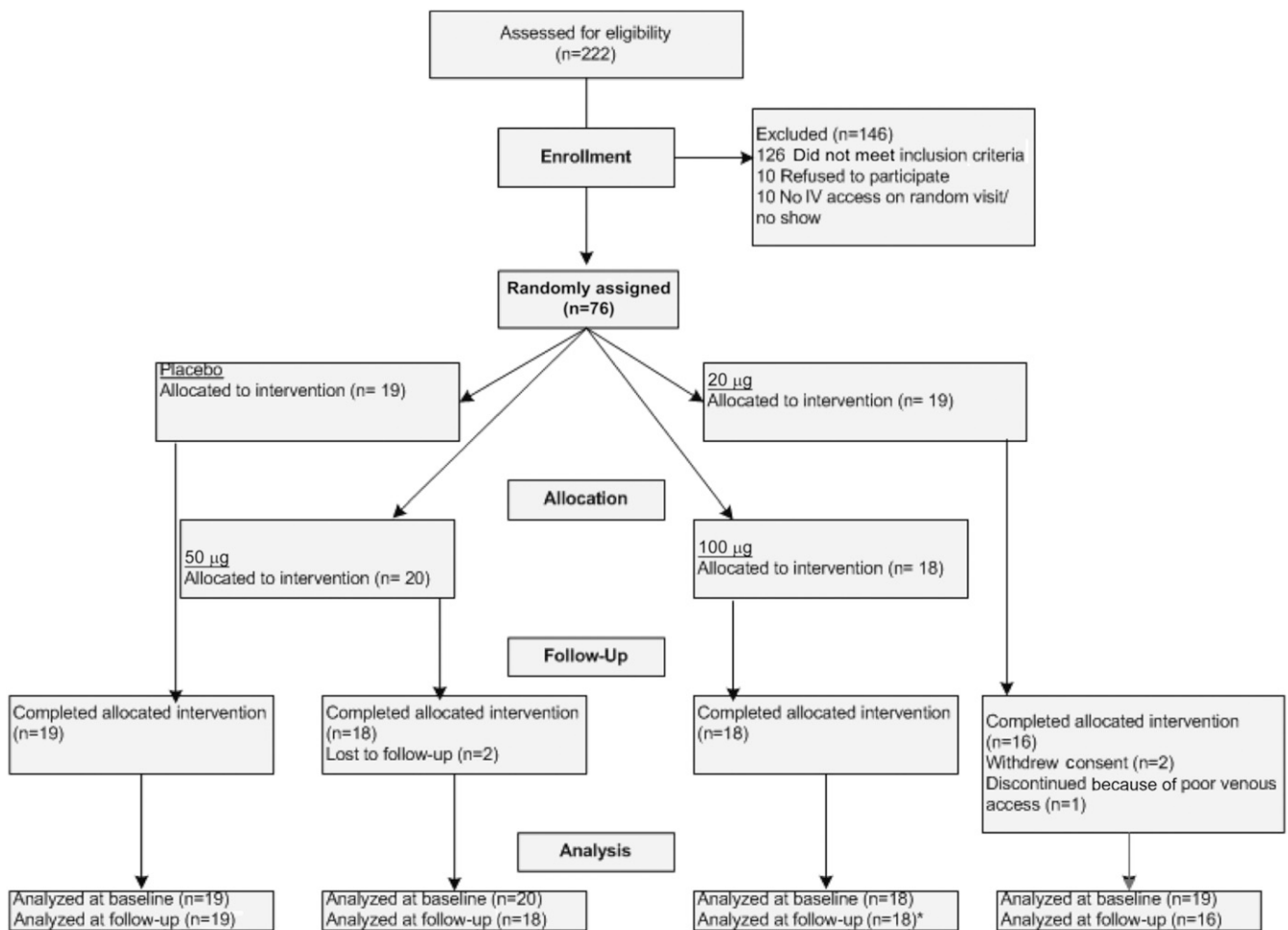


FIGURE 1. Flow chart of the study. IV, intravenous.

