

The Effect of Vitamin D on Calcium Absorption in Older Women

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Context: Vitamin D is often recommended for use with calcium supplements to increase absorption. There are no systematic studies of vitamin D on calcium absorption that indicate what dose should be recommended.

Objective: Our objective was to study the effect of increasing doses of vitamin D₃ on calcium absorption.

Design and Setting: We conducted a randomized double-blind placebo-controlled trial at Creighton University Medical Center, Omaha, NE.

Participants: Participants included 163 postmenopausal Caucasian women with vitamin D insufficiency, defined as a serum 25-hydroxyvitamin D (25OHD) below 20 ng/ml (50 nmol/liter).

Intervention: Participants were randomized to receive one of the vitamin D₃ doses, 400, 800, 1600, 2400, 3200, 4000, or 4800 IU/d, or placebo for 1 yr. Calcium intake was increased to 1200–1400 mg daily by giving daily calcium citrate.

Main Outcome: We evaluated the change in calcium absorption on vitamin D.

Results: Mean serum 25OHD increased from baseline 15.6 ng/ml (39 nmol/liter) to 46.5 ng/ml (112 nmol/liter) in subjects randomized to the highest dose of vitamin D (4800 IU). Calcium absorption was more significantly related to serum 25OHD ($R^2 = 0.50$; $P = 0.001$) than dose ($R^2 = 0.47$; $P = 0.033$). Calcium absorption of a 100-mg dose increased from 52–58% (6 mg) over a serum 25OHD range of 20–66 ng/ml (50–165 nmol/liter).

Conclusions: There was no evidence of a threshold for reduced calcium absorption in the serum 25OHD range of 10–66 ng/ml (25–165 nmol/liter). The increase in absorbed calcium of 6% on high doses of vitamin D is so small that the same amount could be obtained from half a glass of milk (100 ml) or 100 mg elemental calcium. The results challenge assumptions about the value of adding vitamin D to increase calcium absorption except when serum 25OHD is very low that is less than 10 ng/ml (25 nmol/liter). (*J Clin Endocrinol Metab* 97: 3550–3556, 2012)

Vitamin D is a nutrient that is uniquely provided by diet and sunlight. It is converted first in the liver to 25-hydroxyvitamin D (25OHD) and then further hydroxylated in the kidney to the active metabolite 1,25 dihydroxyvitamin D [1,25(OH)₂D, or calcitriol]. A decrease in serum calcium stimulates PTH and increases 1,25(OH)₂D production and calcium absorption, thus linking PTH and

calcitriol to homeostatic regulation of serum calcium (1). Fibroblast growth factor (FGF)-23 is a hormone derived from osteocytes that regulates serum phosphorus in part by stimulating phosphaturia. FGF-23 suppresses serum PTH and 1,25(OH)₂D production and decreases calcium absorption (2). 1,25(OH)₂D increases calcium absorption by binding to the vitamin D receptor in the intestine (3).

Optimal calcium intake is essential for mineralization of bone and plays a role in the prevention of osteoporosis and fractures among the elderly population. But the mean daily intake of calcium in postmenopausal population is estimated to be less than 600 mg/d, and the recommended dietary allowance for this age group is 1200 mg/d together with vitamin D at 800 IU/d (4). The efficiency of calcium absorption determines the amount of calcium absorbed from the diet, and in a normal situation, a low calcium intake leads to higher absorption from the gut, whereas a high calcium intake leads to lower calcium absorption; this adaptive process first described by Nicolaysen *et al.* (5) is now known to be regulated by $1,25(\text{OH})_2\text{D}$.

Calcium absorption occurs by an active saturable system and also by a passive diffusional transport system. $1,25(\text{OH})_2\text{D}$ stimulates and mediates active transcellular calcium absorption (6). In normal people, several factors can affect calcium absorption and $1,25(\text{OH})_2\text{D}$ production by the kidney. There is an age-related impairment of intestinal absorption, although the exact mechanism is unclear (7). In postmenopausal osteoporosis, there is a decrease in calcium absorption and serum $1,25(\text{OH})_2\text{D}$ levels (8). With aging, there is also a decrease in renal production of $1,25(\text{OH})_2\text{D}$ by the kidney associated with the decline in renal function (9) and an impaired response in $1,25(\text{OH})_2\text{D}$ production by the kidney to PTH stimulation (10).

