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Long-Term Effects of Calcium Supplementation on Serum Parathyroid Hormone Level, Bone Turnover, and Bone Loss in Elderly Women

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ABSTRACT

We report a 4-year randomized, double-blind, placebo-controlled clinical trial in 236 normal postmenopausal women (mean age ± SE, 66.3 ± 0.2 years) who were randomized to a calcium (1600 mg/day as the citrate) or placebo group. The women were seen every 6 months; 177 completed the trial. Net percentage changes in each group are given relative to baseline. The differences in net percentage changes (calcium group minus placebo group) in medians were: for lumbar spine bone density, 2.0% ($p < 0.001$) at year 1 and 0.3% (not significant) at year 4; for proximal femur bone density, 1.3% ($p = 0.003$) at year 1 and 1.3% ($p = 0.015$) at year 4; and for total body bone mineral, 0.4% ($p = 0.002$) at year 1 and 0.9% ($p = 0.017$) at year 4. Similar differences at year 4 were: −18.9% ($p = 0.002$) for parathyroid hormone (PTH), −11.9% ($p = 0.026$) for serum osteocalcin, and −32.2% ($p = 0.003$) for urine free pyridinoline. We conclude that long-term administration of calcium supplements to elderly women partially reverses age-related increases in serum PTH level and bone resorption and decreases bone loss. However, the effects on bone loss were weaker than those reported for estrogen, bisphosphonates, or calcitonin therapy, indicating that calcium supplements alone cannot substitute for these in treating established osteoporosis. Nonetheless, because of their safety, high tolerance, and low expense, calcium supplements may be a useful preventive measure for elderly postmenopausal women whose bone mineral density values are normal for their age. (J Bone Miner Res 1998;13:168–174)

INTRODUCTION

Osteoporosis causes more than a million fractures each year in the United States, requiring care at an estimated annual cost of $13.8 billion.[1] Given the magnitude of this problem, public health measures are important and calcium supplements are an attractive option for preventive intervention. With aging and the postmenopausal state, the dietary calcium requirement increases because calcium absorption is impaired[2–4] and renal calcium conservation is decreased.[5,6] Although a National Institutes of Health (NIH) Consensus Development Panel[7] has recommended a calcium intake of 1500 mg/day, a recent national survey[8] found that elderly women consume less than 700 mg/day, which is even less than the RDA for calcium of 800 mg/day established by the National Academy of Sciences.

Clinical studies of the effects of dietary calcium intake on bone loss in postmenopausal women have given conflicting results. During the first few years after menopause—the rapid phase of bone loss—calcium supplements are ineffective in retarding cancellous bone loss from the central skeleton and have only a modest effect in slowing loss of

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cortical bone from the peripheral skeleton,[9–11] suggesting that this phase of postmenopausal bone loss is mediated primarily by estrogen deficiency. In older postmenopausal women, calcium supplements generally have produced a higher bone mineral density (BMD) in the treatment group than in the placebo group only at 1 year, with no significant difference thereafter.[12–17] This indicates that either the initial beneficial effect cannot be maintained or that clinical trials lasting only 2 or 3 years did not have sufficient statistical power to detect a subsequent more modest slowing of bone loss. Positive results, however, were reported by Reid et al.[18] in a 2-year open-label extension of their 2-year trial to 4 years and, more recently, by Recker et al.[19] in a 4-year clinical trial.

Because more information is needed to define the role of calcium supplementation in the prevention of osteoporosis, we conducted a 4-year randomized, prospective, double-blind, placebo-controlled clinical trial in 236 late postmenopausal normal women to assess effects on rates of bone loss from the total skeleton, vertebrae, and hip and on age-related increases in serum parathyroid hormone (PTH) and biochemical markers of bone turnover. Because fracture rates in Olmsted County, Minnesota, resemble those nationally[20] and because the study subjects were normal elderly women from a demographically characterized,[21] age-stratified segment of the Olmsted County population and were not selected on the basis of a low calcium intake, the results are relevant to the general population of American white women.