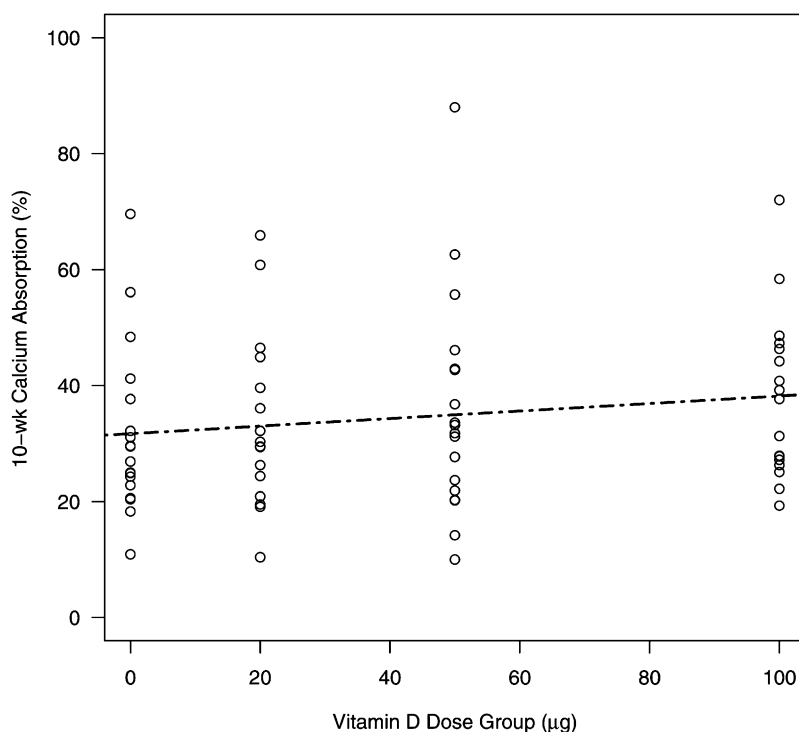


FIGURE 2. Calcium absorption rate at 10 wk (8 wk after randomization) by vitamin D dose group ($n = 70$; univariate P -trend = 0.18). A statistically significant linear dose effect was observed after adjustment for age, weight, and initial calcium absorption (P -trend = 0.03). No evidence of any nonlinear effect was found after examination of change points and higher order terms in the model. Final model: 10-wk calcium absorption = $34.4 - 0.11 \times \text{age} - 0.11 \times \text{weight} + 0.65 \times \text{baseline calcium absorption} + 2.2 \times \text{dose}$.

curvilinear relation between calcium absorption and 25(OH)D at follow-up, linear, quadratic, and cubic spline models with an a priori knot at 80 nmol/L were fitted. Model coefficients and R^2 summary statistics were examined for each model to determine whether the hypothesized change point was supported by the data at hand and whether the model for calcium absorption was improved by the inclusion of more complex, nonlinear terms into the model. The SAS version 9.3 statistical program was used to analyze the data.

RESULTS

Baseline values

Baseline demographic characteristics and laboratory results are provided in **Table 1**. The study flow sheet is shown in **Figure 1**. No statistically significant interactions with treatment arm with respect to baseline variables were found. The declared race was predominantly white; 8% of the participants were black, 6% Hispanic, and 3% Asian. The mean (\pm SD) weight was 70 ± 5.1 kg, mean BMI was 26 ± 4 , and mean age was 58.8 ± 4.9 y. Calcium intake was 1142 ± 509 mg/d. The distribution of BMI values was as follows: 29% <25, 31% from 25 to 29, and 16% ≥ 30 . The mean serum 25(OH)D was 63 ± 14 nmol/L. The distribution of serum 25(OH)D was as follows: 30–49 nmol/L, 24%; 50–75 nmol/L, 54%; and >75 nmol/L, 22%. The baseline calcium absorption efficiency was $32 \pm 14\%$. The dose-response curve for 10-wk calcium absorption showed no evidence of nonlinearity (P -quadratic term in the adjusted model = 0.16). The curve is depicted in **Figure 2**. This was also the case for serum 25

(OH)D (**Figure 3**). Calcium absorption was also related to 24-h urine calcium excretion ($P = 0.002$). Age and weight were considered potential confounders with respect to calcium absorption; all baseline factors were adjusted for these variables in the primary statistical model.

