Calcium and Vitamin D Supplementation in Postmenopausal Women

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Context: Bone health is influenced by the intake of both calcium and vitamin D.

Objective: Our objective was to evaluate the influence of calcium and vitamin D supplementation on PTH and bone turnover.

Setting, Patients, and Design: At an ambulatory research center, 159 postmenopausal healthy white women participated in this double-blind, placebo-controlled parallel, longitudinal factorial study that was 6 months in duration.

Interventions: Subjects were randomly allocated to 4 groups: 1) double placebo, 2) calcium (1200 mg daily) plus placebo, 3) vitamin D_3 (100 μg) plus placebo, and 4) vitamin D_3 and calcium. Serum and urine were collected fasting and 2 hours after a calcium load at baseline and at 3 and 6 months.

Main Outcome Measures: Serum PTH, cross-linked C-telopeptide (CTX), and procollagen type I N-terminal propeptide (P1NP) were measured.

Results: Before study medication, a calcium load resulted in a decline in PTH and CTX and an increase in urinary calcium excretion. Serum CTX and P1NP declined over time with calcium supplementation but did not change with increased vitamin D intake. There was a decline in PTH in the vitamin D groups in the fasting state compared with placebo. Suppression of PTH was greater after a calcium load in the vitamin D groups. A calcium load decreased PTH and CTX and raised urinary calcium.

Conclusions: Fasting PTH declines with vitamin D supplementation. PTH declines after calcium intake. Supplementation of the diet with 1200 mg calcium/d reduces bone turnover markers, whereas supplementation with up to100 μg vitamin D_3/d does not. **(*J Clin Endocrinol Metab* 98: E1702–E1709, 2013)**

The recent Institute of Medicine (IOM) report on dietary reference intakes for calcium and vitamin D recommended a calcium intake of 1200 mg/d for those over 50 years old with a tolerable upper limit (UL) of 2000 mg/d (1–3). Vitamin D intake of 600 IU/d from age 50 to 70, and 800 IU/d over 71 years for the recommended daily allowance (RDA) with an upper limit of 4000 IU/d was recommended. These dietary reference intakes have been challenged in an Endocrine Society Clinical Guideline that implied that vitamin D intake should be substantially higher in much of the population (1500–2000 IU for the RDA and 10,000 IU for the UL) (4). Both conclusions were based on the indicator bone health, a composite of factors including fracture prevention, bone loss, and osteomalacia. Other nonskeletal indicators of vitamin D sufficiency have been proposed, but neither the IOM nor the Endocrine Society found sufficient evidence that informed the formulation of an RDA for vitamin D using these putative biomarkers (1, 2, 4, 5).

Clinical trials of fracture prevention confirm a beneficial effect of calcium with vitamin D supplementation in combination (6). However, it is difficult to discern the

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Abbreviations: Ca/Cr, calcium/creatinine; CTX, cross-linked C-telopeptide; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; P1NP, procollagen type I N-terminal propeptide; RDA, recommended daily allowance; UL, upper limit.

separate contributions of these nutrients. In a previous study, we examined the influence of calcium intake and vitamin D separately as well as their interaction on biomarkers of skeletal nutritional sufficiency, ie, serum PTH and bone turnover markers (7). Because vitamin D enables calcium absorption, studies were also performed after a calcium load to examine the influence of calcium and vitamin D supplementation in the postabsorptive state. Calcium supplementation was found to lower bone turnover markers, but supplementation with 4000 IU vitamin D daily had no effect on bone turnover or PTH levels.

Several weaknesses in this previous study were recognized. The study population was not uniform in gender, ethnicity, or age, and the study duration was only 3 months. As a result of these limitations, we decided to repeat this study in a more uniform population of postmenopausal white women with a larger sample size and greater study duration. Our goal was to reexamine the influence of calcium and/or vitamin D supplementation on bone turnover and PTH levels in the fasting and postprandial states.

Subjects and Methods

A total of 363 healthy postmenopausal white participants were recruited from Winthrop University Hospital and its surrounding area (Figure 1). Participation in this trial began in December 2008 and ended in April 2011. Recruitment was carried out during winter in consecutive years through e-mail, advertisements in the local paper, and a direct mail campaign. Subjects were enrolled in two consecutive winters. Exclusion criteria included any chronic medical illness, osteoporosis, pregnancy, use of medications that influence bone metabolism or interfere with vitamin D metabolism, and unexplained weight loss during the previous year. Participants agreed to refrain from increasing dietary calcium intake or from taking self-prescribed calcium or vitamin D supplements during the study.

The study was approved by the Institutional Review Board of Winthrop University Hospital. All participants gave written consent before participation in any study-related activity. All participants and investigators were blinded to allocation throughout the study except for the research pharmacist and the statistical group. A total of 159 subjects qualified and consented to the study.