Serum $1,25(\text{OH})_2\text{D}$ levels can decrease depending on the substrate supply of 25OHD, but evidence suggests that this is not critical until serum 25OHD decreases below approximately 4 ng/ml (10 nmol/liter) (11, 12).

A study of 319 subjects, age 66 yr, with serum 25OHD below 16 ng/ml (40 nmol/liter) found that calcium absorption was decreased only in a subgroup with serum 25OHD below 4 ng/ml (10 nmol/liter), and in this group, there was a decrease in serum $1,25(\text{OH})_2\text{D}$ (12). This issue of the threshold was thoroughly reviewed in the recent Institute of Medicine (IOM) report (4), but in The Endocrine Society Guidelines, it was suggested that calcium absorption reached a threshold at a serum 25OHD level of 30 ng/ml (13); however, the IOM in a later review discounted the data on serum 25OHD and calcium absorption and showed that it was incorrectly analyzed (14).

The measurement of calcium absorption after vitamin D is not well studied. In this study, we measured calcium absorption before and 1 yr after the administration of increasing doses of vitamin D_3 .

Subjects and Methods

Study design and subject population

The clinical trial was a 1-yr randomized, double-blind placebo-controlled study (ViDOS: Vitamin D Supplementation in

Older Subjects) to determine the effects of increasing doses of vitamin D_3 . The primary outcomes were serum 25OHD and serum PTH. Calcium absorption and the change in absorption was a predetermined secondary outcome. The essential details of the trial are summarized in the following paragraphs, but a more detailed methodology can be obtained from the published primary paper (15).

A total of 163 Caucasian women at least 7 yr postmenopause, ranging in age from 57–90 yr, were recruited from the general population with the help of advertising in local newspapers, churches, and direct mailings. The main inclusion criterion was vitamin D insufficiency defined as serum 25OHD no higher than 20 ng/ml (50 nmol/liter). Exclusion criteria were significant health problems, cancer within the last 10 yr (except skin), previous hip fracture, hemiplegia, uncontrolled type 1 and type 2 diabetes, active kidney stones or history of kidney stones more than twice in their lifetime, body mass index (BMI) greater than 45 kg/m², serum 25OHD below 5 ng/ml (12.5 nmol/liter), and chronic medical conditions involving liver, kidney, alcoholism, or rheumatoid arthritis. Medication exclusions were any use of fluoride, bisphosphonates for more than 3 months, PTH or derivatives within the last 6 months, calcitonin, estrogen, corticosteroid therapy of more than 10 mg/d, drugs interfering with vitamin D metabolism, such as phenytoin or phenobarbital, and high-dose thiazide therapy (> 37.5 mg/d).

Randomization and treatment

Subjects were randomly assigned to one of eight groups, 400, 800, 1600, 2400, 3200, 4000, or 4800 IU vitamin D_3 per day or matching placebo. Calcium tablets contained 200 mg elemental calcium (Citracal; Bayer HealthCare, Morristown, NJ) taken twice daily were given to maintain a total calcium intake of 1200–1400 mg/d based on a baseline 7-d food diary. Subjects were randomized to study medications after completion of all baseline tests.

The statistician provided the randomization schedule using computer-generated codes with SAS software (SAS Institute Inc., Cary, NC). Patients, providers, researchers, and persons who assessed outcomes were blinded to treatment assignment. The blinding was removed on any subject in case of a serious adverse event or other compelling reason. The drug company provided vitamin D and matching placebo (Douglas Labs, Pittsburgh, PA) in appropriately labeled bottles and provided the dose code to the statisticians but had no additional role in the study. The randomization method was randomized blocks with block sizes of 8 and 16, stratified by screening serum 25OHD level below 15 vs. 15 ng/ml or higher (<37.4 vs. \geq 37.4 nmol/liter). Participants were enrolled in winter and spring over 2 yr, April and May 2007 and January to May 2008.