Changes in calcium absorption

The changes in calcium absorption (mean \pm SD) from baseline to follow-up were as follows: placebo, $-2.6 \pm 10.7\%$; 800 IU, $3.9 \pm 10.4\%$; 2000 IU, $5.0 \pm 18.7\%$; and 4000 IU, $6.7 \pm 12\%$. A multivariable model for 10-wk calcium absorption containing the predictor's dose group, baseline calcium absorption, age, and weight (R^2 of multivariable model = 0.41) yielded a statistically significant linear trend across vitamin D dose groups (**Table 2**; $P = 0.03$).

Examination of the relation between 10-wk calcium absorption and 10-wk serum 25(OH)D concentrations showed a similar linear trend, with no evidence of nonlinearity (P -quadratic effect = 0.35). A marginally significant linear effect ($P = 0.05$) due to 10-wk serum 25(OH)D was observed because serum 25(OH)D concentrations increased by 10 nmol/L and mean calcium absorption increased by 1.4%.

Changes in serum 25(OH)D

The response of serum 25(OH)D to increasing doses of vitamin D₃ was linear, and no curvature was found (P -linear trend < 0.001). The only biochemical variable associated with 10-wk serum 25(OH)D was the baseline value for serum 25(OH)D

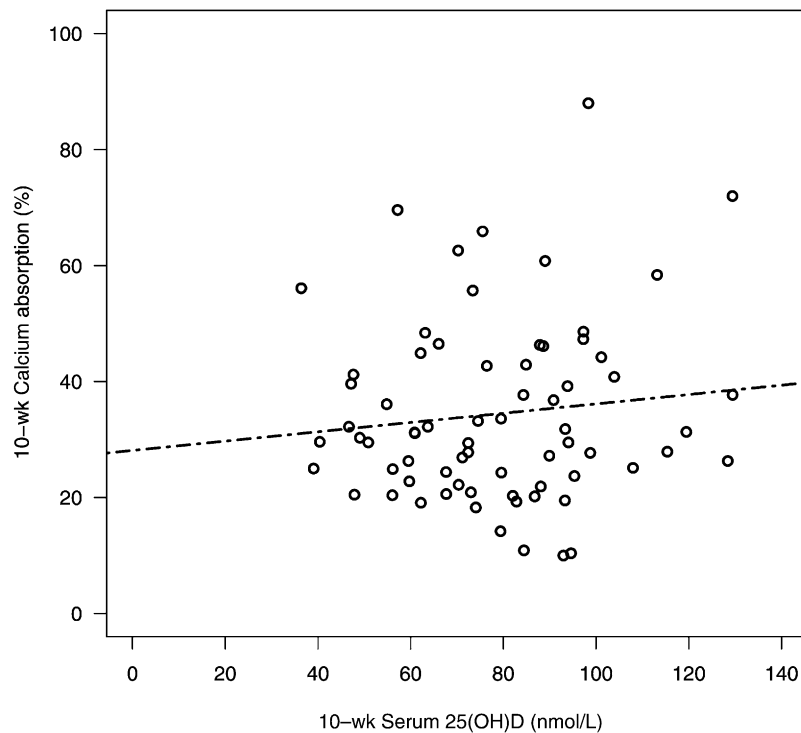


FIGURE 3. Calcium absorption at 10 wk (8 wk after randomization) by 10-wk serum 25(OH)D ($n = 70$). A marginally significant linear effect ($P = 0.05$) was observed in a model adjusted for age, weight, and baseline calcium absorption. No evidence of any nonlinear effect was found after examination of change points and the presence of higher order terms in the model. Final model: 10-wk calcium absorption = $26.8 - 0.16 \times \text{age} - 0.09 \times \text{weight} + 0.66 \times \text{baseline calcium absorption} + 0.14 \times 10\text{-wk serum } 25(\text{OH})\text{D}$. 25(OH)D, 25-hydroxyvitamin D.