MATERIALS AND METHODS

Experimental subjects

All of the 236 women residents of Olmsted County enrolled in this study were fully ambulatory between 61 and 70 years of age, and postmenopausal for 10 years or more. None had a history of osteoporotic fracture or evidence of vertebral fracture by clinical assessment of roentgenograms of the spinal column, as assessed qualitatively by a radiologist, and all had normal values (Z score above -2.0) on bone densitometry of the vertebrae and proximal femur, as adjusted for age and gender. We excluded subjects with a history of renal lithiasis, impaired renal function, hypercalcemia, or hypercalciuria (>300 mg/24 h). As assessed by review of their complete (inpatient and outpatient) medical records and by interview and testing, none of the enrolled women had any disease known to affect bone or calcium metabolism. All had normal values for serum and urinary calcium and serum creatinine. None was receiving estrogen, large doses of vitamin D or calcium, or other drugs known to affect bone, and none had a history of use of fluoride or bisphosphonate drugs. However, women taking supplementary calcium at ≤500 mg/day and/or vitamin D at ≤800 U/day at baseline were eligible for inclusion in the trial.

Subjects first were screened for entry criteria by examination of their medical records at Mayo. For this, we examined 3013 medical records which constituted essentially all women residents of Olmsted County who were between 61 and 70 years of age[21]; 1993 women were not eligible on the basis of the exclusion criteria. Of those remaining, 500 responded positively to a letter requesting an interview, 306 agreed to participate in the clinical trial, and 236 were found to be eligible and were accepted into the trial. All of the women gave written informed consent, and the trial was reviewed and approved by the Mayo Institutional Review Board.

Experimental design

The clinical trial was randomized, controlled, prospective, and double-blind and was conducted under intent-to-treat conditions. Thus, data from all women randomized into the trial were used in data analysis. The women were assigned randomly to the treatment (119 women) or to the control (117 women) group. The subjects were prescribed calcium tablets or matching placebo tablets four times daily—with each meal and at bedtime. The calcium was given as the citrate salt for a total dose of elemental calcium of 1600 mg/day. During the 4 years that the women were treated, each was seen every 6 months. Annually, a blood sample was drawn in the early morning while the subject was fasting, and a 24-h urine collection was turned in at each visit. At baseline and again at the end of the study, roentgenograms of the thoracic and lumbar spinal columns were obtained and an assessment of calcium intake was made by a dietitian from a 7-day food record. Bone densitometry was performed at 6-month intervals. When a serum calcium value >10.4 mg/dl or a urinary calcium value >350 mg/24 h was found, the dosage of calcium (or placebo) was decreased according to a predetermined algorithm. To maintain blinded conditions for the investigators, an unblinded statistician selected a paired woman from the opposite group (placebo or treatment) for whom the dosage was decreased by a comparable number of tablets. The study medications were dispensed by a research pharmacist, and compliance was monitored by pill counting at each visit.

Laboratory studies

All serum samples were obtained in the early morning while the subject was fasting. The serum and urine samples were stored at -70°C until they were analyzed periodically in batches. Biomedical variables were measured annually except for serum 25-hydroxyvitamin D (25(OH)D), which was measured only at baseline, and urinary free pyridinoline, which was measured at baseline and at 1 and 4 years. Serum levels of phosphate, creatinine (Cr), and alkaline phosphatase and urinary levels of Cr were measured by routine methods with an AutoAnalyzer. Serum osteocalcin[22] and bone alkaline phosphatase isoenzyme[23] levels were measured by radioimmunoassays. Free pyridinoline cross-links in a 24-h urine collection were measured with an enzyme immunoassay kit (Pyrilinks, Metra BioSystems, Mountain Home, CA, U.S.A.). Serum intact PTH was measured by a two-site immunochemiluminometric assay.[24] Serum 25(OH)D and 1,25-dihydroxyvitamin D were measured by the methods of Eisman et al.[25] and Kumar et al.,[26] respectively. BMD of the lumbar spine (LS-BMD) and proximal femur (PF-BMD) and total body bone min-