Subjects had a baseline screening visit followed by an initial randomization visit and then visits 15 and 28 weeks later. A complete medical history and physical examination was done. A 3-day dietary questionnaire was filled out by participants to assess daily calcium and vitamin D intake. Body weight and height (using a Harpenden stadiometer) were recorded. Baseline fasting laboratory tests included serum calcium, phosphorus, serum 25-hydroxyvitamin D (25(OH)D), serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), serum PTH, serum cross-linked C-telopeptide (CTX), serum procollagen type I N-terminal propeptide (P1NP), and fasting urine calcium and creatinine (8, 9). After collecting fasting blood and urine samples, participants were given a 600-mg calcium tablet in the form of calcium carbonate with a light breakfast to eat. Breakfast consisted of tea or coffee with a small amount of milk and two slices of white bread (40 mg of calcium per slice) with butter. Two hours after the oral calcium load, serum calcium, $1,25(OH)_2D$, PTH, CTX, and P1NP and urine calcium/creatinine (Ca/Cr) were again measured. The fasting and calcium load studies described above were repeated at each visit.

At the second baseline visit, 159 participants who qualified for the study were assigned to 1 of 4 groups by a simple randomization: 1) placebo vitamin D_3 supplement and active calcium supplement, 2) active vitamin D_3 supplement and placebo calcium supplement, 3) both active vitamin D_3 and active calcium supplements, and 4) both placebo vitamin D_3 and placebo calcium supplements. All subjects took one 600-mg tablet of calcium (or placebo) in the morning and in the evening for a dose of 1200 mg daily (or no calcium). Vitamin D_3 supplements were administered in 50-μg capsules. All subjects took one dose of vitamin D_3 (or placebo) in the morning and one in the evening for a dose of 100 μg (4000 IU) daily (or no vitamin D). In this double-blind, placebo-controlled, parallel-group, longitudinal factorial design, patients and researchers were blinded to group assignment. The trial was recorded with www.ClinicalTrials.gov at http://clinicaltrials.gov/ct2/show/NCT00762775?term_nct0076775&rank_1.

Medications were provided in vials containing enough doses for 12 weeks. The active and placebo calcium supplements and the active and placebo vitamin D supplements had identical appearance and weights. The compositions of the placebos were inert materials. Any con-

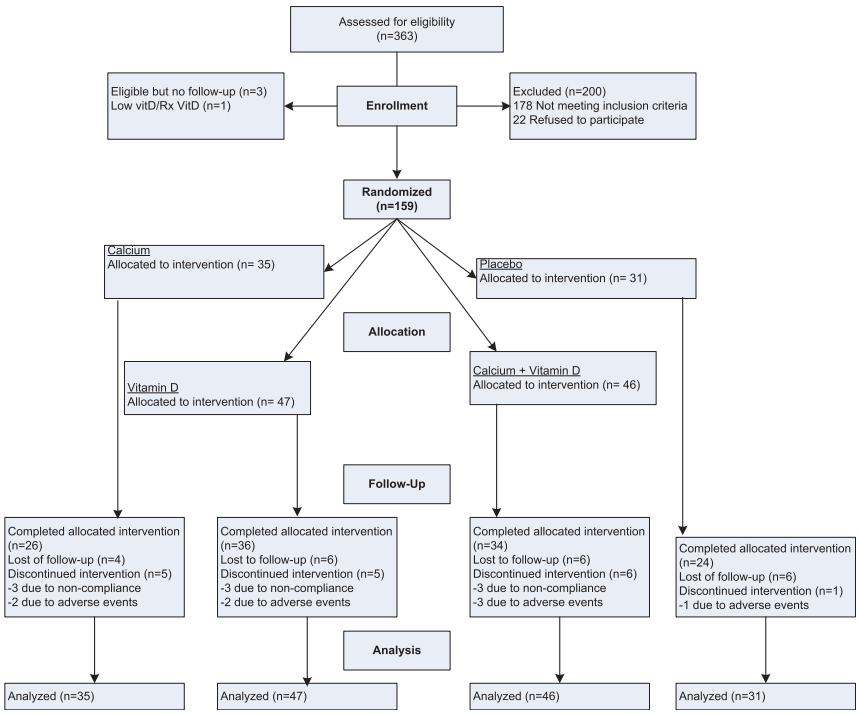

Figure 1. Flow chart of the study.