The Institutional Review Board at Creighton University approved the study protocol, and all subjects were enrolled after signing an informed consent. A Data Safety and Monitoring Board was established at the beginning of the study. The Data Safety and Monitoring Board monitored the study approximately every 6–9 months for adverse events. No interim analysis was performed for safety or efficacy, and there were no pre-defined stopping rules. A central medication log was maintained of all study drugs dispensed to the subjects. Adherence was measured at 3, 6, 9, and 12 months by counting the number of vitamin D and calcium pills returned at each visit, and new bottles of vitamin D and calcium were supplied at each visit. Every 3

months, information was collected on new medications and supplements. Subjects were not allowed to take other vitamin D supplements while on study; those who wanted to take vitamin D supplements were provided with free multivitamins without vitamin D (Kirkman multivitamin without vitamins A and D; Kirkman Labs, Lake Oswego, OR).

Outcomes and follow-up

The primary outcomes of this clinical trial were the changes in serum 25OHD and serum PTH after the dosing. Secondary outcomes of the study were the change in calcium absorption, serum calcium and creatinine, 24-h urine calcium and creatinine, urine bone markers, serum 1,25(OH)₂D, bone mineral density, falls incidence, pulmonary function tests, physical performance tests, blood pressure, and cellular and genetic parameters.

Biochemical measurements

Fasting venous blood specimens were collected from the subjects in the morning, and serum was stored frozen at -70°C until analysis. Serum 25OHD and serum PTH levels were measured at baseline and 6 and 12 months. Serum 25OHD and 1,25(OH)₂D was measured by RIA in the Bone Metabolism Laboratory using kits manufactured by Diasorin, Inc. (Stillwater, MN). The minimum detection range for serum 25OHD in our laboratory is 5 ng/ml (12.5 nmol/liter). Over 3 yr, the interassay variation for serum 25OHD in our laboratory standards was as follows: 13 ng/ml (32.5 nmol/liter), 10.3%; 28 ng/ml (70 nmol/liter), 12.7%; 50 ng/ml (125 nmol/liter), 8.9%. The Bone Metabolism Laboratory participates in the Vitamin D External Quality Assessment Scheme (DEQAS) (9), which is a program that monitors the accuracy and precision of 25OHD assays; our results were within ± 1 SD of the all-laboratory trimmed mean (15). Only baseline serum 1,25(OH)₂D values are available for this paper.

Calcium absorption measurement

Calcium absorption was measured at baseline visit and at 12 months using a single-isotope method. Calcium absorption was measured after an overnight fast by drinking a 5- μCi dose of radioactive calcium (⁴⁵Ca) in 100 mg elemental calcium (calcium chloride) made up to a total volume of 100 ml distilled water. Two hours after dosing, 10 ml venous blood was collected for analysis of ⁴⁵Ca. These analyses were performed in the Creighton University Bone Metabolism Laboratory. Radiocalcium absorption was measured in duplicate samples of 2 ml serum and 18 ml scintillation liquid using a 1900 CA Tri-Carb liquid scintillation counter (Packard Instrument, Meriden, CT). The manufacturer calibrates the liquid counter every 6 months.

A 50- λ aliquot from each dose was taken on the morning of the test dissolved in 20 ml scintillation liquid and counted at the same time as the serum samples of the subject. Samples are counted to 2 sigma (2 SD) (95% probability of correct value). Serum ⁴⁵Ca absorption is expressed as percentage of the actual dose (AD) per liter of plasma. Because the serum absorption value varies with weight (lower in overweight and higher in thin people), the value is corrected for 15% of the body weight for intersubject comparisons.

The single-isotope value at 2 h in percent AD per liter is converted into the percentage of calcium absorbed using a formula derived from a previous double-isotope study (10). The percentage absorbed is specific to the calcium load used in the test, averaging approximately 50% with this 100-mg test. The

amount of calcium absorbed is calculated in a double-isotope study by deconvolutional analysis of serum data after simultaneous administration of oral and iv ⁴⁵Ca/⁴⁷Ca. The 2-h serum ⁴⁵Ca after the oral dose in a double-isotope test is highly correlated with the percentage of calcium absorbed in the double-isotope test: $y = 24 + 14x$ (y = percentage absorbed; x = serum percent AD per liter at 2 h). Pearson correlation $r = 0.705$, and $P = 0.01$ ($n = 79$).