Table 1. Baseline Values in the Two Groups in the Clinical Trial

Variable	Mean ± SD	
	Calcium	*Placebo*
Age (years)	66.2 ± 2.5	66.3 ± 2.6
Time since menopause (years)	16.5 ± 4.4	16.4 ± 4.7
Dietary calcium (mg/day)	711 ± 276	717 ± 295
Serum		
calcium (mg/day)	9.55 ± 0.32	9.58 ± 0.28
intact PTH (pmol/l)	2.72 ± 1.20	2.75 ± 1.09
bone alkaline phosphatase (U/l)	22.3 ± 8.3	24.5 ± 9.5
osteocalcin (ng/dl)	6.36 ± 1.77	6.51 ± 1.86
25(OH)D (ng/ml)	30.4 ± 10.5	29.7 ± 10.3
$1,25(OH)_2D$ (pg/ml)	37.2 ± 10.8	37.6 ± 11.2
estradiol (pg/ml)	13.7 ± 13.0	13.1 ± 11.2
Urine		
calcium (mg/day)	137 ± 63	144 ± 62
free pyridinoline (mmol/mmolCr)	46.9 ± 27.8	45.4 ± 23.2
Creatinine clearance (ml/minute)	78.4 ± 17.0	81.0 ± 15.8
Nephrogenous cAMP (nmol/minute)	3.36 ± 0.82	3.60 ± 1.11

eral (TBBM) were measured by dual-energy X-ray absorptiometry with a Hologic QDR 2000 scanner (Hologic Inc., Waltham, MA, U.S.A.). Vertebral fractures were assessed as described[27] with an eight-point digitization method for the T4 to L5 vertebrae on the lateral roentgenogram. A change of more than 15% from baseline in the anterior, middle, or posterior height of a vertebra was considered to indicate a new fracture.

Statistical analysis

For each subject, all follow-up measurements were related to her corresponding baseline value by expressing any change as a percent of baseline. At each time of observation, the calcium group was compared with the control group using the Wilcoxon rank sum test, since the percent changes did not appear to have a Gaussian distribution. For these time-specific analyses, all subjects providing data at that follow-up time are used in an analysis of data. The reported p values are not corrected for multiple comparisons.

RESULTS

Clinical findings

The major clinical and biochemical characteristics of the subjects at baseline are shown in Table 1. There were no between-group differences in these or in any other covariates, which indicates that the randomization was successful. Of the 236 women enrolled, 177 completed all 4 years of the trial (88 in the calcium and 89 in the placebo group). Thirty-three discontinued treatment for personal reasons or intercurrent illnesses, and 16 discontinued because of side effects (10 in the calcium and 6 in the placebo group). Other reasons for dropping out included BMD below the normal range in two subjects, osteoporotic fractures in five, death in a motor vehicle accident in one, lost to follow-up in one, and taking calcium prescribed by local personal physician in one. The side effects included excessive abdominal cramping, constipation, bloating, or diarrhea (nine in the calcium group; two in the placebo group), arthralgia and depression (one, placebo), renal stone (one, placebo), and hypercalciuria (one, calcium; two, placebo).

In one subject in the calcium group, mild hypercalcemia developed; 44 women in the calcium group and 7 in the placebo group had a urinary calcium exceeding 350 mg/24 h during the study, necessitating a reduction in dosage (calcium or placebo). One patient in the placebo group had hematuria and was found to have a kidney stone. There were no significant differences between groups in the numbers of new vertebral fractures as determined by a fracture algorithm (8 for the calcium group and 9 for the placebo group) or of new nonvertebral fractures (11 for the calcium group and 12 for the placebo group) or total new fractures. As assessed by tablet counts, the mean dose of calcium in the treatment group was 1234 mg/day. There was no significant change in dietary calcium intake in either group over the course of the study.

Laboratory findings

Median BMD values at the three scanning sites in both the calcium treatment and placebo group at baseline and median changes from BMD at 1 and 4 years are given in Table 2. The differences (calcium group minus placebo group) of the net change (follow-up as percent of baseline) in BMD values over time are shown in Fig. 1. For net BMD, the difference in median LS-BMD values was 2.0% ($p < 0.001$) by 1 year which then gradually decreased to 0.3%