Table 1. Baseline Characteristics[a]

Variable	Randomization Group			
	Vitamin D	Calcium and Vitamin D	Calcium	Placebo
Age, y	59.7 (7.1)	57.6 (7.1)	60 (8.)5	58.6 (6.7)
BMI, kg/m^2	26.9 (3.6)	27.4 (3.9)	26.7 (3.3)	26.8 (3.9)
Dietary vitamin D, IU/d	180 (163)	158 (105)	185 (140)	215 (205)
Dietary Ca, mg/d	876 (310)	907 (288)	906 (320)	890 (259)
PTH, pg/mL	37.4 (16)	33.5 (13)	39.1 (17)	35.7 (15)
P1NP, μg/L	55.8 (21)	52.5 (18)	53.8 (21)	56.3 (21)
CTX, ng/mL	0.53 (0.21)	0.47 (0.17)	0.54 (0.25)	0.51 (0.21)
1,25D, pmol/L	113 (42)	108 (37)	115 (29)	113 (34)
25(OH)D, nmol/L	64 (16)	69 (17)	66 (19)	67 (17)
Urinary Ca/Cr	0.11 (0.07)	0.12 (0.06)	0.10 (0.07)	0.11 (0.07)

[a] Reference laboratory ranges are as follows: PTH, 17–72 pg/mL; P1NP, 16–96 μg/L; CTX, 0.142–1.351 ng/mL; 1,25(OH)$_2$D, 39–193 pmol/L; 25(OH)D, 50–125 nmol/L; urinary Ca/Cr, <0.37.

comitant medications or adverse events were recorded. At 4-week intervals, subjects were contacted to encourage compliance and record any adverse events. Participants returned for visits after 15 and 28 weeks. The 3-day dietary record was collected along with the remaining pills. A pill count was done as a means of measuring compliance. Vital signs, height, weight, and travel history were collected. Any concomitant medications and adverse events were recorded. Fasting serum calcium, serum 25(OH)D, serum PTH, serum CTX, and serum P1NP were collected. Fasting urine samples were analyzed for calcium and creatinine. Participants were given a 600-mg calcium tablet (calcium carbonate) again accompanied by a light breakfast. After 2 hours, postprandial blood and urine samples were collected. All unused study medication was collected and counted to evaluate for compliance. Vitamin D$_3$ capsules were custom manufactured by Tishcon Corp, and their content was verified in an independent laboratory (Vitamin D, Skin, and Bone Research Laboratory, Department of Medicine, Boston University School of Medicine, Boston, Massachusetts).

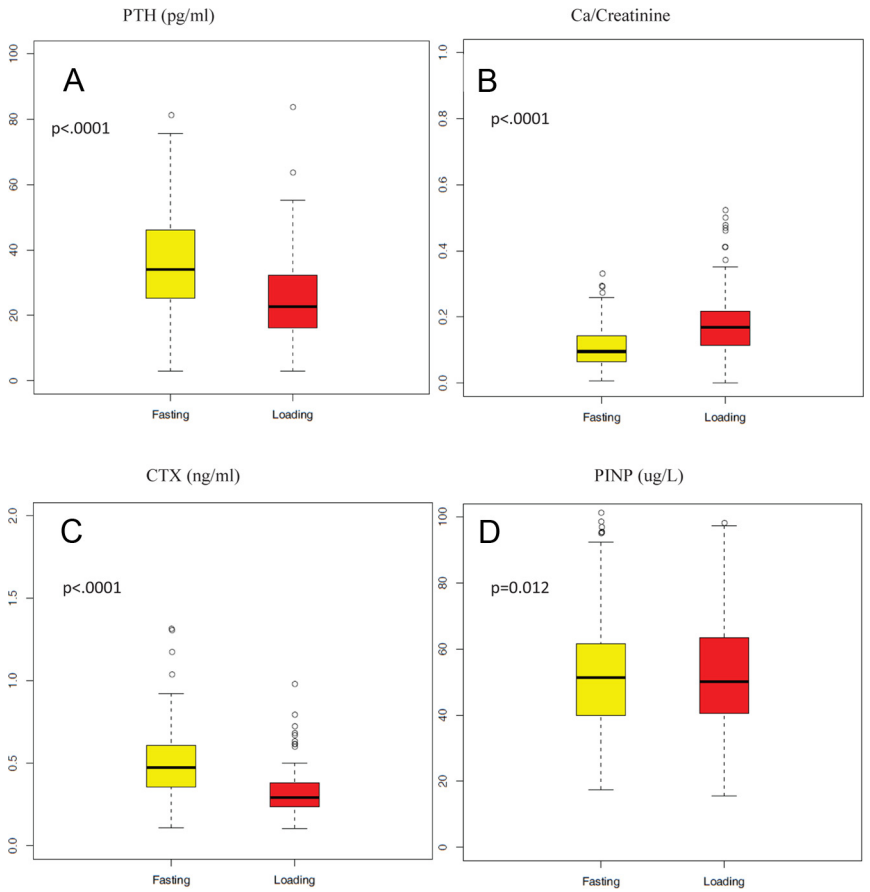

Figure 2. A–D, Response to a calcium load before intervention in 159 subjects for PTH (A), urine Ca/Cr (B), CTX (C), and P1NP (D). Reported *P* values are for comparison between fasting and calcium load.

