Original Investigation

Treatment of Vitamin D Insufficiency in Postmenopausal Women
A Randomized Clinical Trial

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IMPORTANCE Experts debate optimal 25-hydroxyvitamin D (25[OH]D) levels for musculoskeletal health.

OBJECTIVE To compare the effects of placebo, low-dose cholecalciferol, and high-dose cholecalciferol on 1-year changes in total fractional calcium absorption, bone mineral density, Timed Up and Go and five sit-to-stand tests, and muscle mass in postmenopausal women with vitamin D insufficiency.

DESIGN, SETTING, AND PARTICIPANTS This randomized, double-blind, placebo-controlled clinical trial was conducted at a single center in Madison, Wisconsin, from May 1, 2010, through July 31, 2013, and the final visit was completed on August 8, 2014. A total of 230 postmenopausal women 75 years or younger with baseline 25(OH)D levels of 14 through 27 ng/mL and no osteoporosis were studied.

INTERVENTIONS Three arms included daily white and twice monthly yellow placebo (n=76), daily 800 IU vitamin D_3 and twice monthly yellow placebo (n=75), 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 OUTCOMES AND MEASURES Outcome measures were 1-year change in total fractional calcium absorption using 2 stable isotopes, bone mineral density and muscle mass using dual energy x-ray absorptiometry, Timed Up and Go and five 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 for the false discovery rate.

RESULTS After baseline absorption was controlled for, calcium absorption increased 1% (10 mg/d) in the high-dose arm but decreased 2% in the low-dose arm (P = .005 vs high-dose arm) and 1.3% in the placebo arm (P = .03 vs high-dose arm). 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, and Timed Up and Go or five sit-to-stand test scores. Likewise, we found no between-arm differences for numbers of falls, number of fallers, physical activity, or functional status.

CONCLUSIONS AND RELEVANCE High-dose cholecalciferol 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 of 30 ng/mL or higher in postmenopausal women. Instead, we found that low- and high-dose cholecalciferol were equivalent to placebo in their effects on bone and muscle outcomes in this cohort of postmenopausal women with 25(OH)D levels less than 30 ng/mL.

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← Editor's Note

➕ Supplemental content at jamainternalmedicine.com

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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-hydroxyvitamin D (25[OH]D) levels are 30 ng/mL or greater (to convert to nanomoles per liter, multiply by 2.496) and define vitamin D insufficiency (VDI) as 25(OH)D levels less than 30 ng/mL. By contrast, the Institute of Medicine[7] recommends levels of 20 ng/mL or greater. Disagreement continues because many previous clinical trials did not recruit participants based on initial 25(OH)D levels, failed to target or achieve 25(OH)D levels of 30 ng/mL or greater, and/or coadministered calcium supplements.

Defined as a serum 25(OH)D level less than 30 ng/mL, VDI is widespread and affects approximately 75% of postmenopausal US women.[8] Therefore, determining the ideal 25(OH)D level for optimal calcium homeostasis and bone health is important. The aims of this randomized double-blind, placebo-controlled clinical trial were to evaluate the effects of high- and low-dose cholecalciferol on 1-year changes in TFCA, BMD, and muscle fitness in postmenopausal women with VDI. Women with osteoporosis were excluded. On the basis of a prior pilot study,[9] we hypothesized that a high-dose cholecalciferol regimen, administered to achieve and maintain 25(OH)D levels greater than 30 ng/mL for 1 year, would increase TFCA and BMD more than low-dose cholecalciferol or placebo would.

Methods

Study Design

With approval of the University of Wisconsin Institutional Review Board, we conducted a randomized, double-blind, placebo-controlled clinical trial of postmenopausal women from a single center living around Madison, Wisconsin. The study protocol can be found in Supplement 1. Recruitment (**Figure 1**) occurred from May 1, 2010, through July 31, 2013, and the final visit was completed on August 8, 2014. Individuals called in response to local advertisements and were screened by telephone for eligibility. After written consent, we measured eligible participants' serum 25(OH)D, calcium, albumin, creatinine, and parathyroid hormone (PTH) levels.

We enrolled women with a 25(OH)D level of 14 ng/mL through 27 ng/mL, instead of less than 30 ng/mL, to allow for laboratory variability in measurements.[10] Individuals were 5 years or more past menopause or oophorectomy or 60 years or older if they had undergone a prior hysterectomy without oophorectomy. Eligible individuals consuming less than 600 mg or more than 1400 mg/d of calcium identified via questionnaire[11] were counseled to consume 600 to 1400 mg/d by modifying their dietary and/or supplemental calcium intake. We targeted typical calcium intake of postmenopausal US women[12] to ensure generalizability and minimize the harms of high-dose cholecalciferol and because passive calcium ab-

sorption lessens the import of vitamin D–mediated active absorption.[13-15]

We excluded women older than 75 years because increasing age is associated with intestinal resistance to vitamin D.[16,17] We excluded women with hypercalcemia; nephrolithiasis; cancer within 5 years (excluding skin cancer); inflammatory bowel disease; malabsorption; celiac sprue; chronic diarrhea; glomerular filtration rate less than 45 mL/min[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 cholecalciferol at a dosage of more than 400 IU/d.[19] Women with diabetes mellitus were also excluded because the disease and associated medications affect skeletal health.[20] We measured individuals' spine, total-hip, and total-body BMD and excluded those with T scores of −2.5 or less.

