0021-972X/00/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 85, No. 9
Printed in U.S.A.

Effect of Calcium or 25OH Vitamin D_3 Dietary Supplementation on Bone Loss at the Hip in Men and Women over the Age of 60*

MUNRO PEACOCK, GUANGDA LIU, MARK CAREY, RONALD McCLINTOCK, WALTER AMBROSIUS, SIU HUI, AND C. CONRAD JOHNSTON

Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana 46202

ABSTRACT

Dietary supplements that prevent bone loss at the hip and that can be applied safely in the elderly are likely to reduce hip fractures. A daily dietary supplement of 750 mg calcium or 15 μg 25OH vitamin D_3 on bone loss at the hip and other sites, bone turnover and calcium-regulating hormones were studied over 4 yr in elderly volunteers using a randomized, double-blind, placebo-controlled trial. Bone mineral density (BMD) was measured by dual x-ray absorptiometry and bone structure by radiographs. Calcium biochemistry and bone turnover markers were measured in blood and urine. The 316 women entering the trial had a mean age of 73.7 yr and the 122 men of 75.9 yr. Baseline median calcium intake was 546 mg/day, and median serum 25OH vitamin D_3 was 59 nmol/L. On placebo, loss of BMD at total hip was 2% and femoral medulla expansion was 3% over 4 yr. Calcium reduced bone loss, secondary hyperparathyroidism, and bone turnover. 25OH vitamin D_3 was intermediate between placebo and calcium. Fracture rates and drop-out rates were similar among groups, and there were no serious adverse events with either supplement. A calcium supplement of 750 mg/day prevents loss of BMD, reduces femoral medullary expansion, secondary hyperparathyroidism, and high bone turnover. A supplement of 15 μg/day 25OH vitamin D_3 is less effective, and because its effects are seen only at low calcium intakes, suggests that its beneficial effect is to reverse calcium insufficiency. (*J Clin Endocrinol Metab* **85:** 3011–3019, 2000)

AGE-RELATED HIP fracture is common in both men and women (1). The fracture is a stochastic event arising from the interaction between recurrent minor trauma usually from falls and decreased bone strength at the hip (2, 3). In the reduction of bone strength that occurs with age, both low calcium intake (4, 5) and low vitamin D stores (6) have been implicated. Dietary intake of calcium decreases with age (7), and a substantial proportion of the elderly take less than the Recommended Dietary Allowance of 800 mg (8). There is also an age-related reduction in vitamin D status, a major determinant of calcium absorption (9). Thus, dietary supplementation with calcium and/or with vitamin D would seem to be an important health measure in hip fracture prevention.

This is, in part, embodied in the Institute of Medicine's recent recommendations (10) that in men and women over the age of 70 yr daily Adequate Intakes would be 1200 mg calcium and 15 μg (600 IU) vitamin D. However, these recommendations are largely based on studies in younger postmenopausal women. Furthermore, the recommendations do not address the possible interaction between calcium intake and vitamin D status.

The age-related decrease in bone strength at the hip is largely due to a reduction in bone mineral density (BMD) (11–15). But changes in bone structure (16, 17), and perhaps bone turnover (18), also contribute. A number of mechanisms have been implicated in age-related bone loss at the hip. Perhaps the most common biochemical abnormalities considered to be responsible for bone loss are an increase in secondary hyperparathyroidism and in bone turnover (19, 20).

A number of studies of vitamin D and calcium supplementation on femoral BMD (21) and fracture (22, 23) have shown positive effects. However, because of the study design, it is not clear how much of the benefit was due to supplementation with vitamin D or with calcium. Thus, the aims of this study were to study the effect on bone mass and structure at the hip and on bone turnover of maintaining dietary calcium and serum 25-hydroxy vitamin D concentrations in the upper end of the normal ranges in a sample of men and women over the age of 60.

Patients and Methods

Subjects

Women (n = 316) and men (n = 122) aged 60 and over were entered into a randomized, placebo-controlled, double-blind trial on the effect of a daily dietary supplement of 750 mg calcium or 15 μg 25OH vitamin D_3 on bone mass and structure at the upper end of the femur. The study was approved by the Institutional Review Board at the Indiana University School of Medicine. Minorities were not excluded, but all subjects were white and were recruited by advertisements and contact through organizations for retirees from two retirement homes in Franklin, Indiana, and from the town of Franklin and its immediate neighborhood. Over 60% were free-living, and all were independently mobile, able to give informed consent as assessed by the Short Portable Mental Status Test and willing to undertake a 4-yr study. Subjects were excluded if they had a terminal illness; Paget's disease of bone; recurrent urinary stone disease; had been treated with sodium fluoride, bisphosphonate, steroids, or dilantin; had renal disease requiring specific treatment; or were excluded by their primary physician. Low BMD, previous skeletal fracture, and estrogen replacement therapy were not reasons for exclusion.

Received January 3, 2000. Revision received May 3, 2000. Accepted May 19, 2000.

Address correspondence and requests for reprints to: Munro Peacock, M.D., General Clinical Research Center, University Hospital and Outpatient Center, Room 5595, 550 North University Boulevard, Indianapolis, Indiana 46202.

* Supported by Public Health Service Grants PHS-P01-AG05793 and PHS-M01-RR-750.