Biochemistry

Serum 25(OH)D was measured by an RIA from DiaSorin, Inc. The minimum and maximum detectable levels are 3.75 and 250 nmol/L, respectively. The intra-assay precision (coefficient of variation) for the mean of 21.5 nmol/L is 11.7% and for the mean of 122.5 nmol/L is 12.5%. The overall intra-assay variability in our laboratory is 4.1%, and inter-assay variability is 7.0%. Our laboratory participates in and has been certified continuously since 2005 by the Vitamin D External Quality Assessment Scheme, an external quality control program (10). The reference range for serum 25(OH)D is 50 to 125 nmol/L. Serum and urinary calcium were measured by *O*-cresolphthalein complex using automated equipment Dimension-RXL (Dade Behring). Urinary creatinine was measured by Jaffe reaction with automated instrumentation Dimension-RXL. Serum PTH was measured by the Immulite 2000 analyzer for the quantitative measurement of intact PTH (Diagnostic Products Corp). Serum CTX was measured by the Serum Crosslaps ELISA kit made by Nordic Bioscience Diagnostics. Serum P1NP was measured using the UniQ P1NP RIA kit from Orion Diagnostica. Serum CTX provides a measure of bone resorption, and P1NP is a marker of type I collagen synthesis. Serum 1,25(OH)$_2$D

concentrations were measured by an ELISA kit made by Immunodiagnostic Systems. The inter- and intra-assay variability in our laboratory is 17% and 10%, respectively, The reference range for this assay is 39 to 193 pmol/L.

Nutrition assessment

Total daily calcium and vitamin D intake was estimated from a 3-day diet history using Nutrition Pro nutrition analysis software (11).

Statistical methodology

The study consisted of 159 subjects at baseline with observations collected at 3 time points (baseline and 15 and 28 weeks). Simple randomization was performed at baseline using a computer-generated randomization list, and subjects were assigned to 1 of the 4 groups, namely placebo, vitamin D alone, calcium alone, and vitamin D plus calcium. All enrollments occurred in winter. The number of subjects varied at each time point due to dropouts. A total of 120 subjects completed the study.

Descriptive statistics are reported as mean (SD). A repeated-measures mixed-effect model (SAS Proc GLIMMIX) was used for all primary analysis where time, calcium, and vitamin D were taken as fixed effects. The mixed-effect model under the assumption of ignorability (12) allowed us to include all subjects even with the missing data at any time point, thus keeping intact the intent-to-treat principle. We considered both 2-way and 3-way interaction terms between time, calcium, and vitamin D. A subject-specific random slope was also included in the model, and unstructured variance-covariance matrix was used throughout. In each analysis, we were interested to find the significance of the interaction term between calcium and vitamin D with time. A post hoc power analysis revealed that our baseline sample size was large enough to detect this 2-way fixed-effect interaction with sufficient power (>80%) but was not sufficient (<80%) to detect a 3-way interaction between time, calcium, and vitamin D. Consistent with the previous study by our group (7), we calculated this power for a moderate effect size as described in Cohen (13). The significance of each term (interaction and main effects) is reported via a traditional F test obtained from a mixed-effect ANOVA using SAS software version 9.2. A two-tailed P value $<$ .05 was used to determine significance, and α (type-1 error) was kept fixed at 0.05. No statistical correction of P value is reported for multiple comparisons because this has an inflationary effect on the P value (11). Hence, finding a P value close to a .05 threshold should be taken as exploratory in nature and requires confirmation with a larger study.

Results

Demographics

There were no significant differences between the study groups in age, BMI, or dietary intake of calcium (Table 1). There were also no significant differences in baseline laboratory values. Mean dietary calcium intake was less than the recommended intake of the IOM, and mean serum 25(OH)D was above the RDA-associated serum 25(OH)D concentration of 50 nmol/L but below The Endocrine Society's recommended value of 70 to 80 nmol/L. There were no significant group differences in dietary calcium throughout this study.

The distribution of baseline fasting PTH was as follows: >65 pg/mL, n = 6. The baseline 25(OH)D distribution was as follows: <50 nmol/L, n = 35; >50 nmol/L and less than 75 nmol/L, n = 73. Four subjects had serum 25(OH)D <50 nmol/L and PTH >65 pg/mL. Serum fasting CTX and P1NP were above the reference range in 24 and 17 participants, respectively.