Food diary

Dietary intake of calcium and vitamin D is calculated by a dietician from the 7-d food diaries using the Food processor II Plus nutrition and diet analysis system (version 5.1, ESHA Research, Salem, OR). These were done at baseline and at the end of the study. Plastic food models (NASCO, Fort Atkinson, WI) were used to help participants better estimate the quantities consumed.

Statistical methods

Calcium absorption was a prespecified secondary outcome in our trial. Of 163 Caucasian women, baseline analyses were completed on 159 women, one subject did not consent to the baseline and final test, and three women did not have sufficient serum for analysis. At the end of study, 16 women had discontinued from the study, leaving 143 women (88%) for the 12-month analysis.

Baseline characteristics were compared across different dose groups with ANOVA models, and the data are presented as the mean and SD. The variables were tested for normal distribution by using Kolmogorov-Smirnov test. Calcium absorption values showed a normal distribution ($P = 0.99$), with one outlier in the data, but it was included in the analysis. Serum 1,25(OH)₂D ($P = 0.42$) and dietary calcium intake ($P = 0.087$) were distributed normally. Serum 25OHD had a truncated distribution because serum values above 20 ng/ml were ineligible for study; usually, it shows a normal distribution.

Linear regression was used to look at univariate associations between baseline calcium absorption and other baseline variables: age, dietary calcium, weight, and BMI. ANOVA models were used to compare baseline 25OHD categories and baseline serum 1,25(OH)₂D and weight-corrected calcium absorption. Adjustments for multiple comparisons were made with Tukey's method. For the univariate comparisons of calcium absorption, the percent AD per liter was corrected for 15% of body weight based on previously published data (16).

A paired t test was conducted on the placebo group to study the effect of calcium supplementation without vitamin D₃ on the calcium absorption. The effect of vitamin D dose on the 12-month calcium absorption was analyzed through multiple linear regression. Baseline values for serum 25OHD, serum 1,25(OH)₂D, calcium absorption, weight, total calcium intake and tertiles of calcium intake, and age were used as covariates. A similar model examining the effect of 12-month serum 25OHD level with 12-month calcium absorption was also studied by using multiple linear regression analysis, adjusting for the same baseline characteristic. To improve model fit, the vitamin D dose and dietary calcium intake was divided by 1000. Model fit was assessed with residual plots. P values < 0.05 were considered to be statistically significant. SAS software was used for data analysis.

TABLE 1. Baseline characteristics

Vitamin D dose groups	Age (yr)	Serum calcium (mg/dl)	24-h urine calcium (mg)	Calcium intake ^a (mg/d)	Percent calcium absorbed	Serum 25OHD (ng/ml)	Serum 1,25(OH) ₂ D (pg/ml)
All (n = 163)	67 (7.3)	9.5 (0.3)	141 (67)	685 (259)	54.4 (7.6)	15.3 (3.7)	45.2 (18)
Placebo (n = 21)	66 (6.5)	9.4 (0.4)	138 (66)	593 (182)	51.3 (6.5)	15.0 (3.6)	47.8 (18)
400 (n = 20)	68 (8.6)	9.6 (0.3)	123 (70)	606 (212)	54.9 (8.4)	15.1 (4.3)	47.5 (27)
800 (n = 21)	68 (8.1)	9.4 (0.2)	157 (73)	741 (247)	58.1 (7.9)	15.5 (3.8)	43.6 (17)
1600 (n = 20)	66 (7.4)	9.6 (0.3)	133 (57)	754 (244)	55.1 (7.3)	15.0 (4.0)	46.1 (11)
2400 (n = 21)	66 (6.3)	9.5 (0.3)	139 (64)	621 (190)	54.2 (9.5)	15.2 (4.0)	47.9 (16)
3200 (n = 20)	69 (7.7)	9.4 (0.4)	151 (59)	725 (263)	53.9 (6.7)	15.9 (3.2)	46.1 (12)
4000 (n = 20)	66 (7.1)	9.4 (0.4)	148 (68)	673 (324)	55.0 (6.1)	14.9 (3.6)	44.2 (19)
4800 (n = 20)	65 (6.1)	9.5 (0.3)	134 (79)	768 (348)	52.4 (6.8)	15.6 (3.6)	38.3 (19)

Values are reported as mean (SD). Mean dietary vitamin D was 114 IU/d at baseline and 102 IU/d at 12 months. Mean dietary calcium was 593 mg/d at 12 months. Percent AD per liter is the percentage of ⁴⁵Ca dose in 100 mg calcium that is absorbed per liter of serum.