($P < 0.0001$). Serum 25(OH)D concentrations increased from baseline to follow-up on average by 15.7 ± 20.4 nmol/L. Individuals with higher starting 25(OH)D concentrations had smaller changes in 25(OH)D at follow-up ($P = 0.03$). The variability of the 25(OH)D response differed by dose group ($P = 0.002$) and was highest in the 4000-IU/d group. BMI was also negatively correlated with 10-wk serum 25(OH)D ($P = 0.04$). The 10-wk serum 25(OH)D concentrations are depicted in **Figure 4** with the estimated regression. Serum 25(OH)D is expected to increase by 10.6 nmol/L for each increased intake of 400 IU/d ($10 \mu\text{g/d}$).

Correlations between changes in variables

The correlations between changes (10-wk minus baseline) in key variables are presented in **Table 3**. Changes in PTH were inversely associated with changes in serum 25(OH)D ($P = 0.01$). A significant increase in calcium absorption was observed with the increase in serum 1,25(OH)₂D and a trend to increase with serum 25(OH)D ($P = 0.07$). The increase in serum 1,25(OH)₂D was directly correlated with the increase in CTX.

Adverse events

No serious adverse events occurred. Three adverse events were reported: sinusitis, seasonal allergy, and jaw pain. None were judged to be related to the intervention. No instances of hypercalcemia or hypercalciuria occurred.

DISCUSSION

Our study showed that, at serum 25(OH)D concentrations of 40 to 130 nmol/L, no evidence of a “threshold” for calcium absorption was found. However, none of our participants had very low 25(OH)D concentrations, so we cannot comment on whether there is a “threshold” in the vitamin D deficiency range. On the basis of our results and those of the other studies cited, we conclude that there is no evidence in reference to calcium absorption that vitamin D should be increased above the Institute of Medicine (IOM) recommendation (Recommended Dietary Allowance) of a serum 25(OH)D concentration of 50 nmol/L (2–6). Gallagher et al (4) found no evidence of a calcium absorption threshold with serum 25(OH)D concentrations ranging from 25 to 165 nmol/L. They concluded that the additional 6% of calcium absorbed from the high dose of vitamin D could be achieved by increasing calcium intake with half a glass of milk. In a recent study in

TABLE 2
Baseline predictors of 10-wk follow-up calcium absorption¹

Factor	Model estimate	SE	<i>P</i> value
Age (y)	-0.11	0.31	0.71
Weight at baseline (lb)	-0.11	0.06	0.06
Calcium absorption at baseline (%)	0.65	0.11	<0.0001
Vitamin D dose group ²	2.21	1.00	0.03

¹ Predictors are based on a multivariable linear regression model; model $R^2 = 0.41$.

² Dose group: 0.80 for 800 IU, 2.0 for 2000 IU, and 4.0 for 4000 IU.