A total of 363 women were screened, and 159 were enrolled. Final analyses were done on 159 subjects.

Influence 2 hours after a calcium load before intervention (n = 159)

The influence of the calcium load performed on all subjects (n = 159) before intervention may be seen in Figure 2. There was no change in serum creatinine or 25(OH)D, as expected. Serum calcium increased slightly ($P < .0001$) accompanied by a decline in serum PTH ($P < .0001$). Serum CTX and P1NP declined significantly, and there was a significant increase in urine calcium.

Values after intervention assignment in the 4 groups in the fasting state

Data are presented in Table 2. There were no significant interaction terms (time × vitamin D and time × calcium) in the fasting state for serum creatinine or calcium and for urine Ca/Cr. There was a significant decline in bone turnover markers for calcium supplementation: P1NP ($P < .002$) and CTX ($P < .001$), but no interaction was observed for the vitamin D interventions. Findings were similar when baseline PTH or the bone turnover markers were used as covariates. There was a significant decline ($P < .03$) in serum $1,25(OH)_2D$ for the calcium but not the vitamin D intervention.

Fasting serum PTH trended downward for the vitamin D intervention ($P = .075$) and for the calcium intervention ($P = .096$). The variability in the fasting serum PTH values was notable. Because of the significant correlation between baseline and subsequent values of PTH, baseline adjusted analysis of PTH was performed (Figure 3). In this analysis, there was a significant decline in PTH over time as captured by the interaction between time and vitamin D ($F_{1,113} = 4.07$, $P = .046$) and a nonsignificant decline in the calcium ($F_{1,113} = 2.26$, $P = .13$) groups. The slope of change of PTH over time was significant for the combined treatment group ($P < .0003$). A separate analysis of PTH was also done comparing individual groups with placebo alone. There were significant differences in the vitamin D ($P < .05$) and combined groups ($P = .02$) and a trend with the calcium group ($P = .07$).

Table 2. Fasting and Calcium Load Values With Treatment Assignments[a]

	Urine Ca/Cr			Serum Ca, mg/dL			$1,25(OH)_2D$, pmol/L		
Group	Fasting	Load	Change	Fasting	Load	Change	Fasting	Load	Change
Placebo									
Base	0.11 (0.07)	0.17 (0.09)	0.06 (0.08)	9.6 (0.37)	9.9 (0.48)	0.28 (0.3)	113.1 (34.2)	111.9 (36.9)	−1.14 (19.7)
15 wk	0.09 (0.05)	0.15 (0.08)	0.07 (0.06)	9.6 (0.41)	9.8 (0.31)	0.19 (0.28)	117.7 (42.3)	113.5 (34.7)	−2.5 (24.2)
28 wk	0.12 (0.1)	0.22 (0.26)	0.1 (0.2)	9.4 (0.29)	9.6 (0.46)	0.2 (0.33)	108.5 (33.2)	112.2 (32.9)	2.6 (22.3)
Calcium									
Base	0.1 (0.07)	0.17 (0.09)	0.07 (0.07)	9.5 (0.39)	9.7 (0.43)	0.21 (0.26)	115.1 (28.9)	119.5 (31.7)	4.47 (19.6)
15 wk	0.09 (0.05)	0.19 (0.1)	0.09 (0.1)	9.5 (0.44)	9.8 (0.44)	0.33 (0.33)	108.7 (29)	108.9 (34)	0.29 (20.1)
28 wk	0.12 (0.09)	0.21 (0.12)	0.1 (0.08)	9.4 (0.45)	9.7 (0.53)	0.22 (0.32)	108.3 (39.8)	102 (33.9)	−6.3 (26.7)
Vitamin D									
Base	0.11 (0.07)	0.16 (0.09)	0.05 (0.08)	9.7 (0.32)	9.9 (0.37)	0.22 (0.3)	112.9 (42.2)	116.5 (45)	4.4 (24.5)
15 wk	0.1 (0.09)	0.16 (0.11)	0.05 (0.08)	9.6 (0.39)	9.9 (0.46)	0.27 (0.24)	128.9 (38.5)	141.8 (46.2)	11.4 (39.4)
28 wk	0.08 (0.05)	0.18 (0.1)	0.09 (0.07)	9.5 (0.33)	9.8 (0.38)	0.28 (0.3)	126.9 (41)	133.3 (43.8)	5.9 (28.3)
Calcium + vitamin D									
Base	0.12 (0.06)	0.21 (0.11)	0.1 (0.1)	9.6 (0.41)	9.9 (0.38)	0.34 (0.28)	107.6 (36.8)	112.7 (33)	5.1 (20.7)
15 wk	0.14 (0.09)	0.24 (0.15)	0.11 (0.11)	9.7 (0.33)	9.9 (0.38)	0.25 (0.23)	100.5 (25.9)	105.8 (27.8)	5.3 (21)
28 wk	0.13 (0.07)	0.27 (0.15)	0.14 (0.12)	9.6 (0.32)	9.9 (0.35)	0.23 (0.24)	96.1 (30.3)	102.2 (36.4)	6.2 (20.1)
P values	.671,[b] .08[c]	.329,[b] .127[c]	.533,[b] .898[c]	.374,[b] .084[c]	.372,[b] .164[c]	.943,[b] .873[c]	.306,[b] .03[c]	0.067,[b] <.001[c]	.361,[b] .082[c]

