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A randomized, double-blind, placebo-controlled clinical trial on the treatment of vitamin D insufficiency in postmenopausal women

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Abstract

Importance—Experts debate optimal 25(OH)D levels for musculoskeletal health.

Objective—To compare effects of placebo, low-dose and high-dose vitamin D on one-year changes in total fractional calcium absorption, bone mineral density, Timed-Up-and-Go and 5-sit-to-stand tests and muscle mass in postmenopausal women with vitamin D insufficiency.

Design—Randomized, double-blind, placebo-controlled, clinical trial conducted from May 2010 to August 2014.

Setting—Single-center trial conducted in Madison, Wisconsin.

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Study supervision: Hansen supervised the study, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Participants—230 postmenopausal women ≤75 years old with baseline 25(OH)D levels 14-27 ng/mL and no osteoporosis.

Intervention—Three arms included daily white and twice monthly yellow placebo (n=76), daily 800 IU vitamin D_3 and twice monthly yellow placebo (n=76), and daily white placebo and twice monthly 50,000 IU vitamin D_3 (n=79). The high-dose vitamin D regimen achieved and maintained 25(OH)D levels ≥30 ng/mL.

Main Outcome Measures—One year change in total fractional calcium absorption using two stable isotopes, bone mineral density and muscle mass using dual energy x-ray absorptiometry, Timed-Up-and-Go and 5-Sit-to-Stand tests, functional status (Health Assessment Questionnaire) and physical activity (Physical Activity Scale for the Elderly), with Benjamini-Hochberg correction of p-values to control the false discovery rate.

Results—After controlling for baseline absorption, calcium absorption increased 1% (10 mg/day) in the high-dose arm, but decreased by 2% in low-dose (p=0.005 vs. high-dose) and by 1.3% placebo (p=0.03 vs. high-dose) arms. We found no between-arm changes in spine, mean total hip, mean femoral neck or total body bone mineral density, trabecular bone score, muscle mass, 5-sit-to-stand or Timed-Up-and-Go test scores. Likewise, we found no between-arm differences for numbers of falls, number of fallers, physical activity or functional status.

Conclusion and Relevance—High-dose vitamin D therapy increased calcium absorption, but the effect was small and did not translate into beneficial effects on bone mineral density, muscle function, muscle mass or falls. We found no data to support experts' recommendations to maintain serum 25(OH)D levels ≥30 ng/mL in postmenopausal women. Instead, we found that low and high-dose vitamin D were equivalent to placebo, in their effects on bone and muscle outcomes in this cohort of postmenopausal women with 25(OH)D levels <30 ng/mL.

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Keywords

bone mineral density; calcium absorption; clinical trial; postmenopausal women; sarcopenia; vitamin D

INTRODUCTION

Nearly half of postmenopausal women sustain an osteoporotic fracture.[1,2] Low vitamin D levels contribute to osteoporosis via decreased total fractional calcium absorption (TFCA), secondary hyperparathyroidism, increased bone resorption and decreased bone mineral density (BMD).[3] Unfortunately, experts disagree on the optimal vitamin D level for skeletal health. Some[4-6] contend that optimal serum 25(OH)D levels are ≥30 ng/mL and define vitamin D insufficiency (VDI) as <30 ng/mL. By contrast, the Institute of Medicine[7] recommends levels ≥20 ng/mL. Disagreement continues, as many previous clinical trials did not recruit subjects based on initial 25(OH)D levels, failed to target or achieve 25(OH)D levels ≥30 ng/mL, and/or co-administered calcium supplements.

VDI, defined as a serum 25(OH)D <30 ng/mL, is widespread and affects ~75% of postmenopausal American women.[8] Therefore, determining the ideal 25(OH)D level for

optimal calcium homeostasis and bone health is of utmost clinical import. The aims of this double-blind, placebo-controlled trial were to evaluate the effects of high and low-dose vitamin D on one-year changes in TFCA (the fraction of ingested calcium absorbed in the intestine), BMD and muscle fitness in postmenopausal women with VDI. Women with osteoporosis were excluded. Based on our prior pilot study,[9] we hypothesized that a high-dose vitamin D regimen, administered to achieve and maintain 25(OH)D >30 ng/mL for one year, would increase TFCA and BMD more than low-dose vitamin D or placebo.

METHODS

Study Design

With IRB approval, we conducted a single-center, randomized, double-blind, placebo-controlled trial involving postmenopausal women living around Madison, Wisconsin (latitude 43°N). Recruitment (**Figure 1**) occurred from May 2010 to July 2013 and the final visit was completed in August 2014. Individuals called in response to local advertisements, and were phone-screened for eligibility. After written consent, we measured eligible subjects' serum 25(OH)D, calcium, albumin, creatinine and parathyroid hormone levels.