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

We purchased low-dose cholecalciferol (800 IU, white capsules), high-dose cholecalciferol (50 000 IU, yellow capsules), and identical placebo capsules (Tischon Corporation) and independently verified capsule content before use. Participants randomized to high-dose cholecalciferol received a loading dose (50 000 IU/d for 15 days) to quickly raise their 25(OH)D levels to greater than 30 ng/mL,[22] with sham loading of yellow placebo capsules in other arms to maintain masking. After loading, participants in the high-dose arm took one 50 000-IU capsule every 15th day for the next 11.5 months. Participants in the low-dose arm took 800 IU/d of cholecalciferol and yellow placebo capsules every 15th day. Participants in the placebo arm ingested white placebo capsules daily and yellow placebo capsules every 15th day (eFigure in Supplement 2). We dispensed prefilled 31-day pill boxes and counted remaining capsules at postrandomization visits to monitor adherence.

The University of Wisconsin Pharmaceutical Research Center (PRC) personnel randomized eligible individuals into treatment arms in forced blocks of 6 (eFigure in Supplement 2), stratifying by high PTH level and calcium intake greater than 1000 mg/d. Stratification by PTH level occurred because secondary hyperparathyroidism occurs in only 10% to 33% of people with VDI,[9,23-25] 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 participants, knew the treatment allocation. We dispensed Total Block sunscreen to participants for use between April and October.[22,26]

Outcome Measures

The 1-year change in TFCA was the primary outcome, and change in BMD was the secondary outcome. Additional out-

Figure 1. Participant Flow Diagram
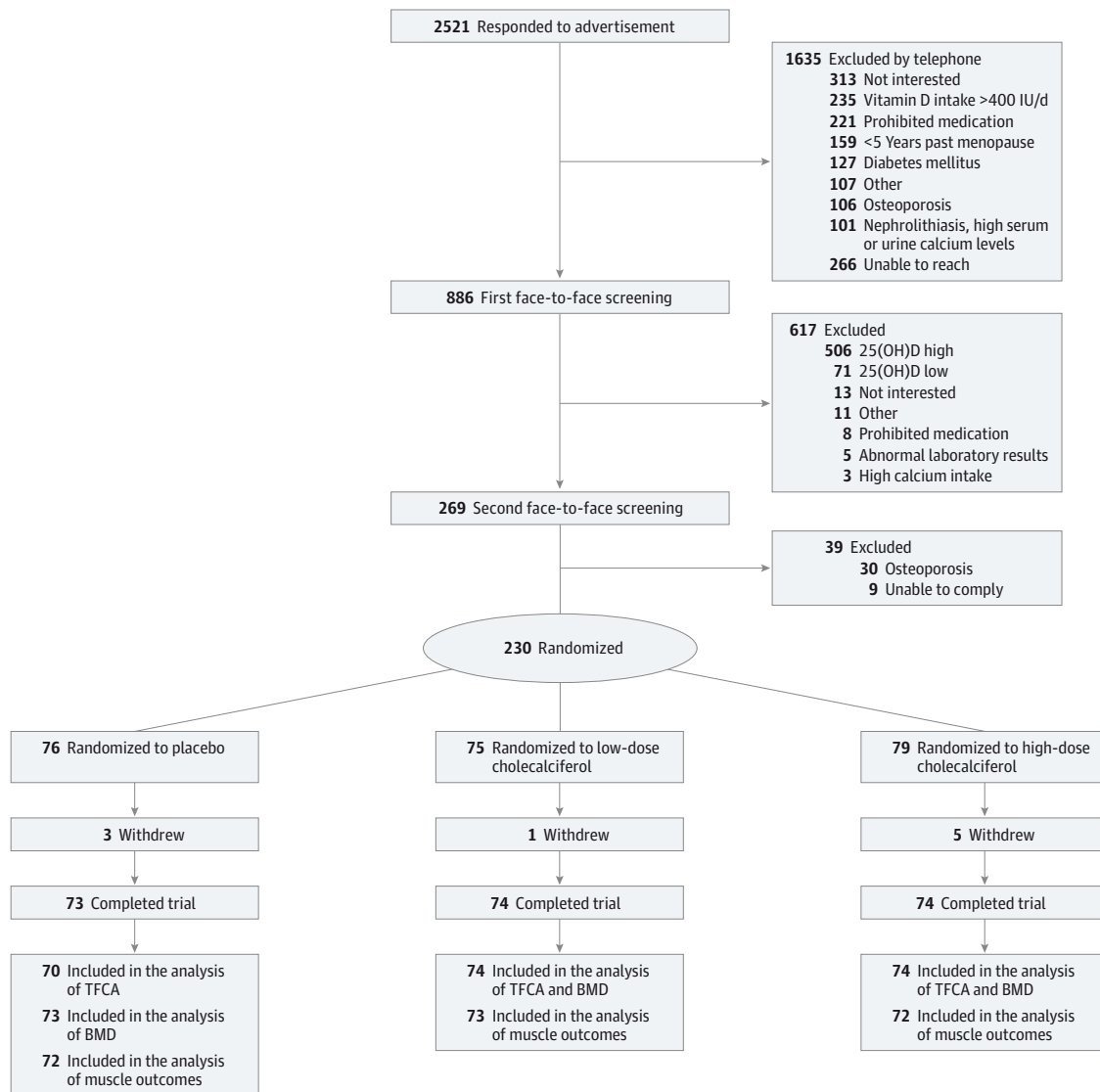
The calcium isotope doses were not recorded in 2 individuals, and a urine sample was mishandled in 1 individual. Muscle tests were not performed in 4 individuals because of pain and/or an injury. 25(OH)D indicates 25-hydroxyvitamin D; TFCA, total fractional calcium absorption; BMD, bone mineral density.