^a Obtained from 7-d food diary.

Results

Baseline characteristics of study group

The baseline characteristics are shown in Table 1. There were no significant differences between the groups at baseline. The mean (\pm SD) age of our study population was 67 (\pm 7.3) yr, and the range was 57–90 yr. The mean daily intake of calcium from food and supplements was 685 (\pm 259) mg and mean vitamin D intake was 114 (\pm 69) IU/d. Calcium absorption data are shown in Table 1.

Baseline analyses

At baseline, calcium absorption was inversely related to weight ($R^2 = 0.042$; $P = 0.009$) and BMI ($R^2 = 0.033$; $P = 0.022$). Calcium absorption (adjusted by 15% of body weight) showed a nonsignificant decrease with increasing age ($R^2 = 0.017$; $P = 0.099$).

At baseline, the range for serum 25OHD was 5–20 ng/ml and for serum 1,25(OH)₂D was 16–92 pg/ml. Using linear regression and weight as a covariate, calcium absorption is correlated with serum 1,25(OH)₂D ($P = 0.063$) but not serum 25OHD ($P = 0.46$). When the baseline data are divided into three groups based on serum 25OHD levels of 5–10, 11–15, and 16–20 ng/ml, mean serum 1,25(OH)₂D is significantly lower in the 5- to 10-ng/ml group (36.8 ± 13.1 pg/ml), compared with 46.5 ± 14.9 pg/ml in the 11- to 15-ng/ml group and 44.5 ± 17.2 pg/ml in the 16- to 20-ng/ml group ($P = 0.034$). But mean calcium absorption was not lower in the 5- to 10-ng/ml serum 25OHD group ($P = 0.65$). The numbers in each group are 15, 48, and 78, respectively.

Effect of vitamin D on calcium absorption

Overall adherence for study medication was 94% for vitamin D and 91% for calcium.

Transformation of the serum ⁴⁵Ca absorption data to percent calcium absorbed was calculated using the for-

mula described in *Subjects and Methods*. Mean calcium absorption changed in the placebo group from 51.3% at baseline to 49.1% at 12 months (1.95% AD/liter to 1.79% AD/liter; paired t test, $P = 0.65$). In the multiple regression model, the change in the mean percent calcium absorbed was 52.4–55.5 (2.03% AD/liter to 2.25% AD/liter), which is equivalent to 3 mg calcium over 12 months ($P = 0.033$) (Fig. 1A). The results expressed as % AD/liter are shown in Supplemental Fig. 1A, published on The Endocrine Society's Journals Online web site at <http://jcem.endojournals.org>. Of the covariates, only baseline calcium absorption had a significant effect on the 12-month absorption value ($P < 0.0001$), whereas baseline serum 1,25(OH)₂D ($P = 0.73$), baseline serum 25OHD ($P = 0.33$), age ($P = 0.62$), weight ($P = 0.57$), total calcium intake (dietary calcium) ($P = 0.87$), or tertiles of calcium intake had no predictive value on final calcium absorption. There was no effect of baseline dietary calcium on 12-month absorption. The adjusted R^2 for the regression model was 0.48 (Table 2).

Because subjects demonstrated variability in the response of serum 25OHD to vitamin D doses, changes in calcium absorption were analyzed as a function of 12-month serum 25OHD levels. Percent calcium absorbed increased significantly with increasing serum 25OHD ($P = 0.0019$) (Fig. 1B). In the multiple regression model the percent calcium absorbed increased by 6 percent from 52 to 58%, (1.98% AD/liter to 2.44% AD/liter; Supplemental Fig. 1B) over a serum 25OHD range of 20 to 66 ng/ml. Because the calcium load in the test is 100 mg, this corresponds to an increase in the amount of calcium absorbed of 6 mg per 100 mg calcium intake.