We enrolled women with a 25(OH)D >14 ng/mL and ≤27 ng/mL, instead of <30 ng/mL, to allow for laboratory variability in measurements.[10] Subjects were ≥5 years past menopause or oophorectomy, or ≥60 years old if prior hysterectomy without oophorectomy. Eligible subjects consuming <600 mg or >1400 mg calcium/day via questionnaire[11] were counseled to consume 600-1400 mg/day by modifying their dietary and/or supplemental calcium intake. We targeted typical calcium intake of postmenopausal American women[12] to ensure generalizability, minimize harms of high-dose vitamin D, and because passive calcium absorption lessens the import of vitamin D-mediated active absorption.[13-15]

We excluded women >75 years old as increasing age associates with intestinal resistance to vitamin D.[16,17] We excluded women with hypercalcemia, nephrolithiasis, cancer within five years (excluding skin cancer), inflammatory bowel disease, malabsorption, celiac sprue, chronic diarrhea, glomerular filtration rate (GFR) <45 mL/minute,[18] adult fragility fracture of the hip, spine or wrist and use of bone-active medications within the past 6 months including bisphosphonates, estrogens, calcitonin, teriparatide, oral corticosteroids, anticonvulsants, or vitamin D >400 IU/day.[19] Women with diabetes were also excluded, as the disease and associated medications affect skeletal health.[20] We measured subjects' spine, bilateral hip and total body BMD and excluded those with T-scores ≤-2.5.

Subjects completed 4-7 day food diaries within one month of TFCA studies, using scales and household measuring tools to record intake. Food diaries were analyzed using Food Processor® Software (ESHA Research, Salem, OR) to calculate subjects' customary intake of nutrients (**Table 1**), caffeine and alcohol. Dietary intake, except alcohol, was reproduced during TFCA studies.

We purchased low-dose vitamin D (800 IU white capsules), high-dose vitamin D (50,000 IU yellow capsules) and identical placebo capsules (Tischon, Westbury, NY, USA) and independently verified capsule content prior to use. Subjects randomized to high-dose

vitamin D received a loading dose (50,000 IU daily for 15 days) to quickly raise 25(OH)D >30 ng/mL,[21] with sham loading of yellow placebo capsules in other arms to maintain blinding. After loading, subjects in the high-dose arm took one 50,000 IU capsule every 15th day for the next 11.5 months. Subjects in the low-dose arm took vitamin D 800 IU daily and yellow placebo capsules every 15th day. Subjects in the placebo arm ingested daily white placebo and every 15th day yellow placebo capsules (**eFigure 1).** We dispensed pre-filled 31-day pill boxes and counted remaining capsules at post-randomization visits to monitor adherence.

UW Pharmaceutical Research Center (PRC) personnel randomized eligible subjects into treatment arms in forced blocks of six (**eFigure 1**), stratifying by high PTH and calcium intake >1,000 mg/day. Stratification by PTH occurred because secondary hyperparathyroidism occurs in only 10% to 33% of people with VDI,[22-25] and individuals without it might not benefit from vitamin D. Stratification by high calcium intake occurred because passive calcium absorption, facilitated by high calcium intake, lessens the import of vitamin D-mediated active absorption.[13-15] Only PRC personnel, who had no direct contact with subjects, knew treatment allocation. We dispensed Total Block® sunscreen to subjects for use between April and October.[26,21]

Outcome Measures

The one-year change in TFCA was the primary outcome and change in BMD was the secondary outcome. Additional outcomes were the effect of placebo, low and high-dose vitamin D on muscle function, muscle mass, muscle mass, trabecular bone score and bone turnover. We also evaluated pain, functional status and physical activity during the study.

We measured TFCA using the gold-standard dual stable calcium isotope method, in which the intravenous isotope tracks renal re-absorption and endogenous fecal calcium excretion.[27,28] Isotopes were purchased as calcium carbonate powder (Trace Sciences International, Wilmington, DE); purity and enrichment were confirmed by mass spectrometry. Waisman Clinical Biomanufacturing Facility personnel reconstituted isotopes[25] and tested solutions for sterility and pyrogenicity.[29] Solutions were stored and dispensed by the PRC.

For TFCA measurements, women fasted from midnight and attended the UW Clinical Research Unit (CRU) at ~0700. After phlebotomy, subjects consumed breakfast with ≤50 mL calcium-fortified orange juice containing ~8 mg of ^{44}Ca, for a total oral calcium load of ~300 mg. The glass was rinsed with de-ionized water, which subjects also drank. Simultaneously, nurses infused ~3 mg of ^{42}Ca over 5 minutes followed by ≤50 mL normal saline. Nurses weighed isotope syringes and recorded ^{42}Ca and ^{44}Ca doses. Subjects remained on the CRU during the 24-hour urine collection, consuming meals that replicated usual nutrient intake based on food diaries. Subjects continued outpatient medications and supplements, and began study capsules on discharge.

Wisconsin State Laboratory of Hygiene personnel quantified concentrations and ratios of calcium isotopes in 24-hour urine specimens by high-resolution inductively coupled plasma mass spectrometry as described.[30,31,25] Subjects' baseline and final urine samples were

analyzed simultaneously. We calculated TFCA as the dose-corrected ratio of the two calcium isotopes in a 24-hour urine collection.[25,32]

Subjects returned for study visits ~30, 60, 120, 240 and 365 days following randomization. At each visit, we measured 25(OH)D, calcium, Timed Up and Go (TUG)[33] and five sit-to-stand (STS)[34] tests. Subjects reported pain over the prior week (10 cm scale), functional status (modified Stanford Health Assessment Questionnaire) and activity (Physical Activity for the Elderly Scale).[35] Subjects reported all adverse events, specifically nephrolithiasis, fracture, fall, infection and hospitalization. At 0, 60, 120 and 365 days, subjects' 24-hour urine calcium were measured.