[a] Results are shown as mean (SD). Reference laboratory ranges are as follows: urine Ca/Cr, <0.37; serum Ca, 9.1–10.4 mg/dL; $1,25(OH)_2D$, 39–193 pmol/L; P1NP, 16–96 μg/L; CTX, 0.142–1.351 ng/mL; PTH, 14–72 pg/mL. *P* values reported are for second-order interaction terms.

[b] *P* values for time × vitamin D interaction effect.

[c] *P* values for time × calcium interaction effect.

Values after intervention in the 4 groups after a calcium load

Response to the oral calcium load for each relevant parameter is also given in Table 2. Again, as expected, there was no change in fasting creatinine or serum calcium. Calcium intervention resulted in declines after ingestion of a calcium load for PTH, ($P < .038$), P1NP ($P < .002$), and CTX ($P < .003$). Serum PTH declined from the fasting levels in the vitamin D intervention ($P = .038$). During supplementation, the decline in PTH after a calcium load was higher in both vitamin D treatment groups (for combined $P = .03$). The placebo and calcium-alone groups did not differ significantly from the fasting results.

Response of vitamin D metabolites

The response of serum 25(OH)D in the vitamin D-supplemented groups is depicted in Supplemental Figure 1 (published on The Endocrine Society's Journals Online website at http://jcem.endojournals.org). The dose response is curvilinear. In the fasting state, the calcium intake group experienced a significant decline in calcitriol over time ($F_{1,259} = 4.74$, $P = .03$). A similar response was seen in the calcium-loaded state, with an even lower *P* value ($F_{1,260} = 11.26$, $P < .001$), suggesting a larger effect size. Serum calcitriol in the vitamin D treatment group showed a trend toward increase (Supplemental Figure 2).

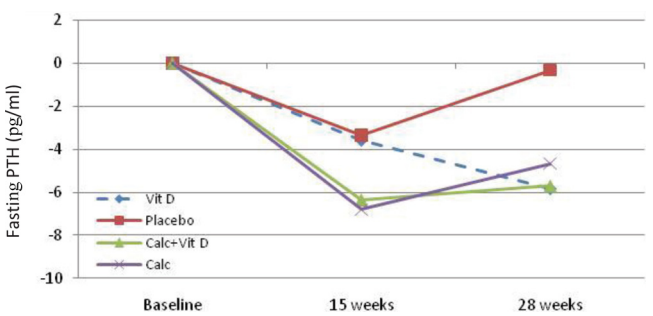

Figure 3. Groupwise mean plot of 4 groups for response to fasting PTH after baseline adjustment. A mixed-effects model revealed that the interaction term time × vitamin D was significant ($F_{1,113} = 4.07$, $P = .046$), and time × calcium was nonsignificant ($F_{1,113} = 2.26$, $P = .13$).

Compliance and adverse events

Compliance (pill count) was 78% for vitamin D and 78% for calcium. There were no differences in compliance for any group. There were no serious adverse events in the study. Reported adverse events are given in Table 3 using a scheme similar to MedDRA. The patients who dropped out because of adverse events were as follows: placebo group, 1 for flank pain; vitamin D group, 1 for a sprained ankle and 1 for arthralgia; calcium plus vitamin D group, 1 for constipation, 1 for upset stomach, 1 for palpitations and headaches; and calcium group, 1 for upset stomach and 1 for leg cramps and weakness. No change in mean serum calcium or phosphate was seen in the fasting state. One incident of transient hypercalcemia was noted in the calcium plus vitamin D group.