comes were the effect of placebo, low-dose cholecalciferol, and high-dose cholecalciferol on muscle function, 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 reabsorption and endogenous fecal calcium excretion.[27,28] Isotopes were purchased as calcium carbonate powder (Trace Sciences International); purity and enrichment were confirmed by mass spectrometry. Waisman Clinical Biomanufacturing Facility personnel reconstituted isotopes[9] 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 University of Wisconsin Clinical Research Unit

(CRU) at approximately 7 AM. After phlebotomy, participants consumed breakfast along with 50 mL or less of calcium-fortified orange juice that contained approximately 8 mg of ^{44}Ca for a total oral calcium load of approximately 300 mg. The glass was rinsed with deionized water, which participants also drank. Simultaneously, nurses infused approximately 3 mg of ^{42}Ca during 5 minutes followed by 50 mL or less of normal saline. Nurses weighed isotope syringes and recorded ^{42}Ca and ^{44}Ca doses. Participants remained in the CRU during the 24-hour urine collection, consuming meals that replicated usual nutrient intake based on food diaries. Participants continued taking outpatient medications and supplements and began taking study capsules on discharge.

Wisconsin State Laboratory of Hygiene personnel quantified concentrations and ratios of calcium isotopes in 24-hour

Table 1. Baseline Characteristics of the Randomized Study Participants

Characteristic	All Participants (N = 230)	Placebo (n = 76)	Low-Dose Cholecalciferol (n = 75)	High-Dose Cholecalciferol (n = 79)	P Value[a]
Demographic characteristics					
Age, mean (SD), y	61 (6)	61 (6)	60 (6)	60 (5)	.78
Weight, mean (SD), kg	81 (18)	81 (19)	82 (18)	80 (18)	.91
Height, mean (SD), cm	163 (6)	163 (6)	164 (6)	162 (5)	.91
BMI, mean (SD)	30.8 (6.8)	30.6 (6.6)	31.2 (7.4)	30.7 (6.5)	.91
Race, No. (%)					
White	207 (90.0)	68 (89.5)	67 (89.3)[b]	72 (91.1)	.74
Black	14 (6.1)	6 (7.9)	7 (9.3)	1 (1.3)	
Asian	5 (2.2)	1 (1.3)	1 (1.3)	3 (3.8)	
American Indian/Alaskan	2 (0.9)	0	0	2 (2.5)	
Hispanic/Latina	2 (0.9)	1 (1.3)	0	1 (1.3)	
Bone mineral density measures, median (IQR)					
Spine, g/cm^2	1.155 (1.055 to 1.286)	1.143 (1.048 to 1.228)	1.145 (1.080 to 1.275)	1.163 (1.044 to 1.280)	.91
Spine T score	−0.2 (−1.1 to 0.9)	−0.3 (−1.1 to 0.9)	−0.3 (−0.8 to 0.8)	−0.2 (−1.2 to 0.9)	.91
Hip, g/cm^2	0.961 (0.900 to 1.038)	0.954 (0.882 to 1.025)	0.961 (0.905 to 1.038)	0.966 (0.911 to 1.032)	.91
Hip T score	−1.0 (−1.5 to −0.5)	−1.0 (−1.7 to −0.6)	−1.0 (−1.4 to −0.4)	−1.1 (−1.6 to −0.5)	.78
Dietary habits, median (IQR)					
Caloric intake, kcal/d	1842 (1539 to 2198)	1943 (1651 to 2258)	1782 (1558 to 2045)	1839 (1497 to 2196)	.78
Carbohydrates, g/d	222 (175 to 266)	231 (194 to 274)	215 (171 to 261)	205 (171 to 261)	.74
Protein, g/d	75 (62 to 86)	74 (59 to 86)	75 (65 to 89)	76 (64 to 86)	.91
Fat, g/d	72 (60 to 91)	77 (58 to 96)	72 (60 to 88)	68 (61 to 90)	.89
Fiber, g/d	19 (14 to 25)	21 (15 to 28)	19 (15 to 24)	17 (14 to 24)	.74
Dietary calcium, mg/d	905 (703 to 1099)	929 (777 to 1110)	890 (678 to 1101)	896 (706 to 1077)	.91
Calcium supplement, mg/d	0 (0 to 0)	0 (0 to 29)	0 (0 to 0)	0 (0 to 0)	.78