TABLE 2. MEDIAN CHANGES AND 25TH TO 75TH PERCENTILE RANGE IN BMD FROM BASELINE AT 1 AND 4 YEARS AFTER TREATMENT

	Lumbar spine		*Proximal femur*		*Total body*	
Interval	*Ca*	*Placebo*	*Ca*	*Placebo*	*Ca*	*Placebo*
Baseline	0.90	0.92	0.81	0.81	1.02	1.03
Change, 1 year (*n*)	(103)	(110)	(102)	(110)	(103)	(109)
Median	0.018	0.001	0.013	0.003	0.008	0.004
Range	−0.001, 0.039	−0.015, 0.019	−0.001, 0.026	−0.012, 0.017	−0.002, 0.018	−0.005, 0.011
p	<0.001		0.002		0.003	
Change, 4 years (*n*)	(88)	(89)	(87)	(89)	(88)	(88)
Median	0.024	0.021	0.003	−0.010	0.011	0.002
Range	−0.002, 0.055	−0.014, 0.043	−0.023, 0.024	−0.031, 0.005	−0.0004, 0.023	−0.014, 0.021
p	0.127		0.016		0.019	

Results are shown as grams per square centimeter. Interquartile range is 25th to 75th percentiles.
p values based on Wilcoxon rank-sum test comparing calcium and placebo groups.

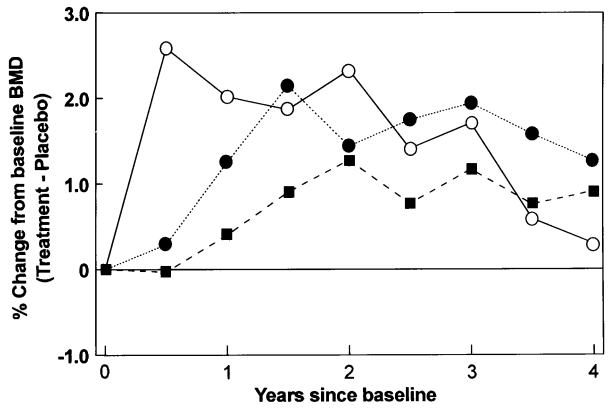

FIG. 1. Group differences (treatment group minus placebo group) in median BMD values as net from baseline (percent change from baseline) for measurements at lumbar spine (open circles and solid line), proximal femur (closed squares and dashed line), and TBBM (closed circles and dotted line).

(not significant) by 4 years; the difference in median PF-BMD values was 1.3% ($p = 0.003$) by 1 year and remained at 1.3% ($p = 0.015$) at 4 years; the difference in median TBBM values was 0.4% ($p = 0.002$) by 1 year and then increased to 0.9% ($p = 0.017$) by 4 years.

Values for the change in serum and urine variables are given in Table 3. Differences (calcium group vs. the placebo group) in median values were −18.9% ($p = 0.002$) for serum PTH, −11.9% ($p = 0.026$) for osteocalcin, a marker for bone formation, and −32.2% ($p = 0.003$) for free pyridinoline, a marker for bone resorption.

For both BMD and biochemical measurements, when the significance of changes in the treatment versus the placebo group is analyzed using data for women completing the full 4 years of the trial, the results are similar to those obtained using intent-to-treat conditions.

DISCUSSION

Our data show that daily administration of 1600 mg of calcium to elderly women for 4 years decreases the age-related increases in serum PTH and bone resorption and decreases the rate of bone loss. We found that the calcium supplements were safe and well-tolerated. The only side effect of the calcium preparation was the occurrence of minor gastrointestinal symptoms in a small number of subjects. However, to maintain values for urinary calcium at <350 mg/day, it was necessary to decrease the dose of calcium citrate in about one-third of the subjects in the calcium treatment group. Because patients with achlorohydria hyperabsorb calcium as citrate in the fasting state and about 10% of elderly women are achlorohydric,[28] this might explain the hypercalciuria that developed during treatment in some subjects. Also, mild hypercalcemia occurred in one subject in the calcium group; none of the subjects in the calcium group experienced nephrolithiasis, nephrocalcinosis, or a decrease in glomerular filtration rate.

Although the retardation in the rate of bone loss was relatively small, this effect was more substantial than that found in most of the previously reported trials that were conducted for only 2 or 3 years. The longer duration of our study is probably responsible for our greater statistical power. Our results were not greatly different from those of Reid et al.[18] who followed a 2-year study with an open-label extension for 2 more years in most of the original study patients. However, our results were less favorable than those of Recker et al.[19] who recently reported results of a 4-year clinical trial of the effects of calcium at 1200 mg/day compared with placebo in 197 elderly women. They found that the change in forearm BMD was −1.2% in the placebo group and +0.3% in the calcium group but did not measure changes in BMD at other sites.