A significant covariate on the 12-month calcium absorption value with serum 25OHD as a predictor was baseline calcium absorption ($P < 0.0001$). There was no significant effect of baseline serum 1,25(OH)₂D ($P =$

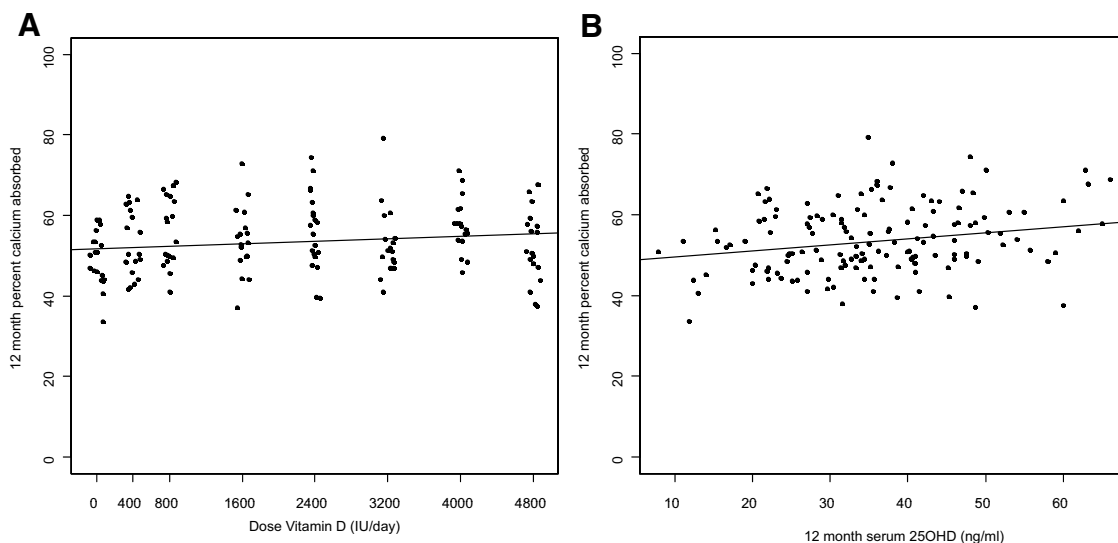


FIG. 1. A, Multiple regression analysis of 12-month calcium absorption (percent absorbed) on vitamin D₃ doses of 400–4800 IU daily. Calcium absorption was significantly correlated with dose after adjustment for age, body weight, daily total calcium intake, serum 1,25(OH)₂D, and baseline serum 25OHD (adjusted $R^2 = 0.48$; $P = 0.033$). B, Multiple regression analysis of 12-month calcium absorption (percent absorbed) vs. 12-month serum 25OHD. Final calcium absorption was significantly correlated with final serum 25OHD after adjustment for age, body weight, daily total calcium intake, serum 1,25(OH)₂D, and baseline serum 25OHD (adjusted $R^2 = 0.51$; $P = 0.0019$).

0.58), baseline serum 25OHD ($P = 0.56$), age ($P = 0.60$), weight ($P = 0.85$), total calcium intake ($P = 0.96$), or tertiles of calcium intake. There was no effect of baseline dietary calcium on 12-month absorption. Overall, 12-month calcium absorption was more highly correlated with 12-month serum 25OHD ($P = 0.0019$) than with the

dose of vitamin D ($P = 0.033$). The adjusted R^2 for the regression model was 0.51 (Table 3).

Discussion

This is the first randomized placebo-controlled study to study the long-term effect of increasing doses of vitamin D₃ on calcium absorption. Although there was a positive correlation between vitamin D₃ dose and final 12-month calcium absorption, the relationship between final calcium absorption was more highly correlated with final serum 25OHD than with vitamin D dose and can be attributed to the wide variation in an individual's response to a vitamin D dose.