PRC reviewed 25(OH)D levels at ~30, 60, 120 and 240 days. If a woman in the high-dose treatment arm had a 25(OH)D level <30 ng/mL, PRC adjusted her vitamin D dose. For example, a woman whose 25(OH)D level was 25 ng/mL received vitamin D_3 50,000 IU/day for 7 days, then 50,000 IU once weekly to achieve and maintain repletion. To preserve blinding, ~8% of subjects in the other arms received sham adjustments of yellow placebo capsules.

One year after randomization, BMD was again measured using the same Lunar bone densitometry machine (GE Healthcare, Madison, WI). The trabecular bone score was determined using TBS iNsight version 2.1.0.0 (Medimaps Group, Switzerland). Muscle mass was calculated as the appendicular lean mass in kg, divided by height in m^2.[36] Serum 25(OH)D was measured at UW using an HPLC assay[10] with between-run coefficients of variation (CV) of 3.2-13% for $25(OH)D_2$ and 2.6-4.9% for $25(OH)D_3$. Methods for other laboratory tests are described in **Table 1**.

Sample Size

The primary outcome was the effect of vitamin D on TFCA. With high-dose vitamin D,[25] the standard deviation (SD) for absolute change in TFCA was 1%. With low-dose vitamin D,[37] the SD for change in TFCA was 7%. Without intervention, the SD for monthly change in TFCA was 1%.[38] Thus, recruitment of 70 women/arm (n=210) provided ~90% power to detect a 3% difference in the change in TFCA between high-dose and placebo arms, and ~80% power to detect a 3% difference between high-dose and low-dose vitamin D arms, with a two sided alpha of 0.05. To compensate for attrition, we planned to randomize up to 250 women.

Statistical Analysis

Data were graphed to determine distribution and outliers. Normal data were summarized using the mean ± standard deviation (SD) and analyzed by analysis of variance. Skewed data were summarized using the median (25^{th}, 75^{th} interquartile range) and analyzed using the Kruskal-Wallace rank sum test. To control the false discovery rate, we corrected p-values using the Benjamini and Hochberg method[39] for subjects' baseline characteristics (**Table 1**), subjects' paired changes in dietary habits (**eTable 2**), between-arm changes in absolute and percent BMD (**Figure 3, eTable 4**), trabecular bone score (**eTable 4**), bone turnover (**eTable 5**) and adverse events (**eTables 6, 7 and 9**). Between-arm one-year changes in

muscle outcomes were summarized using means and 95% confidence intervals corrected for multiple comparisons using the Tukey honest significant difference test (**Table 2)**. All outcomes were analyzed by intent-to-treat principle, using R (The R Project for Statistical Computing, http://www.r-project.org). LASSO and StepAIC R programs were used for modeling.

A data safety monitoring board (DSMB) met every 18 months to monitor the trial's progress and safety. Withdrawal occurred for three predefined events: nephrolithiasis, hypercalcemia (defined as a serum calcium ≥10.4 mg/dL twice over ~2 weeks) or fragility fracture (spine, wrist or hip). If subjects developed hypercalciuria (defined as >400 mg/24 hours), we repeated the test. For persistent hypercalciuria, we counseled subjects to reduce calcium intake. As hypercalciuria is common and often asymptomatic,[34] its presence did not require withdrawal. All adverse events were categorized by system in Oncore.

We reported serious adverse events (death, hospitalization or predefined event) to the DSMB within 24 hours, and cumulative adverse events at DSMB meetings. To prepare reports, the team submitted subjects' adverse events to the PRC, who entered treatment assignment and forwarded reports to the DSMB. We defined an excess harm Z value > −3.0[40,41] as an indication to prematurely stop the study.

RESULTS

Figure 1 summarizes subject recruitment, randomization and completion. Nine women (4%) who withdrew from the study were similar to the remaining 221 subjects in age, race and 25(OH)D levels; all withdrew for personal reasons. Baseline demographics did not differ across treatment arms (**Table 1**). Serum 25(OH)D levels were significantly different between arms at all post-randomization visits (p<0.001, **Figure 2**). From 30 days to 365 days following randomization, average 25(OH)D levels were 19±5 ng/mL in placebo, 28±5 ng/mL in low-dose, and 56±12 ng/mL in high-dose treatment arms (p<0.001). Five subjects (7%) in the high-dose arm required additional vitamin D to maintain 25(OH)D ≥30 ng/mL. Adherence to therapy was ~100% across all arms (**eTable 1**, n=221). Subjects exhibited no significant pair-wise changes in dietary habits during the study (**eTable 2**).