There were important differences between our study and that by Recker et al. that may have contributed to their more-favorable results. First, their subjects were almost a decade older than ours. Next, they selected their subjects on

Table 3. Median Changes and 25th to 50th Percentile Range in Serum and Urine Data Between Baseline and at 4 Years of Treatment

	Calcium (n = *88*)			*Placebo* (n = *89*)			
Variable	*Bsl*	*Change*	*Range*	*Bsl*	*Change*	*Range*	p
Serum							
calcium (mg/dl)	9.50	0.10	−0.10, 0.27	9.60	−0.10	−0.30, 0.17	<0.001
PTH (pmol/l)	2.51	0.02	−0.64, 0.60	2.54	0.48	−0.13, 1.29	0.001
bone AP (U/l)	21.30	2.70	−4.40, 8.17	22.80	3.75	−2.48, 10.45	0.590
osteocalcin (ng/ml)	6.10	−0.70	−1.30, 0.63	6.40	0.10	−1.28, 1.30	0.035
Urine							
calcium (mg/24 h)	134.0	57.55	15.00, 106.00	137.0	4.30	−43.00, 40.50	<0.001
free pyridinoline (nmol/mmol Cr)	39.5	3.05	−6.88, 17.78	41.1	13.40	2.35, 27.10	0.001
Cr clearance (ml/minute)	75.8	−4.72	−16.56, 8.27	80.5	−2.33	−13.39, 8.39	0.604

"Change" is the median change: interquartile range is 25th to 75th percentiles.
Cr, creatinine; AP, alkaline phosphatase; Bsl, baseline.
p values based on Wilcoxon rank-sum test comparing calcium and placebo groups.

the basis of a low calcium intake, whereas we did not. The average habitual dietary intake of calcium of their study subjects was about 450 mg/day while that of subjects in our study was about 700 mg/day. Dawson-Hughes et al.[11] demonstrated that a calcium supplement of 500 mg/day significantly retarded bone loss in elderly postmenopausal women with a calcium intake of less than 400 mg/day but not in those with an intake of 400–650 mg/day. Taken together, these three clinical trials indicate that the lower the habitual calcium intake, the greater the benefit of calcium supplements. Finally, almost half of the subjects in the Recker et al. study had prevalent vertebral fractures at baseline screening, whereas we excluded subjects who had either vertebral fractures or low BMD. They also found that calcium supplementation decreased the occurrence of new fractures in the subgroup with prevalent vertebral fractures at baseline but not in the subgroup without them. Because a history of previous vertebral fractures is a strong risk factor for the occurrence of new fractures, our inability to detect an antifracture effect of calcium treatment relates to inadequate statistical power to detect a change in fracture rates in this lower risk population.

The relatively modest statistical significance of changes in bone density between the calcium and placebo groups also points out the difficulty of detecting even a halving of the rate of bone loss by densitometry when the rate of bone loss in the placebo group is expected to be only about 1%/year.[29] This contrasts with the effects of more potent antiresorptive drugs, such as estrogen,[30] calcitonin,[31] and alendronate,[32] which cause a moderate increase in bone density during the initial 2 or 3 years of therapy. This also may explain the difficulty of most of the previous studies in demonstrating a significant effect of calcium supplementation in retarding bone loss, particularly in those studies with a smaller sample size and in those in which the duration of treatment was less than 4 years. In addition, although no abnormalities were detected in the quality-control measurements, the increase in BMD in the placebo group may indicate an upward drift in densitometer readings from baseline values. However, because of the randomization format, it was possible to detect the beneficial effect of calcium supplementation by comparing the treatment group with the placebo group.