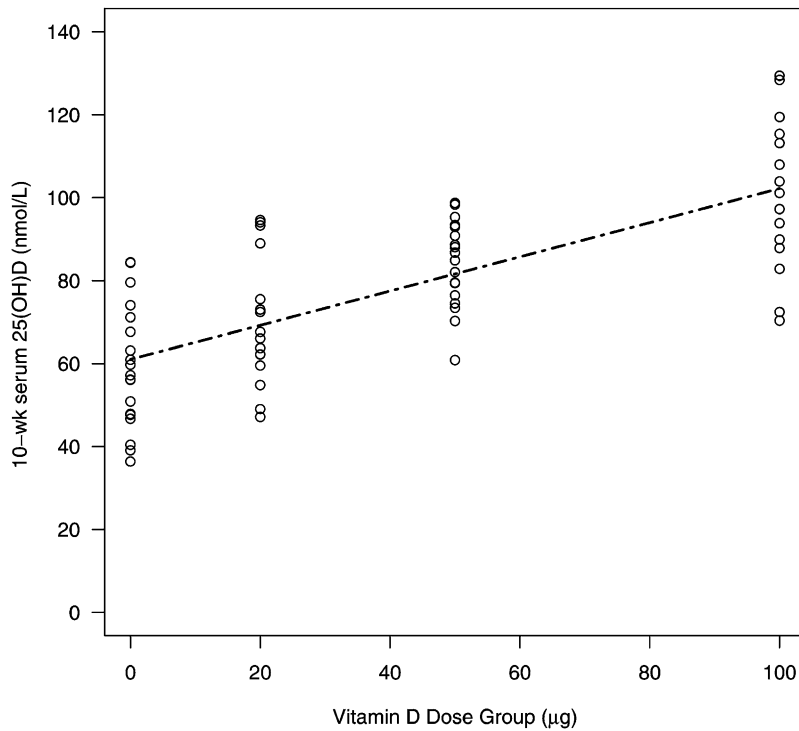


FIGURE 4. Serum 25(OH)D concentrations at 10 wk (8 wk after randomization) by vitamin D dose group ($n = 70$). A strong linear effect was observed in both the univariate model and after adjustment for age, weight, and initial calcium absorption ($P < 0.0001$). No evidence of any nonlinear effect was found after examination of change points and the presence of higher order terms in the model. Final model: 10-wk 25(OH)D = $24 + 0.19 \times \text{age} - 0.11 \times \text{weight} + 0.65 \times \text{baseline } 25(\text{OH})\text{D absorption} + 10.6 \times \text{dose}$. 25(OH)D, 25-hydroxyvitamin D.

children, no increase in calcium absorption was noted from 1000 IU vitamin D₃/d, despite a decrease in serum PTH (6).

Our study design differed from that of Gallagher et al (4) in that we used the more accurate method for calcium absorption, the dual-isotope technique. We also used a higher carrier. The low-carrier method is thought to better reflect transcellular calcium transport, whereas the method we used more accurately reflects the response to a calcium-rich meal. Despite the differences in the design or methods of the studies by Hansen et al (2), Shapses et al (3), and Gallagher et al (4) and our study, the results are consistent. Gallagher et al (4) noted an absolute increase in calcium absorption of 6% at a dose of 4800 IU/d compared with our increase of 6.7%. Hansen et al (2) observed an absolute increase of 3%, and Shapses et al (3) observed an increase of 3.7%.

Perhaps the most important finding from these studies is that there was a small linear response of calcium absorption to increasing vitamin D intakes. There was no evidence of a threshold at 50 or 80 nmol/L. It is likely that serum calcitriol and calcium absorption continue to increase with even higher intakes of vitamin D. We observed in this study that even when PTH declined in response to increased vitamin D intakes, serum calcitriol did not decrease. Vitamin D intoxication is thought to result from direct effects of 25(OH)D on bone, but elevated calcitriol concentrations may persist even in the case of severe hypercalcemia (14).

Although our study did not include a sufficient number of subjects with very low concentrations of serum 25(OH)D (<10 to 12 nmol/L), previous literature suggests that very low 25(OH)D

TABLE 3
Correlations between changes (follow-up minus baseline) in key laboratory markers¹

	Spearman correlation coefficient					
	Serum 25(OH)D	Calcium absorption	Serum 1,25(OH) ₂ D	Serum PTH	Serum CTX	Serum PINP
Serum 25(OH)D	1.0					
Calcium absorption	0.22	1.0				
Serum 1,25(OH) ₂ D	0.23 ²	0.03	1.0			
Serum PTH	-0.30 ²	0.04	-0.05	1.0		
Serum CTX	0.19	0.30 ²	0.28 ²	-0.001	1.0	
Serum PINP	0.16	-0.12	0.01	-0.17	-0.06	1.0