All calcium intake, mg/d	967 (752 to 1215)	1007 (808 to 1306)	961 (699 to 1202)	962 (739 to 1174)	.78
Iron, mg/d	13 (10 to 16)	14 (11 to 16)	12 (9 to 16)	13 (11 to 16)	.78
Magnesium, mg/d	306 (247 to 370)	335 (261 to 405)	289 (244 to 337)	305 (247 to 354)	.47
Vitamin D, IU/d	196 (115 to 266)	190 (138 to 299)	176 (115 to 254)	207 (107 to 263)	.91
Oxalate, servings/d	0.9 (0.4 to 1.8)	1.1 (0.5 to 2.1)	0.9 (0.4 to 1.9)	0.6 (0.3 to 1.4)	.46
Serum laboratory measures, median (IQR)					
Calcium, mg/dL	9.1 (0.4)	9.1 (0.3)	9.2 (0.4)	9.1 (0.4)	.91
Albumin, g/dL	3.9 (0.3)	4.0 (0.3)	3.9 (0.3)	3.9 (0.3)	.78
Creatinine, mg/dL	0.8 (0.2)	0.8 (0.2)	0.8 (0.2)	0.8 (0.1)	.78
GFR, mL/min	79 (17)	79 (17)	77 (17)	80 (16)	.78
PTH, pg/dL	41 (30 to 54)	40 (29 to 53)	42 (31 to 56)	42 (33 to 52)	.78
25(OH)D, ng/mL	21 (3)	21 (3)	21 (3)	21 (3)	.91
1,25(OH)D$_2$, pg/mL[c]	41 (31 to 54)	41 (32 to 51)	42 (32 to 55)	40 (31 to 53)	.91
Estradiol, pg/mL[c]	48 (40 to 56)	49 (40 to 58)	47 (42 to 54)	48 (39 to 55)	.78

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)D$_2$, 1,25-dihydroxyvitamin D; BMI, body mass index (calculated as the weight in kilograms divided by height in meters squared); GFR, glomerular filtration rate; IQR, interquartile range; PTH, parathyroid hormone.

SI conversion factors: To convert 25(OH)D to nanomoles per liter, multiply by 2.496; 1,25(OH)$_2$D to picomoles per liter, multiply by 2.6; albumin to grams per liter, multiply by 10; calcium to millimoles per liter, multiply by 0.25; to creatinine to micromoles per liter, multiply by 88.4, estradiol to picomoles per liter, multiply by 3.671; PTH to nanograms per liter, multiply by 1.

[a] P values were adjusted for multiple comparisons using the Benjamini and Hochberg method[21] to control for the false discovery rate.

[b] Percentages do not equal 100 because of rounding.

[c] Denotes measurement of laboratory studies at randomization rather than screening. Data with a normal distribution are summarized using mean (SD) and analyzed using analysis of variance. Data with outliers are summarized using median (IQR) and analyzed using the Kruskal-Wallis test.

urine specimens by high-resolution inductively coupled plasma mass spectrometry as previously described.[9,30,31] Participants' baseline and final urine samples were analyzed simultaneously. We calculated TFCA as the dose-corrected ratio of the 2 calcium isotopes in a 24-hour urine collection.[9,32]

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

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

One year after randomization, the BMD was again measured using the same Lunar bone densitometry machine (GE Healthcare). The trabecular bone score was determined using TBS iNsight software, version 2.1.0.0 (Medimaps Group). Muscle mass was calculated as the appendicular lean mass in kilograms divided by height in square meters.[36] Serum 25 (OH)D was measured at the University of Wisconsin using a high-performance liquid chromatography assay[10] with between-run coefficients of variation of 3.2% to 13% for 1.25-dihydroxyvitamin D (1,25[OH]D_2) and 2.6% to 4.9% for 25(OH)D_3. Methods for other laboratory tests are listed in Table 1.