Main Outcome Measures

TFCA is summarized in **eFigure 2a**. TFCA increased 0.6% in the high-dose arm and decreased by 4.5% in low-dose (p=0.009) and by 0.9% in the placebo arms (p=0.46 vs high-dose). By chance, the low-dose arm had higher baseline TFCA. In models controlling for baseline calcium absorption, TFCA increased 1% (10 mg/day) in the high-dose arm, but decreased 2% in low-dose (p=0.005 vs. high-dose) and 1.3% placebo (p=0.03 vs. high-dose) arms (**eFigure 2b**). In models (**eTable 3**), the one-year change in TFCA was inversely associated with baseline TFCA, baseline 25(OH)D and dietary sodium, and positively associated with body mass index, serum estradiol, GFR and 60-day 25(OH)D levels.

We found no between-arm differences for the absolute or annualized percent change in lumbar spine, mean total hip or total body BMD (**Figure 3, eTable 4**). Likewise, we found no significant between-arm differences for absolute or annualized percent changes in

trabecular bone score (**eTable 4**). High-dose vitamin D had a small, beneficial effect on femoral neck BMD. The absolute change in mean femoral neck BMD with high-dose vitamin D was −0.003 g/cm^2 (IQR −0.012, 0.005 g/cm^2), with low-dose vitamin D was −0.009 g/cm^2 (−0.02, 0.001 g/cm^2) and with placebo was −0.008 g/cm^2 (−0.016, −0.001 g/cm^2). The overall p-value for between-arm changes was 0.03, but with adjustment to control the false discovery rate, the p-value was no longer significant (p=0.12). Annualized changes in hip BMD were associated with change in TFCA, but only in subjects randomized to high-dose vitamin D (rho 0.24, p=0.04).

The within-arm and between-arm one-year changes in muscle outcomes are summarized in **Table 2**. All treatment arms experienced slightly faster Timed-Up-and-Go and 5-Sit-to-Stand tests during the study. However, we found no between-arm differences for the degree of improvement in either of these tests. We likewise detected no between-arm differences in muscle mass, number of falls or number of fallers. Finally, we found no between-arm differences for the one-year change in Health Assessment Questionnaire score or Physical Activity for the Elderly score.

We measured bone turnover markers in subjects who attended all study visits before 10 am, fasting since midnight (n=149, 65%). We found no consistent between-arm differences in CTX or BSAP, when analyzed as changes from baseline (**eTable 5 7**) or in models (data not shown).

Predefined adverse events are summarized in **eTable 6 8**. Nephrolithiasis was incidentally detected in a woman in the low-dose arm who underwent abdominal imaging for other reasons; lack of prior imaging precluded ability to determine timing of the stone. Falls, fractures and hospitalizations were evenly distributed across arms. Two subjects in the low-dose vitamin D arm experienced transient asymptomatic hypercalcemia. Hypercalciuria occurred nine times, seven in the high-dose (4 subjects), once in low-dose, and once in the placebo arm (p=0.19). Serum calcium and phosphorus levels were similar between all arms (**eTable 7**). At 60 days, the high-dose arm had higher urine calcium levels than the low-dose (p=0.007) or placebo arms (p=0.001) arms (**eTables 7 and 8**). Likewise at 120 and 365 days, the high-dose arm experienced higher urine calcium levels than the placebo arm (**eTables 7 and 8**). We found no other differences in adverse effects across treatment arms (**eTable 9**).

COMMENT

Experts have heatedly debated optimal 25(OH)D levels needed to optimize musculoskeletal health. While some groups[4-6,42] advocate levels ≥30 ng/mL, the Institute of Medicine[7] defines vitamin D repletion as a level ≥20 ng/mL. We designed a clinical trial to directly address ongoing controversy about optimal vitamin D levels for musculoskeletal health. We found that compared to placebo, high-dose vitamin D had a very small effect on calcium absorption (1% or 10 mg/day) that did not translate into meaningful changes in lumbar spine, mean total hip, femoral neck or total body BMD, trabecular bone score, Timed Up and Go test score, 5-Sit-to-Stand score, muscle mass, falls or number of fallers. Study results do not support the recommendation to maintain serum 25(OH)D levels ≥30 ng/mL.

In a retrospective study[43] of 316 postmenopausal women with serum 25(OH)D levels <17 ng/mL, women with levels ≤4 ng/mL had lower calcium absorption than those with higher 25(OH)D levels. Interestingly, $1,25(OH)_2D$ levels were low only in women with 25(OH)D ≤4 ng/mL. Authors concluded that profound vitamin D deficiency must exist, in order to impair calcium absorption. However, the study did not test changes in calcium absorption with vitamin D therapy, limiting the ability to conclude that calcium absorption was "optimal" in women with 25(OH)D levels ≥5 ng/mL.

Two recent randomized clinical trials[44,45] found that when controlling for baseline calcium absorption, high-dose vitamin D increased calcium absorption in postmenopausal women. In 163 women with 25(OH)D <20 ng/mL,[44] calcium absorption increased in the 4800 IU/day arm compared to placebo. However, actual difference in calcium absorption between placebo and high-dose vitamin D was only 6 mg a day. In another trial, researchers[45] randomized 67 women with 25(OH)D <30 ng/mL to 0, 800, 2000 or 4000 IU of vitamin D_3 daily for 8 weeks. Calcium absorption decreased by 2.6% in the placebo arm and increased 6.7% in the 4000 IU arm. In both studies, baseline calcium absorption was a strong independent predictor of change in calcium absorption with vitamin D therapy.