Using 0.23 for the upper limit for the urine Ca/Cr ratio in the fasting state during the intervention periods, the frequency of values exceeding this concentration was highest in the combined treatment group (10 instances, compared with 1–2 in the other groups). Using the less sensitive value for defining hypercalciuria of a Ca/Cr greater than 0.37, there was only 1 episode of hypercal-

Table 2. Continued

	P1NP, μg/L			CTX, ng/mL			PTH, pg/mL		
Group	**Fasting**	**Load**	**Change**	**Fasting**	**Load**	**Change**	**Fasting**	**Load**	**Change**
Placebo									
Base	56.3 (21.1)	54.3 (22)	−1.5 (9.1)	0.51 (0.21)	0.31 (0.12)	−0.2 (0.13)	35.7 (15.1)	26.9 (14.9)	8.8 (9.20)
15 wk	56.1 (19.7)	53.9 (19.9)	−2.7 (8.2)	0.55 (0.23)	0.34 (0.14)	−0.23 (0.15)	35.9 (14.2)	25.8 (13.1)	−6.7 (9.7)
28 wk	55.5 (22.3)	53.4 (21.8)	−2.6 (7.3)	0.57 (0.24)	0.35 (0.12)	−0.22 (0.14)	36.7 (18.8)	30.5 (18.9)	−5.9 (13.5)
Calcium									
Base	53.7 (21.3)	54.5 (23.2)	0.35 (6.5)	0.54 (0.25)	0.34 (0.15)	−0.23 (0.16)	39.1 (16.9)	26.3 (12.3)	−12.8 (10.1)
15 wk	46.3 (22.2)	48.9 (25.1)	−0.16 (6)	0.44 (0.22)	0.29 (0.16)	−0.17 (0.1)	31.7 (14)	25 (11)	7.8 (7.5)
28 wk	45.1 (22.9)	42.9 (17.8)	−2.2 (9.8)	0.5 (0.27)	0.31 (0.15)	−0.19 (0.15)	32.9 (17.1)	23.7 (14.7)	−9.2 (11.8)
Vitamin D									
Base	55.8 (21.3)	51.2 (16.2)	−2.9 (4.5)	0.53 (0.21)	0.32 (0.13)	−0.18 (0.14)	37.4 (16)	26.7 (13.8)	11.1 (10.4)
15 wk	56.1 (19)	51.5 (16.7)	−3.6 (5.2)	0.57 (0.2)	0.33 (0.12)	−0.24 (0.11)	34.3 (17.2)	22.4 (12.2)	−9.9 (6.9)
28 wk	54 (17.3)	50.9 (15.8)	−3.1 (5.7)	0.58 (0.19)	0.34 (0.13)	−0.25 (0.11)	32.8 (10.6)	24.1 (15.6)	−9 (11)
Calcium + vitamin D									
Base	52.5 (17.8)	55 (18.2)	−1.29 (4.5)	0.47 (0.17)	0.32 (0.16)	−0.17 (0.13)	33.5 (13)	22 (10.6)	−11.5 (9.5)
15 wk	48.7 (15.1)	47.2 (15.2)	−2.6 (7.2)	0.44 (0.21)	0.27 (0.11)	−0.18 (0.13)	23.7 (10.3)	18 (8)	−5.8 (6.4)
28 wk	50.1 (16)	45.9 (14.3)	4.2 (7.8)	0.45 (0.17)	0.27 (0.12)	−0.18 (0.11)	24 (11.6)	19.1 (9.5)	−5 (9.6)
P values	.686,[b] .002[c]	.802,[b] <.001[c]	.994,[b] .198[c]	.437,[b] <.001[c]	.225,[b] .003[c]	.045,[b] .022[c]	.075,[b] .036[c]	.038,[b] .412[c]	.725,[b] .266[c]

ciuria in each group. No adverse events were believed to be related to supplementation other than hypercalciuria.

Discussion

This study provides insight into the response to dietary supplementation with calcium and/or vitamin D. Before group assignment, a calcium load resulted in significant declines in PTH and bone turnover markers (CTX and P1NP) and an increase in urinary calcium excretion. The increase in urinary calcium excretion presumably reflects the calcium load that is not used. These responses are likely seen after each ingestion of a calcium supplement (or calcium-rich meal) and result in a postprandial decrease in PTH and bone resorption. After long-term dietary supplementation, a decline in fasting PTH is observed with vitamin D supplementation, likely as a result of genomic effects of calcitriol on the parathyroid (14). Although calcium supplementation appropriately suppresses calcitriol levels with high vitamin D intake (in the absence of calcium supplementation), calcitriol levels do not decline from baseline even when a calcium load suppresses PTH. Calcium loading is more effective in suppressing PTH in the presence of vitamin D supplementation.

These findings are consistent with our previous study that was not age-, race-, or gender-specific and found that calcium supplementation reduced bone turnover markers but vitamin D supplementation did not (7). This finding is consistent with a recent food fortification study with a similar design and other studies as well (15).