The most consistent finding in previous clinical trials of calcium supplementation was an early transient increase in BMD in the lumbar spine, and we found this effect in the present study as well. The explanation for this is not clear, although it is possible that it may represent an enrichment of calcium-deficient apatite as has been reported by Burnell et al.[33] If so, it would explain why this increase occurred predominantly in the cancellous bone of the vertebrae which has a greater surface-to-volume ratio. However, in our study, the effect on retarding bone loss at the proximal femur, a site containing a mixture of cortical and cancellous bone, continued over the 4 years of the study. Moreover, the inhibition of bone loss over 4 years found in the clinical trial by Recker et al.[19] occurred at the distal forearm, a site that is almost entirely cortical bone. Thus, the protracted effect of calcium supplementation over several years appears to be best detected at sites containing mostly cortical bone.

Although we had marginal statistical power for detecting the small decreases in bone loss induced by calcium supplements, we had sufficient power to detect decreases in serum PTH of 5% and urinary free pyridinoline, a marker for bone resorption, of 10%. In fact, decreases associated with calcium supplementation far exceeded these amounts. The high baseline values for serum PTH and urinary pyridinium cross-links in the elderly women in our study were comparable to findings in a large population-based, age-stratified sample of women residing in Olmsted County, Minnesota,[34] a population that is generally representative of the population of white women of the United States.[21]

We have previously published[35] an interim study of

circadian changes in biochemical markers of bone turnover in a sample of 28 women from this clinical trial who had received either placebo or calcium supplements for 3 years. In these subjects, the double-blind format was maintained by having the patients selected by an unblinded statistician. In this sample, values for serum PTH and biochemical markers for bone turnover were similar to those found in the completed clinical trial, except that the magnitude of the changes induced by the calcium supplements were even greater than for the entire trial. These better results are probably attributable to several differences between the two studies. These include the selection of subjects in the substudy from those in the clinical trial who had the highest and lowest calcium intake (diet plus calcium or placebo) at baseline, more accurate assessment of parathyroid gland function by using 24-h integrated values for serum PTH based on sampling every 2 h in a metabolic ward rather than by using only a single early morning serum sample, and measurement of total pyridinium cross-links by high-pressure liquid chromatography. Because the secondary hyperparathyroidism and increased bone resorption are major determinants of age-related bone loss,[36] their reduction by calcium supplementation is consistent with calcium deficiency playing an important role in age-related bone loss.

The general view is that the rapid phase of bone loss in the early postmenopause is due to loss of the direct action of estrogen on bone cells and that this phase is poorly responsive or nonresponsive to calcium supplementation. The subsequent slower phase of bone loss has been thought to be caused, at least in part, by age-related changes in nonskeletal calcium homeostasis, including impaired calcium absorption[3,4,37] and enhanced renal losses,[5,6] leading to increases in serum PTH and increases in bone resorption. We recently found that long-standing estrogen replacement will also return the secondary hyperparathyroidism to normal and decrease bone resorption.[32,38] This suggests that estrogen deficiency may not only cause the rapid postmenopausal phase of bone loss through a direct effect on bone cell function but also may be permissive for the subsequent slower phase of bone loss indirectly by adversely affecting external calcium homeostasis. The findings that estrogen replacement in postmenopausal women increases calcium absorption[39,40] and enhances renal calcium conservation[5,6] may explain why only estrogen can decrease bone loss during the rapid, early postmenopausal phase whereas either estrogen replacement or large doses of calcium supplements can retard bone loss in the slower, late postmenopausal phase.

Our data do not allow us to estimate the threshold of dietary calcium intake that prevents a negative calcium balance and bone loss in elderly postmenopausal women. Because the women in our study with increased values for serum PTH and bone resorption at baseline had a calcium intake that was only slightly lower than the current RDA of 800 mg/day, it is obvious that this dietary intake level is too low. Heaney et al.[41] used regression analysis of calcium balance data to estimate that postmenopausal women should have an intake of about 1500 mg/day to prevent negative calcium balance. Prospective dose-ranging studies assessing bone loss should now be made to establish the optimal level of calcium intake for estrogen-deficient postmenopausal women.

We have found that long-term calcium supplementation decreases serum PTH, bone resorption, and bone loss in elderly women. However, these effects were weaker than those reported after treatment with estrogen, calcitonin, or bisphosphonates, indicating that calcium cannot substitute for them in treating established osteoporosis. Nonetheless, because of their safety, high tolerance, and low expense, calcium supplementation may be a useful preventive measure for elderly postmenopausal women whose BMD values are normal for their age.

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