Few studies have evaluated the relationship between calcium absorption and BMD. Most cross-sectional studies[46-48] report no association. In the prospective "Study of Osteoporotic Fractures," calcium absorption (measured by single serum radioisotope level) in 5,453 Caucasian postmenopausal women[49] was weakly but significantly associated with femoral neck BMD ($r = 0.06$, $p<0.001$). Researchers subsequently recorded incident fractures for ~5 years. In models adjusting for age, each SD (7.7%) decrease in calcium absorption was associated with a 1.24 fold (95% CI, 1.05 to 1.48) increase in hip fracture, but not with fractures at other skeletal sites. The study, along with our own data, suggests that large increases in calcium absorption are needed to increase BMD and reduce fracture risk.

Even if high-dose vitamin D did not increase BMD, its use would be warranted if such therapy reduced falls, which almost always precede an osteoporotic fracture. A recent randomized clinical trial[50] of 409 women ages 70-80 was specifically designed to evaluate the effect of vitamin D or placebo on falls risk. Authors detected no reduction in falls with vitamin D therapy, administered as 800 IU daily for two years.

Sanders and colleagues[51] reported that vitamin D 500,000 IU intramuscular once yearly caused more fractures and falls than placebo. In a post-hoc analysis of a subset of subjects,[52] those randomized to vitamin D had higher $1,25(OH)_2D$ levels and bone resorption three months after randomization, potentially explaining the higher fracture rate. While we found no significant increase in bone resorption or declines in BMD associated with high-dose vitamin D, the benefits of high-dose vitamin D were too small to justify its routine use.

Our trial has several strengths. We recruited a large number of highly motivated subjects. Adherence to study medication was excellent, and attrition was low (4%). We replicated typical dietary habits during TFCA study visits. We used the gold-standard method to measure TFCA and subjects remained inpatients, permitting a complete 24-hour urine collection. Subjects received sunscreen to minimize sun-mediated increases in vitamin D

levels. Vitamin D study capsule content was independently verified prior to study use. We measured covariates that could influence TFCA, BMD and/or muscle tests besides 25(OH)D, including subjects' dietary habits, serum PTH, estradiol and $1,25(OH)_2D$ levels, pain and activity. 25(OH)D levels were measured by HPLC, one of two gold-standard assays.[53] Finally, PRC adjusted vitamin D doses to maintain 25(OH)D >30 ng/mL in the high-dose arm, with sham adjustments in other arms to maintain blinding.

We also note some study limitations. Few African American women participated, limiting our ability to detect differential responses to vitamin D based on race. Results cannot be used to guide vitamin D therapy for young adults, men or women >75 years old. Subjects participated for only one year; perhaps longer exposure to high-dose vitamin D through more remodeling cycles would yield greater effects on BMD.[54]

In conclusion, one year of high-dose vitamin D given to postmenopausal women with 25(OH)D levels <30 ng/mL (21 ± 3 ng/mL at baseline) had a trivial effect on calcium absorption, and no clinically meaningful beneficial effects on bone mineral density, muscle function or falls. Study results do not justify the common and frequently touted[4-6,42] practice of administering high-dose vitamin D to older adults, in order to maintain serum 25(OH)D levels ≥30 ng/mL. Rather, study results support the Institute of Medicine's conclusion that vitamin D repletion is a serum 25(OH)D level of ≥20 ng/mL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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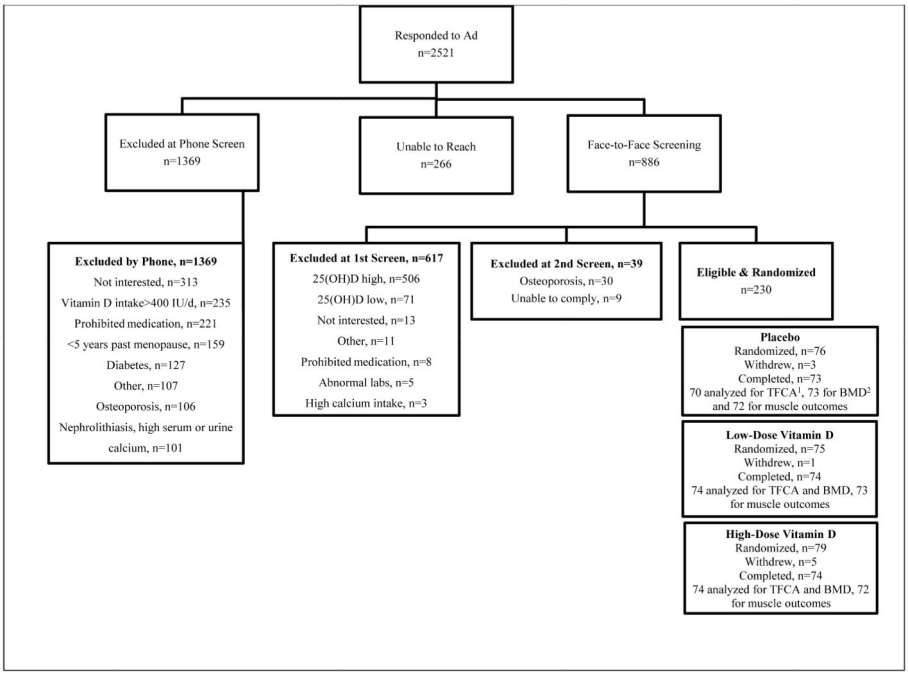
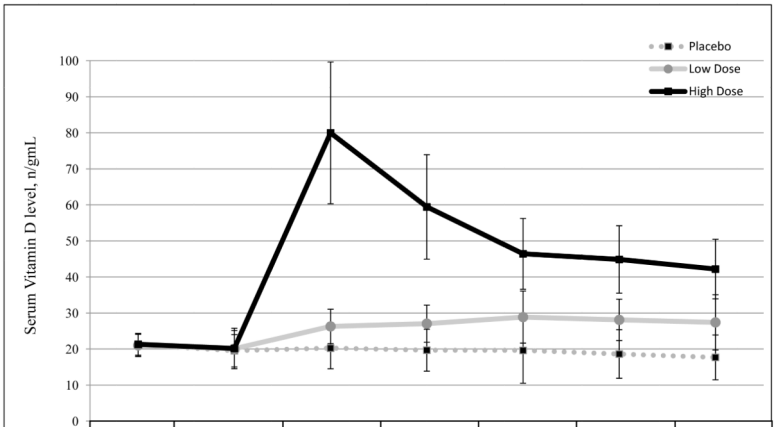