The decline in PTH under high-calcium/high-25(OH)D conditions may represent a pharmacologic effect. Sai et al (14) noted that serum PTH declines continuously up to 150 nmol/L. They propose that this decline may be the result of pharmacologic effects of higher serum 25(OH)D or calcitriol on the vitamin D response element in the PTH gene (14). In concert with earlier studies, we found that serum calcitriol concentration rises with increasing doses of vitamin D supplementation (see Supplemental Figure 2) (16).

The mean baseline dietary calcium intake in the current study was over 900 mg/d, and baseline serum 25(OH)D was above 45 nmol/L, yet calcium supplementation did reduce bone turnover despite some studies that would not have predicted this (17, 18). Because this was not a dose-response study, we can only conclude that 1200 mg/d of calcium supplements is more effective in reducing bone turnover than an unsupplemented intake of 900 mg/d. The calcium intake in the current study was at the UL (2000 mg/d) recommended by the IOM. The UL value was based on an increased risk of kidney stones observed in the calcium supplementation arm of the Women's Health Initiative (19).

In our previous study, bone turnover markers showed a trend to increase in the high vitamin D group (3). In the current, more homogeneous study, an increase in bone turnover was not observed in the high vitamin D group. Thus, with intakes of vitamin D at the current UL (4000 IU/d), we found no evidence for adverse effects on bone health in terms of increased bone turnover. The risk of

Table 3. Adverse Events by Randomization Groups

System Organ Class (MedDRA)	Number of Subjects With ≥1 Event			
	Vitamin D Group	Calcium Group	Calcium + Vitamin D Group	Placebo Group
Cardiac disorders	0	1	3	0
Congenital, familial, and genetic disorders	0	0	0	0
Gastrointestinal disorders	7	3	6	4
General disorders and administration site conditions	0	0	0	1
Immune system disorders	0	0	0	0
Infections and infestations	4	2	5	4
Injury, poisoning, and procedural complications	0	0	0	0
Investigations	0	0	0	0
Metabolism and nutrition disorders	2	1	1	0
Musculoskeletal and connective tissue disorders	4	5	6	3
Neoplasms: benign, malignant, and unspecified (including cysts and polyps)	0	0	0	0
Nervous system disorder	3	1	5	2
Psychiatric disorders	0	0	0	0
Respiratory, thoracic, and mediastinal disorders		1	1	
Skin and sc tissue disorders	0	0	1	0
Surgical and medical procedures	0	0	0	0
Vascular disorders	0	0	0	0
Total	20	14	26	14

increased bone turnover at the UL proposed by The Endocrine Society (10 000 IU/d) is unknown.

We speculate that increasing calcium intake after the calcitriol-dependent process is saturated will result in transcellular transport and a decline in postabsorptive PTH levels. The decline in PTH after the calcium load at baseline in our study was notable. The effect of increased calcium intake can best be detected in the postabsorptive rather than the fasting state. The intermittent effect of calcium intake can be readily detected on the bone turnover markers, indicating a sustained effect on bone turnover. In contrast, vitamin D may have a genomic effect on PTH secretion but at high levels may act directly to increase bone remodeling, thereby counteracting any beneficial influence of PTH secretion on bone turnover (5). A recent study found that standard vitamin D therapy decreased bone turnover to a greater extent than very high doses (the equivalent of 6500 IU/d) (15).

There are several limitations of this study. A 24-hour measurement of PTH would have been preferable as would a larger sample size. There has been a great deal of discussion in recent years about the most accurate assay for serum 25(OH)D (20). However, our laboratory was certified by the Vitamin D External Quality Assessment Scheme when these analyses were performed and was within the all-laboratory trimmed mean (ALTM). Furthermore, this study was performed in subjects who had baseline serum 25(OH)D levels actually above the serum 25(OH)D-linked RDA recommendations of the IOM. Nonetheless, the conditions of this study are similar to advice that may be given to women currently, and it is clear that increased calcium supplementation reduced bone turnover, whereas increasing serum 25(OH)D (above 50 nmol/L) did not.

Our study supports the benefit of high calcium intake in reducing bone turnover in postmenopausal white women. The placebo group had a mean daily calcium intake of 946 mg. Bone turnover was significantly reduced by the addition of 1200 mg/d of calcium supplements and further reduced by a calcium load. Current recommendations for nutritional intake of calcium heavily relied on calcium balance studies (1). It is unknown whether this effect would be sustained or whether it only reflects the remodeling transient, although it has been sustained in some studies (21–23). Moreover, it is unknown whether this is a pharmacologic effect and what the long-term safety of very high calcium intake may be (24). Our combined calcium/vitamin D group had more instances of hypercalciuria. Hypercalciuria has been observed in other studies as well (5, 13, 16). Studies using a less sensitive definition of hypercalciuria (ie, 0.37 Ca/Cr) may not have noted its presence (25).

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