Sample Size

The primary outcome was the effect of cholecalciferol on TFCA. With high-dose cholecalciferol,[9] the SD for absolute change in TFCA was 1%. With low-dose cholecalciferol,[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 per arm (n = 210) provided approximately 90% power to detect a 3% difference in the change in TFCA between high-dose and placebo arms and approximately 80% power to detect a 3% difference between high-dose and low-dose cholecalciferol arms, with a 2-sided α of .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 (SD) and analyzed by analysis of variance. Skewed data were summarized using the median (interquartile range [IQR]) and analyzed using the Kruskal-Wallis test. To control for the false discovery rate, we corrected P values using the Benjamini and Hochberg method[21] for participants' baseline characteristics (Table 1), participants' paired changes in dietary habits, between-arm changes in absolute and percentage of BMD, trabecular bone score, bone turnover, and adverse events. Between-arm 1-year changes in muscle outcomes were summarized using means and 95% CIs corrected for multiple comparisons using the Tukey honest sig-

nificant difference test (**Table 2**). All outcomes were analyzed by the 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 3 predefined events: nephrolithiasis, hypercalcemia (defined as a serum calcium level ≥10.4 mg/dL twice during approximately 2 weeks), or fragility fracture (spine, wrist, or hip). If participants developed hypercalciuria (defined as a calcium level >400 mg/24 h), we performed the test again. For persistent hypercalciuria, we counseled participants to reduce calcium intake. Because hypercalciuria is common and often asymptomatic,[34] its presence did not require withdrawal. All adverse events were categorized by system in the OnCore Database of the University of Wisconsin.

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 participants' adverse events to the PRC, whose staff entered treatment assignment and forwarded reports to the DSMB. We defined an excess harm z value greater than –3.0[39,40] as an indication to prematurely stop the study.

Results

Figure 1 summarizes participant recruitment, randomization, and completion. Nine women (3.9%) who withdrew from the study were similar to the remaining 221 participants 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 among the arms at all postrandomization visits (P < .001, **Figure 2**). From 30 days to 365 days after randomization, the mean (SD) 25(OH)D levels were 19 (5) ng/mL in the placebo arm, 28 (5) ng/mL in the low-dose cholecalciferol arm, and 56 (12) ng/mL in the high-dose cholecalciferol arm (P < .001). Five participants (6.3%) of the 79 in the high-dose cholecalciferol arm required additional cholecalciferol to maintain 25(OH)D levels of 30 ng/mL or greater. Adherence to therapy was approximately 100% across all arms (n = 221; eTable 1 in Supplement 2). Participants exhibited no significant pairwise changes in dietary habits during the study (eTable 2 in Supplement 2).

Main Outcome Measures

eFigure 2 in Supplement 2 summarizes TFCA, which increased 0.6% in the high-dose arm and decreased 4.5% in the low-dose arm (P = .009) and 0.9% in the placebo arms (P = .46 vs high-dose arm). By chance, the low-dose arm had a higher baseline TFCA. In models controlling for baseline calcium absorption, TFCA increased 1% in the high-dose arm but decreased 2% in the low-dose arm (P = .005 vs high-dose arm) and 1.3% in the placebo arm (P = .03 vs high-dose arm) (eFigure 2 in Supplement 2). In models (eTable 3 in Supplement 2), the 1-year change in TFCA was inversely associated with the baseline TFCA, 25(OH)D level, and dietary sodium level and