Figure 1. Participant Flow Diagram

[a]TFCA denotes total fractional calcium absorption. [b]BMD denotes bone mineral density. The calcium isotope doses were not recorded in 2 subjects and a urine sample was mishandled in a 3rd. Muscle tests were not performed in 4 subjects due to pain and/or an injury.



	Screening	Randomization	30 days	60 days	120 days	240 days	365 days
Placebo	21 ± 3	20 ± 4	20 ± 6	20 ± 6	20 ± 9	19 ± 7	18 ± 6
Low Dose	21 ± 3	20 ± 5	26 ± 5	27 ± 5	29 ± 7	28 ± 6	27 ± 8
High Dose	21 ± 3	20 ± 6	80 ± 20	59 ± 15	46 ± 10	45 ± 9	42 ± 8

Figure 2. Serum Vitamin D Levels by Treatment Assignment

Serum vitamin D levels were summarized using the mean and standard deviation, and compared across treatment arms by analysis of variance, with correction of p-values to control the false discovery rate using the Benjamini and Hochberg method. Vitamin D levels were not significantly different across treatment groups at the screening (p=0.89) and randomization (p=0.89) visits. At all subsequent visits, serum vitamin D levels were significantly different (p<0.001) across all three treatment arms. Pairwise comparisons likewise showed p-values <0.001. To convert 25(OH)D from ng/mL to mmol/L, multiply values by 2.496.

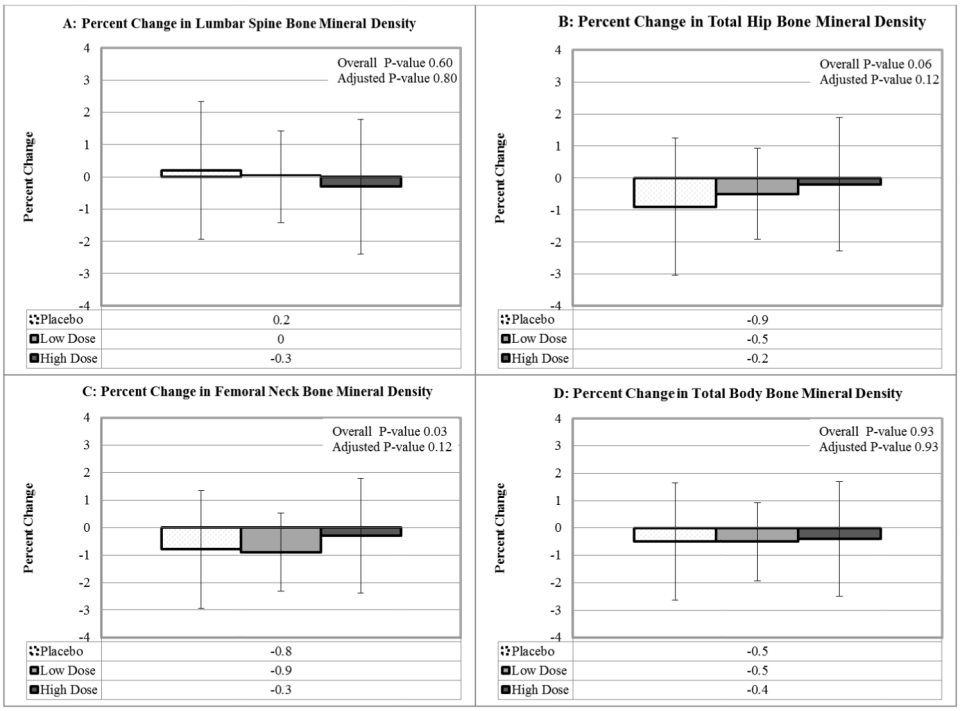


Figure 3. Annualized Percent Change in Bone Mineral Density by Treatment Assignment

We found no significant between-arm differences for the change in spine, mean total hip, mean femoral neck or total body bone mineral density. Kruskal-Wallis tests were used to calculate the overall p-value, with correction of p-values to control the false discovery rate using the Benjamini and Hochberg method.