In this study, if serum 25OHD increased from 20 to 66 ng/ml, then the increase in absorbed calcium would be 6%, corresponding to an increase of 6 mg per 100 mg calcium intake. In a typical normal population, serum 25OHD values usually lie between 15 and 45 ng/ml, so the increase in absorbed calcium is even smaller, 4 mg of a 100-mg calcium load. In a short-term study, administration of 50,000 IU vitamin D₂ daily for 2 wk increased calcium absorption measured by a double-isotope method by 3% of a 300-mg calcium load over a serum 25OHD range similar to that in our study (17). Although the 6% increase in calcium absorption in our study is larger than the 3% in that study, it is because results of calcium absorption depend on the amount of calcium used in the test; lower calcium loads result in higher absorption as shown in other studies (16, 18). We used a smaller calcium carrier of 100 mg without

TABLE 2. Multiple regression model of 12-month calcium absorption with vitamin D dose as a predictor

Effect	Estimate	SE	P value
Intercept	0.56340	0.61070	0.36
Age (yr)	-0.00271	0.00547	0.62
Food diary calcium (g/d), tertiles			
≤577 mg	0.01938	0.10290	0.78
577–735 mg	-0.05013	0.09821	
>735 mg	Reference		
Total calcium intake (g/d)	-0.04666	0.28920	0.87
Weight (kg)	-0.00150	0.00263	0.57
Baseline calcium absorption (% AD/liter)	0.76800	0.07363	<0.0001
Baseline serum 1,25(OH) ₂ D (pg/ml)	-0.00103	0.00293	0.73
Baseline serum 25OHD (ng/ml)	0.01085	0.01116	0.33
Dose (IU/d)	0.05331	0.02471	0.033

The adjusted R^2 for this model is 0.48. Vitamin D dose was divided by 1000 to fit the models. To estimate the outcome variable, use dose 0 in the models above to correspond to placebo; 0.4 for vitamin D 400 IU/d; 0.8 for vitamin D 800 IU/d; 1.6 for vitamin D 1600 IU/d; 2.4 for vitamin D 2400 IU/d; 3.2 for vitamin D 3200 IU/d; 4.0 for vitamin D 4000 IU/d; and 4.8 for vitamin D 4800 IU/d. Percent AD per liter is the percentage of the dose absorbed per liter of serum.

TABLE 3. Multiple regression model of 12-month calcium absorption with serum 25OHD as a predictor

Effect	Estimate	SE	P value
Intercept	0.2035	0.6154	0.74
Age (yr)	−0.00281	0.005349	0.60
Food diary calcium (g/d), tertiles			
≤577 mg	0.03678	0.101	0.91
577–735 mg	−0.00449	0.09785	
>735 mg	Reference		
Total calcium intake (g/d)	−0.01581	0.2823	0.96
Weight (kg)	0.000505	0.002642	0.85
Baseline calcium absorption (% AD/liter)	0.7591	0.07184	<0.0001
Baseline serum 1,25(OH) ₂ D (pg/ml)	−0.00157	0.002859	0.58
Baseline serum 25OHD (ng/ml)	0.006387	0.01099	0.56
12-Month serum 25OHD (ng/ml)	0.01047	0.003289	0.0019

The adjusted R² for this model is 0.51. Vitamin D dose was divided by 1000 to fit the models. Percent AD per liter is the percentage of the dose absorbed per liter of serum.

food, whereas in the above study (17), the calcium load was 300 mg of calcium in a breakfast meal that is a more physiological test. However, whichever absorption method is used, it is clear that calcium absorption is weakly related to serum 25OHD within the normal range and supports the finding in humans that the threshold at which calcium absorption is decreased is very low, approximately 4ng/ml as previously discussed (12).

The small change in calcium absorption on vitamin D₃ contrasts with the potent action of 1,25(OH)₂D. In a study in osteoporotic women, synthetic 1,25(OH)₂D 0.5 μg daily increased calcium absorption by 17% over 1 yr using the same calcium carrier of 100 mg and a double-isotope method (19). In the same patients, calcium absorption increased by 20% measured by metabolic balance studies, indicating that the 100-mg test is physiologically meaningful. The comparison between the very modest effect of vitamin D and the more potent effect of 1,25(OH)₂D is not that surprising because, as noted above, calcium absorption is maximal at a serum 25OHD between 4–10 ng/ml and because 1,25(OH)₂D is the primary regulator of calcium absorption. In normal metabolism, an increasing level of serum 25OHD increases the conversion of 1,25(OH)₂D to the inactive metabolite 24,25(OH)₂D as well as through a negative feedback loop that limits conversion of 25OHD to 1,25(OH)₂D by FGF-23 to maintain a constant level of 1,25(OH)₂D and avoid excessive calcium absorption (20), but as seen above, direct administration of 1,25(OH)₂D can overcome the regulatory systems and will lead easily to hypercalcemia unless dietary