Table 2. One-Year Changes in Muscle Outcomes

Measure	Placebo (n = 73 of 76)	Low-Dose Cholecalciferol (n = 73 of 75)	High-Dose Cholecalciferol (n = 74 of 79)	Mean (95% CI)[a] High vs Low	High vs Placebo	Low vs Placebo
Timed Up and Go Test						
Baseline, mean (SD)	8.28 (1.69)	8.04 (1.56)	8.03 (1.70)	0.05 (−0.42 to 0.53)	−0.03 (−0.50 to 0.44)	−0.08 (−0.56 to 0.39)
12 Months, mean (SD)	7.92 (1.59)	7.60 (1.55)	7.65 (1.77)			
Change, mean (95% CI)[b]	−0.35 (−0.70 to −0.01)	−0.44 (−0.66 to −0.22)	−0.38 (−0.66 to −0.11)			
P value				.97	.99	.91
Five Sit-to-Stand Test						
Baseline, mean (SD)	10.32 (2.88)	9.86 (2.50)	9.83 (2.27)	−0.06 (−0.83 to 0.72)	−0.49 (−1.26 to 0.29)	−0.43 (−1.21 to 0.34)
12 Months, mean (SD)	9.77 (3.02)	8.88 (2.50)	8.78 (2.09)			
Change, mean (95% CI)	−0.55 (−1.02 to −0.07)	−0.98 (−1.49 to −0.47)	−1.04 (−1.44 to −0.63)			
P value				.98	.30	.39
Health Assessment Questionnaire						
Baseline, mean (SD)	0.13 (0.25)	0.14 (0.33)	0.05 (0.14)	0.04 (−0.04 to 0.12)	0.01 (−0.08 to 0.09)	−0.03 (−0.11 to 0.05)
12 Months, mean (SD)	0.14 (0.33)	0.12 (0.32)	0.06 (0.21)			
Change, mean (95% CI)	0.01 (−0.03 to 0.05)	−0.02 (−0.09 to 0.04)	0.02 (−0.02 to 0.05)			
P value				.48	.99	.58
Physical Activity Scale for the Elderly						
Baseline, mean (SD)	169 (96)	167 (85)	177 (83)	17.6 (−13.4 to 48.6)	13.2 (−17.8 to 44.3)	−4.4 (−35.5 to 26.8)
12 Months, mean (SD)	153 (86)	146 (69)	173 (74)			
Change, mean (95% CI)	−17.25 (−39.08 to 4.58)	−21.64 (−37.66 to −5.63)	−4.04 (−21.40 to 13.33)			
P value				.38	.57	.94
Muscle Mass[c]						
Baseline, mean (SD)	7.24 (1.05)	7.35 (1.24)	7.29 (1.14)	−0.05 (−0.23 to 0.14)	−0.1 (−0.29 to 0.08)	−0.06 (−0.24 to 0.13)
12 Months, mean (SD)	7.35 (1.32)	7.40 (1.40)	7.30 (1.28)			
Change, mean (95% CI)	0.1 (−0.03 to 0.24)	0.05 (−0.05 to 0.14)	0.002 (−0.09 to 0.10)			
P value				.83	.39	.74
Falls						
No. of falls per arm	33	36	35			
P value				.92		
Fallers						
No. (%) of fallers	23 (30.3)	24 (32.9)	22 (29.7)			
P value				.92		

[a] To control for the false discovery rate for multiple comparisons, we used the Tukey honest significant difference method to adjust the CIs and P values.

[b] We summarized within-arm 1-year changes in continuous muscle outcomes using the mean (95% CI).

[c] Muscle mass was calculated as the appendicular lean mass in kilograms divided by height in meters.^{2}
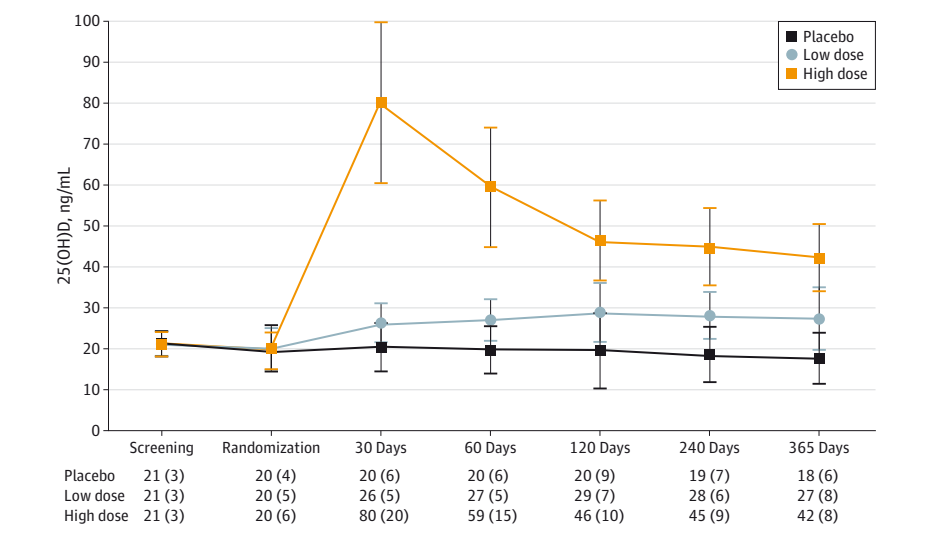
positively associated with body mass index, serum estradiol level, glomerular filtration rate, and 60-day 25(OH)D level.

We found no between-arm differences for the absolute or annualized percentage change in lumbar spine, mean total-hip, or total-body BMD (**Figure 3** and eTable 4 in Supplement 2). Likewise, we found no significant between-arm differences for absolute or annualized percentage changes in trabecular bone score (eTable 4 in Supplement 2). High-dose cholecalciferol had a small, beneficial effect on femoral neck BMD. The absolute change in median (IQR) femoral neck BMD was −0.003 g/cm² (−0.012 to 0.005 g/cm²) with high-dose cholecalciferol, −0.009 g/cm² (−0.02 to 0.001 g/cm²) with low-dose cholecalciferol, and −0.008 g/cm² (−0.016 to −0.001 g/cm²) with placebo. The overall P value for between-arm changes was .03,

but with adjustment to control for the false discovery rate, the P value was no longer significant (P = .12). Annualized changes in hip BMD were associated with change in TFCA, but only in participants randomized to high-dose cholecalciferol (ρ = 0.24, P = .04).