Table 1

Baseline Characteristics of Randomized Subjects

	All Subjects n=230	**Placebo n= 76**	**Low-Dose Vitamin D n= 75**	**High-Dose Vitamin D n= 79**	***P* value[a]**
Demographic Characteristics					
Age, years	61 ± 6	61 ± 6	60 ± 6	60 ± 5	0.78
Weight, kg	81 ± 18	81 ± 19	82 ± 18	80 ± 18	0.91
Height, cm	163 ± 6	163 ± 6	164 ± 6	162 ± 5	0.91
Body Mass Index, kg/m^2	30.8 ± 6.8	30.6 ± 6.6	31.2 ± 7.4	30.7 ± 6.5	0.91
Race					
White	207 (90%)	68 (90%)	67 (89%)[b]	72 (91%)	
Black	14 (6%)	6 (8%)	7 (9%)	1 (1%)	
Asian	5 (2%)	1 (1%)	1 (1%)	3 (4%)	0.74
American Indian/Alaskan	2 (1%)	0 (0%)	0 (0%)	2 (3%)	
Hispanic/Latina	2 (1%)	1 (1%)	0 (0%)	1 (1%)	
Bone Mineral Density Measures					
Spine, g/cm^2	1.155 (1.055, 1.286)	1.143 (1.048, 1.228)	1.145 (1.080, 1.275)	1.163 (1.044, 1.280)	0.91
Spine T-Score	−0.2 (−1.1, +0.9)	−0.3 (−1.1, +0.9)	−0.3 (−0.8, +0.8)	−0.2 (−1.2, +0.9)	0.91
Hip, g/cm^2	0.961 (0.900, 1.038)	0.954 (0.882, 1.025)	0.961 (0.905, 1.038)	0.966 (0.911, 1.032)	0.91
Hip T-Score	−1.0 (−1.5, −0.5)	−1.0 (−1.7, −0.6)	−1.0 (−1.4, −0.4)	−1.1 (−1.6, −0.5)	0.78
Dietary Habits					
Kilocalories, kcal/day	1842 (1539, 2198)	1943 (1651, 2258)	1782 (1558, 2045)	1839 (1497, 2196)	0.78
Carbohydrates, g/day	222 (175, 266)	231 (194, 274)	215 (171, 261)	205 (171, 261)	0.74
Protein, g/day	75 (62, 86)	74 (59, 86)	75 (65, 89)	76 (64, 86)	0.91
Fat, g/day	72 (60, 91)	77 (58, 96)	72 (60, 88)	68 (61, 90)	0.89
Fiber, g/day	19 (14, 25)	21 (15, 28)	19 (15, 24)	17 (14, 24)	0.74
Dietary Calcium, mg/day	905 (703, 1099)	929 (777,1110)	890 (678,1101)	896 (706,1077)	0.91
Calcium Supplement, mg/day	0 (0, 0)	0 (0, 29)	0 (0, 0)	0 (0, 0)	0.78
All Calcium Intake, mg/day	967 (752, 1215)	1007 (808,1306)	961 (699,1202)	962 (739,1174)	0.78
Iron, mg/day	13 (10, 16)	14 (11, 16)	12 (9, 16)	13 (11, 16)	0.78
Magnesium, mg/day	306 (247, 370)	335 (261, 405)	289 (244, 337)	305 (247, 354)	0.47
Vitamin D, IU/day	196 (115, 266)	190 (138, 299)	176 (115, 254)	207 (107, 263)	0.91
Oxalate, servings/day	0.9 (0.4, 1.8)	1.1 (0.5, 2.1)	0.9 (0.4, 1.9)	0.6 (0.3, 1.4)	0.46
Serum Laboratory Measures					
Calcium, mg/dL	9.1 ± 0.4	9.1 ± 0.3	9.2 ± 0.4	9.1 ± 0.4	0.91
Albumin, g/dL	3.9 ± 0.3	4.0 ± 0.3	3.9 ± 0.3	3.9 ± 0.3	0.78
Creatinine, mg/dL	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.1	0.78
GFR, mL/minute	79 ± 17	79 ± 17	77 ± 17	80 ± 16	0.78

	All Subjects n=230	**Placebo n= 76**	**Low-Dose Vitamin D n= 75**	**High-Dose Vitamin D n= 79**	***P* value[a]**
PTH, pg/dL	41 (30, 54)	40 (29, 53)	42 (31, 56)	42 (33, 52)	0.78
25(OH)D, ng/mL	21 ± 3	21 ± 3	21 ± 3	21 ± 3	0.91
$1,25(OH)_2D$, pg/mL[c]	41 (31, 54)	41 (32, 51)	42 (32, 55)	40 (31, 53)	0.91
Estradiol, pg/mL[c]	48 (40, 56)	49 (40, 58)	47 (42, 54)	48 (39, 55)	0.78