¹ CTX, C-terminal telopeptides of type I collagen; PINP, procollagen type 1 N-terminal propeptide; PTH, parathyroid hormone; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

² $P \leq 0.05$ (for nonzero Spearman correlation coefficient).

leads to calcium malabsorption and osteoporosis (15). It is believed that the reduction in calcium absorption is caused by reduced levels of calcitriol that result from a substrate deficiency of 25(OH)D. It is calcitriol that regulates intestinal calcium absorption rather than 25(OH)D (4, 15–17).

The decrease in serum PTH in response to higher serum 25(OH)D concentrations is of interest particularly in this group, which is presumably calcium-sufficient (Table 3). The construct of a “threshold” has also been applied to a serum 25(OH)D concentration above which PTH is no longer suppressed to determine vitamin D sufficiency. Many statistical models have been applied to this conceptual “threshold,” with the most publicized articles suggesting a threshold >70 – 80 nmol/L (18–24). However, a review of the literature indicated a large heterogeneity of “thresholds” or no threshold (25). Most of these studies were cross-sectional in design. In our admittedly small prospective study, no evidence of nonlinearity of the decline in PTH with increasing 25(OH)D concentrations was found.

Another interesting finding in our study was the positive association between the increase in serum calcitriol and serum CTX (Table 3). As serum calcitriol rises with increasing vitamin D exposure, it may increase bone resorption independently of PTH. The actions of vitamin D on bone apparently differ with low, sufficient, and high vitamin D exposures (25). Osteoporosis is observed in sarcoidosis, for which the primary defect in the calcium economy is high calcitriol concentrations. Evaluations of the effects of high doses of vitamin D, which are recommended for prevention and treatment of a variety of disorders, should assess whether increased bone resorption occurs.

We and others have previously noted that the dose-response curve of the increment in serum 25(OH)D in response to increasing doses of vitamin D intake is curvilinear and is related to the baseline concentration. However, in this study we found only a linear relation. We observed a $0.6\text{-nmol} \cdot \text{L}^{-1} \cdot \mu\text{g}^{-1}$ vitamin D₃ response in this study, which is similar to our previous reports (26).

We recognized several limitations of our study. Dietary calcium intake was not controlled, and the group was almost sufficient for calcium intake (and vitamin D status) according to IOM recommendations. Whereas no threshold for vitamin D was found, there were an insufficient number of vitamin D–deficient participants (<30 nmol/L) to confirm that there is indeed a decrease in calcium absorption at these very low levels, as previously reported. It can be questioned whether 8 wk is sufficient to reach a plateau in the vitamin D/25(OH)D dose-response curve. However, evidence from the available literature suggests that the plateau is reached before this time (27–30). Our findings are also confined to postmenopausal women, 83% of whom were white. Nevertheless, our study had many strengths. It was a randomized, double-blind, placebo-controlled, dose-response design that used vitamin D₃. The dual-isotope method was used to measure calcium absorption. A physiologic calcium intake was used for the carrier. The dose-response design included the IOM recommended ranges of vitamin D intake, up to the upper limit.

In conclusion, the results of our study and those of previous isotopic studies make it clear that increasing concentrations of 25(OH)D may minimally increase intestinal calcium absorption, but there is no apparent threshold up to the upper limit of 4000 IU/d. The small increase in calcium absorption attained with vitamin D supplementation does not support increasing the

Recommended Dietary Allowance above the recommendation of the IOM committee.

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The authors' responsibilities were as follows—JFA: designed the study, wrote the manuscript, and supervised the study; RD and AS: worked with JFA to draft the protocol; MM: supervised the study; LR: supervised the laboratory studies; SAA: performed the isotope studies; and MF: performed the statistical analyses, interpreted the data, and produced the tables and figures. All authors read and approved the final manuscript. None of the authors had a conflict of interest to declare.

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