The within-arm and between-arm 1-year changes in muscle outcomes are summarized in Table 2. All treatment arms experienced slightly faster TUG and STS test results 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 1-year change in Health Assessment Questionnaire score or Physical Activity for the Elderly score.

Figure 2. Serum 25-Hydroxyvitamin D (25[OH]D) Levels by Treatment Assignment

	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)

Serum 25(OH)D levels were summarized using mean (SD) and compared across treatment arms by analysis of variance, with correction of P values to control for the false discovery rate using the Benjamini and Hochberg method.[21] The 25(OH)D levels were not significantly different across treatment groups at the screening (P = .89) and randomization (P = .89) visits. At all subsequent visits, serum 25(OH)D levels were significantly different (P < .001) across all 3 treatment arms. Pairwise comparisons likewise had P < .001. To convert 25(OH)D to nanomoles per liter, multiply by 2.496.

We measured bone turnover markers in individuals who attended all study visits before 10 AM, fasting since midnight (n = 149 [64.8%]). We found no consistent between-arm differences in C-telopeptide or bone-specific alkaline phosphatase, when analyzed as changes from baseline (eTable 5 in Supplement 2) or in models.

Predefined adverse events are summarized in eTable 6 in Supplement 2. 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 participants in the low-dose cholecalciferol arm experienced transient asymptomatic hypercalcemia. Hypercalciuria occurred 9 times: 7 times in the high-dose arm (4 participants), once in the low-dose arm, and once in the placebo arm (P = .19). Serum calcium and phosphorus levels were similar in all arms (eTable 7 in Supplement 2). At 60 days, the high-dose arm had higher urine calcium levels than the low-dose (P = .007) and placebo (P = .001) arms (eTable 7 and eTable 8 in Supplement 2). Likewise, at 120 and 365 days, the high-dose arm experienced higher urine calcium levels than the placebo arm (eTable 7 and eTable 8 in Supplement 2). We found no other differences in adverse effects across treatment arms (eTable 9 in Supplement 2).

Discussion

Experts have debated the optimal 25(OH)D levels needed to optimize musculoskeletal health. Although some groups[4-6,41] advocate levels of 30 ng/mL or greater, the Institute of Medicine[7] defines vitamin D repletion as a level of 20 ng/mL or greater. We designed a clinical trial to directly address ongoing controversy about optimal vitamin D levels for musculoskeletal health. We found that compared with placebo, high-dose cholecalciferol had a very small effect on calcium absorption (1%) that did not translate into meaningful changes in lumbar spine, mean total-hip, femoral neck, or total-body BMD, trabecular bone score, TUG score, STS test score, muscle mass, number of falls, or number of fallers. Study results do not support the recommendation to maintain serum 25 (OH)D levels at 30 ng/mL or greater.

In a retrospective study[42] of 316 postmenopausal women with serum 25(OH)D levels less than 17 ng/mL, women with levels of 4 ng/mL or less had lower calcium absorption than those with higher 25(OH)D levels. Of interest, 1,25(OH)D$_2$ levels were low only in women with 25(OH)D levels of 4 ng/mL or less. Those study authors concluded that profound vitamin D deficiency must exist 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 of 5 ng/mL or greater.

Two randomized clinical trials[43,44] found that when controlling for baseline calcium absorption, high-dose cholecalciferol increased calcium absorption in postmenopausal women. In 163 women with 25(OH)D levels less than 20 ng/mL,[43] calcium absorption increased in the 4800-IU/d arm compared with placebo. However, the actual difference in calcium absorption between the placebo and high-dose cholecalciferol arms was only 6 mg/d. In another trial, researchers[44] randomized 67 women with 25(OH)D levels less than 30 ng/mL to 0, 800, 2000, or 4000 IU/d of cholecalciferol for 8 weeks. Calcium absorption decreased 2.6% in the placebo arm and increased 6.7% in the 4000-IU/d arm. In both studies, baseline calcium absorption was a strong independent predictor of change in calcium absorption with cholecalciferol therapy.