SI conversion factors: To convert calcium to mmol/L, multiply values by 025. To convert albumin to g/L, multiply values by 10. To convert creatinine to μmol/L, multiply values by 88.4. To convert PTH to ng/L, multiply values by 1. To convert 25(OH)D to nmol/L, multiply values by 2.496. To convert $1,25(OH)_2D$ to pmol/L, multiply values by 2.6. To convert estradiol to pmol/L, multiply values by 3.671. At the UW Primate Center, serum $1,25(OH)_2D$ was extracted, samples were evaporated and derivatized (Amplifex Diene, AB Sciex, Framington, MA) and 30 uL was injected for liquid chromatography-tandem mass spectrometry analysis using a Shimazdu LC system (Columbia, MD) coupled to a QTRAP 5500 equipped with a Turbo V Ion source (AB Sciex) as previously published with an intra and inter-assay variability of 9.3% and 14.1%, respectively. The UW Primate Center measured serum estradiol using an in-house assay. Samples were extracted with ethyl ether, the ether was evaporated and antibody (Holly Hill Biologicals) and trace (Perkin Elmer) were added with overnight incubation. The following day a charcoal solution was added, followed by incubation for 15 minutes, centrifugation, removal of the supernatant, addition of scintillation cocktail and then analysis using a beta counter. For lower estradiol levels typical of postmenopausal women, the intra and inter-assay variability for the assay is 4.5% and 5.8%, respectively. The UW Clinical Laboratory measured PTH using a chemiluminescent immunoassay performed on the Siemens ADIVA Centaur XP. The intra and inter-assay variability for the assay is 4.4% and 6.5%.

[a] P-values were adjusted for multiple comparisons using the Benjamini and Hochberg method to control the false discovery rate.

[b] Percentages do not equal 100 due to rounding.

[c] Denotes measurement of laboratory studies at randomization rather than screening. Data with a normal distribution are summarized using mean and standard deviation, and analyzed using analysis of variance. Data with outliers are summarized using the median (1^{st}, 3^{rd} IQR) and analyzed using the Kruskal-Wallis test.

Table 2

One-Year Changes in Muscle Outcomes

Measure		Placebo n=73 of 76	Low-Dose Vitamin D n=73 of 75	High-Dose Vitamin D n=74 of 79	High vs. Low[c]	High vs. Placebo[c]	Low vs. Placebo[c]
Timed Up and Go Test	Baseline	8.28 ± 1.69	8.04 ± 1.56	8.03 ± 1.70	0.05 (−0.42, 0.53) p=0.97	−0.03 (−0.50, 0.44) p=0.99	−0.08 (−0.56, 0.39) p=0.91
	12 months	7.92 ± 1.59	7.60 ± 1.55	7.65 ± 1.77			
	Change[a]	−0.35 (−0.70, −0.01)	−0.44 (−0.66, −0.22)	−0.38 (−0.66, −0.11)			
Five Sit-to-Stand Test	Baseline	10.32 ± 2.88	9.86 ± 2.50	9.83 ± 2.27	−0.06 (−0.83, 0.72) p=0.98	−0.49 (−1.26, +0.29) p=0.3	−0.43 (−1.21, 0.34) p=0.39
	12 months	9.77 ± 3.02	8.88 ± 2.50	8.78 ± 2.09			
	Change	−0.55 (−1.02, −0.07)	−0.98 (−1.49, −0.47)	−1.04 (−1.44, −0.63)			
Health Assessment Questionnaire	Baseline	0.13 ± 0.25	0.14 ± 0.33	0.05 ± 0.14	0.04 (−0.04, 0.12) p=0.48	0.01 (−0.08, 0.09) p=0.99	−0.03 (−0.11, 0.05) p=0.58
	12 months	0.14 ± 0.33	0.12 ± 0.32	0.06 ± 0.21			
	Change	0.01 (−0.03, 0.05)	−0.02 (−0.09, 0.04)	0.02 (−0.02, 0.05)			
Physical Activity Scale for the Elderly	Baseline	169 ± 96	167 ± 85	177 ± 83	17.6 (−13.4, 48.6) p=0.38	13.2 (−17.8, 44.3) p=0.57	−4.4 (−35.5, 26.8) p=0.94
	12 months	153 ± 86	146 ± 69	173 ± 74			
	Change	−17.25 (−39.08, 4.58)	−21.64 (−37.66, −5.63)	−4.04 (−21.40, 13.33)			
Muscle mass[b]	Baseline	7.24 ± 1.05	7.35 ± 1.24	7.29 ± 1.14	−0.05 (−0.23,0.14) p=0.83	−0.1 (−0.29,0.08) p=0.39	−0.06 (−0.24,0.13) p=0.74
	12 months	7.35 ± 1.32	7.40 ± 1.40	7.30 ± 1.28			
	Change	0.1 (−0.03, 0.24)	0.05 (−0.05, 0.14)	0.002 (−0.09, 0.10)			
Falls	n per Arm	33 falls	36 falls	35 falls	p=0.92		
Fallers	subjects	23 (30%)	24 (32%)	22 (32%)	p=0.92		

[a] We summarized within-arm one-year changes in continuous muscle outcomes using the mean (95% confidence interval).

[b] Muscle mass was calculated as the appendicular lean mass (kg) divided by height in meters^2.

[c] To control the false discovery rate for multiple comparisons, we used Tukey's method to adjust the confidence intervals and p-values.