calcium is restricted. In a study of treatment with 15 μg synthetic 25OHD to elderly women for 4 yr, there was no increase in serum 1,25(OH)₂D when measured at 6 months and then annually (21).

In the baseline data, there was a nonsignificant decrease in calcium absorption in our subjects when serum 25OHD was less than 10 ng/ml. However, serum 1,25(OH)₂D level was significantly lower when the serum 25OHD levels were below 10 ng/ml, suggesting that calcium absorption in vitamin D deficiency is reduced secondary to substrate deficiency of 25OHD and reduced 1,25(OH)₂D production as discussed earlier (10, 11). Other cross-sectional data on almost 1000 subjects have shown significant correlations only between calcium absorption and serum 1,25(OH)₂D and not with serum 25OHD, supporting the more important role of 1,25(OH)₂D in absorption in the normal physiological range of serum 25OHD (22, 23). Recently, it was suggested in The Endocrine Society Guidelines that calcium absorption reached a normal threshold at a serum 25OHD of 30 ng/ml (75 nmol/liter), and this was one of the reasons to treat people to that level with vitamin D (6). Based on a review of the literature, the IOM was not able to substantiate those data (5). When the findings from this study are combined with other results, it is clear that the threshold for normal calcium absorption occurs at a much lower level of serum 25OHD level, 5–10 ng/ml (12.5–25 nmol/liter).

The strength of this study is that this is the first long-term prospective study to study the effect of increasing doses of vitamin D supplementation on calcium absorption over a wide range of achieved serum 25OHD values in older women. Other strengths are a large sample size, the use of a 7-d food diary to record nutritional intake, adjustment for known covariates, and excellent compliance from our study population. Our study has some limitations. We did not measure calcium absorption using a double-isotope method, which is a more precise method; however, the single-isotope values correlate highly with the double isotope results. We measured baseline calcium absorption on the usual calcium intake and not on the study calcium intake. Although the comparison at the end of the study is between placebo/calcium and vitamin D/calcium, it is possible that the increased calcium intake after the baseline absorption test could suppress the absorption response to vitamin D, and this might be relevant in those with lower calcium absorption. However, we did not find an effect of baseline dietary calcium on calcium absorption in the models in Tables 2 and 3. In studies of calcium absorption in rats, there is no difference in the calcium absorption response to 1,25(OH)₂D in those with low *vs.* high calcium intake (24). In a previous analysis of calcium absorption data on 489 women, we examined the

relation between serum 25OHD and calcium absorption in a multivariate analysis correcting for calcium intake and found no effect of calcium intake (22). Our results may not apply to other age groups or ethnic groups because calcium absorption and 1,25(OH)₂D metabolism are different in older women.

In summary, our results show that the increase in calcium absorption is very small in women given vitamin D doses between 400 and 4800 IU daily. Even though serum 25OHD increases to 66 ng/ml (165 nmol/liter), the increase in calcium absorbed is only 6 mg from a 100-mg carrier dose. Malabsorption occurs only when serum 25OHD is very low, as in severe vitamin D deficiency, defined as serum 25OHD below 10 ng/ml (10–25 nmol/liter), and the lack of substrate causes a reduction in 1,25(OH)₂D production (11, 12). This explains why the vitamin D₃ effect is small compared with the known effects of oral 1,25(OH)₂D therapy because in normal physiology, the most important regulator of calcium absorption is 1,25(OH)₂D.

These results challenge the widespread assumption that vitamin D should be used to increase calcium absorption; the same amount of calcium could be absorbed by drinking a small glass of milk (100 ml) or taking a 100-mg elemental calcium tablet.

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