Few studies have evaluated the association between calcium absorption and BMD. Most cross-sectional studies[45-47] report no association. In the prospective Study of Osteoporotic Fractures, calcium absorption (measured by single serum radioisotope level) in 5453 white postmenopausal women[48] was weakly but significantly associated with fem-

Figure 3. Annualized Percent Change in Bone Mineral Density by Treatment Assignment
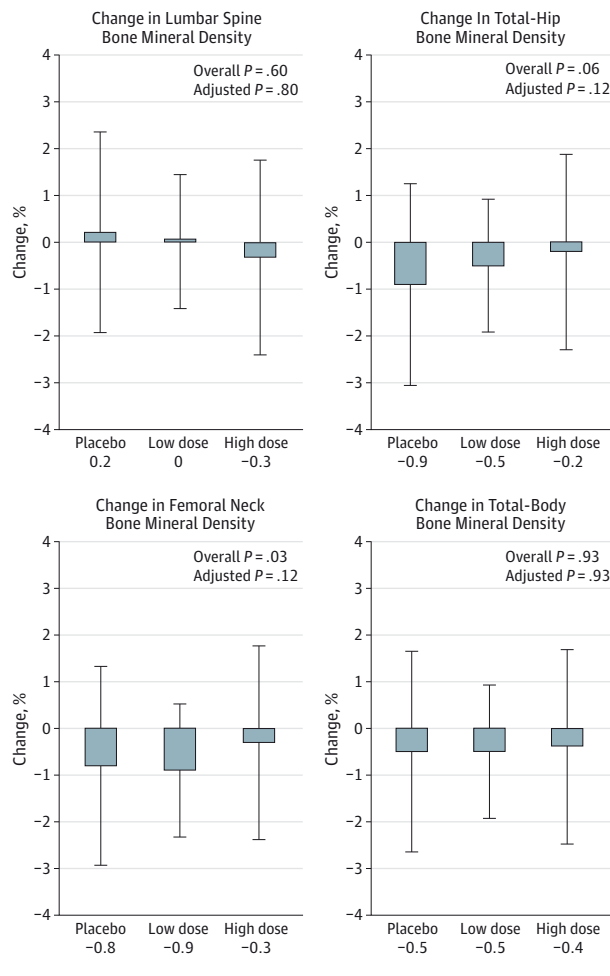
We found no significant between-arm differences for the change in spine, mean total-hip, mean femoral neck, or total-body bone mineral density. Kruskal-Wallis tests were used to calculate the overall *P* value, with correction of *P* values to control for the false discovery rate using the Benjamini and Hochberg method.[21]

oral neck BMD (r = 0.06, P < .001). Researchers subsequently recorded incident fractures for approximately 5 years. In models adjusting for age, each SD decrease (7.7%) in calcium absorption was associated with a 1.24-fold (95% CI, 1.05-1.48) increase in hip fracture but not with fractures at other skeletal sites. That 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 cholecalciferol did not increase BMD, its use would be warranted if such therapy reduced falls, which almost always precede an osteoporotic fracture. A randomized clinical trial[49] of 409 women aged 70 to 80 years was specifically designed to evaluate the effect of cholecalciferol or

placebo on the risk of falls. The authors detected no reduction in falls with cholecalciferol therapy, administered as 800 IU/d for 2 years.

Sanders and colleagues[50] reported that 500 000 IU of cholecalciferol administered intramuscularly once yearly caused more fractures and falls than placebo. In a post hoc analysis of a subset of participants,[51] those randomized to cholecalciferol had higher 1,25(OH)$_2$D levels and bone resorption 3 months after randomization, potentially explaining the higher fracture rate. Although we found no significant increase in bone resorption or decreases in BMD associated with high-dose cholecalciferol, the benefits of high-dose cholecalciferol were too small to justify its routine use.

Our trial has several strengths. We recruited a large number of highly motivated participants. Adherence to study medication was excellent, and attrition was low (4%). We replicated typical dietary habits during the TFCA study visits. We used the gold standard method to measure TFCA and participants remained inpatients, permitting a complete 24-hour urine collection. Participants received sunscreen to minimize sun-mediated increases in vitamin D levels. Cholecalciferol study capsule content was independently verified before study use. We measured covariates that could influence TFCA, BMD, and/or muscle tests besides 25(OH)D, including participants' dietary habits; serum PTH, estradiol, and 1,25 (OH)$_2$D levels; pain; and activity. The 25(OH)D levels were measured by high-performance liquid chromatography, 1 of 2 gold standard assays.[52] Finally, the PRC adjusted cholecalciferol doses to maintain 25(OH)D levels greater than 30 ng/mL in the high-dose arm, with sham adjustments in other arms to maintain masking.

We also note some study limitations. Few African American women participated, limiting our ability to detect differential responses to cholecalciferol based on race. Results cannot be used to guide cholecalciferol therapy for young adults, men, or women older than 75 years. Individuals participated for only 1 year; perhaps longer exposure to high-dose cholecalciferol through more remodeling cycles would yield greater effects on BMD.[53]

Conclusions

One year of high-dose cholecalciferol given to postmenopausal women with 25(OH)D levels less than 30 ng/mL (mean [SD], 21 [3] ng/mL at baseline) had a negligible effect on calcium absorption and no clinically meaningful beneficial effects on BMD, muscle function, or falls. Study results do not justify the common and frequently touted[4-6,41] practice of administering high-dose cholecalciferol to older adults to maintain serum 25(OH)D levels of 30 ng/mL or greater. 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 or greater.

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