Study design

The study was designed as a double-blind, controlled-trial comparing a calcium supplement and a supplement of 25OH vitamin D_3 with appropriate placebos. After baseline studies, subjects were randomized to 1 of 16 strata by age (60–74 and ≥75), sex, serum 25OH vitamin D concentration (<60 nmol/L and ≥60 nmol/L), and dietary calcium intake (<480 mg/day and ≥480 mg/day), which from previous studies were estimated to be close to the medians for this population. Assessment of dietary calcium intake was available within 24 h and serum 25OH vitamin D concentration within 2 weeks of the baseline visit and did not delay randomization into the study. Subjects were randomized into the study over a 17-month period. Subjects were seen every 6 months and had BMD measured, blood and urine collected, and questionnaire data gathered. Radiographs were performed every 12 months. At each clinic visit, fresh supplement and placebo were supplied, and returned tablets and capsules were recorded. At each visit a diary of fractures was collected, food frequency questionnaires entered into the dietary database, and prescription and nonprescription medications recorded.

Medication

Calcium supplement was given as calcium citrate malate (Procter and Gamble, Cincinnati, OH). Tablets containing 250 mg elemental calcium or placebo and capsules containing 5 μg 25OH vitamin D_3 or placebo (Bone Care) were taken three times per day with meals. The 25OH vitamin D_3 content of the capsules was analyzed every 6 months, and there was no significant decrease seen over the 4 yr of study. Placebos were of the same shape, color, and consistency as the active supplements.

Methods

Bone mass. BMD of the right hip was measured by dual-energy x-ray absorptiometry (Lunar Corp., Madison, WI). After subject repositioning the measurements were repeated. The coefficient of variation of BMD at the total hip was 2.13% and at femoral neck was 2.52% (17). Where there was a history of fracture or replacement by a prosthesis on the right (n = 13), the left hip was measured. Data were acquired with Lunar Software version 1.2, and analysis was performed using software version 4.1, which provides measurements at total hip, shaft, femoral neck, Ward's triangle, and greater trochanter. All scans were analyzed using the auto search mode. BMD of the lumbar spine in the anteroposterior position and total body were also measured, but not in duplicate. Quality control was monitored using an external spine phantom.

Bone structure. Radiographs of the lower pelvis to include both upper femurs in 15-degree internal rotation were taken on standard x-ray equipment using a focal distance of 101.6 cm. All measurements were made on the same hip as that measured by DXA. The width of the femur and the medulla at the mid shaft were measured directly from radiographs by one observer using a Digimatic Caliper (Mitutoyo) as described previously (17). The coefficient of variation (CV) of femur shaft width was 0.58% and femur medulla width was 1.96% (17).

Blood and urine biochemistry. A 24-h urine and blood and urine taken after an overnight fast were collected at each visit. Calcium and creatinine (Cr) were measured in all urines, and cross-linked N-telopeptide of type I collagen (NTX) in all fasting urines. Serums from all visits were measured for calcium, Cr, intact PTH, $1,25(OH)_2$ vitamin D, 25OH vitamin D, alkaline phosphatase, and osteocalcin. In addition, hematology and biochemistry for monitoring safety were measured at each visit. All serum samples were subaliqoted after allowing the blood to clot and stored at −70 C until assayed. All urine was subaliqoted and stored at −30 C until assayed.

Serum osteocalcin was measured by RIA using a rabbit polyclonal antiserum raised against bovine osteocalcin (24). The interassay CV is 11.0% at a serum concentration of 3.9 ng/mL with a normal range of 4–19 ng/100 mL.

NTX in urine was measured by an enzyme-linked immunoabsorbant assay using a monoclonal antibody to human NTX (Ostex International). The interassay CV is 12.6% at a concentration of 124 nM/mM bone collagen equivalents with a normal range for NTX/Cr of 2–89 nM/mM. Serum intact PTH was measured by a double antibody method (Nichols Institute). The interassay CV is 9.8% at a serum concentration of 17.5 pg/mL, with a normal range of 10–65 pg/mL.

Serum 25OH vitamin D was assayed using the vitamin D-binding protein from rat serum and $1,25(OH)_2$ vitamin D was assayed using the vitamin D receptor protein from calf thymus after extraction from serum and purification on HPLC. The interassay CV for 25OH vitamin D is 10.3% at a serum concentration of 30.3 nmol/L and for $1,25(OH)_2$ vitamin D is 10.4% at a serum concentration of 63.4 pmol/L.

Serum calcium (CV = 2.2% at 2.4 mmol%), urine calcium (CV = 2.2% at 3.9 mmol%), and serum Cr (CV = 2.0% at .4 mmol%) and urine Cr (2.8% at 8.3 mmol%) were measured by standard methods on a Roche autoanalyzer.

Fractures. Lifetime history of fracture was obtained at the first visit. Each subject kept a diary of fractures. At each visit fractures were recorded along with the type of trauma responsible, whether the fracture was confirmed by radiography and whether it required splinting or surgery. The occurrence of vertebral fractures from L4 to T4 was assessed from lateral thoracic and lumbar radiographs taken at the first and last visit for each subject. A fracture was defined as a reduction of the anterior vertebral height of 20% or greater (25).

Diet history. The prevailing dietary calcium intake was assessed at each visit by the National Cancer Institute *Health, Habits, and History* (26).

Statistics and analysis

Descriptive statistics for all variables were calculated. The three groups were compared at baseline to evaluate the success of the randomization. Because the BMD at total hip showed the lowest variance, the primary outcome of the study was the change in total hip BMD from baseline to 48 months using an intent-to-treat analysis. If no measurement was made at 48 months, the last observation was carried forward. The change in BMD was compared using ANOVA. The differences from baseline were used as the outcomes for this analysis, allowing for treatment and visit effects and their interactions. The data were also analyzed using repeated measures ANOVA, which accounted for the correlation over time within a subject. In this analysis, the missing data were assumed to occur at random. To allow for the possibility that the subject's probability of dropout was related to her/his true BMD, the repeated-measures analysis was extended to include modeling the probability of dropout (27, 28). The results of this analysis did not differ from the primary analysis described above. Interactions between calcium and vitamin D were examined by splitting the sample into high and low calcium intake groups at the median calcium intake and using analysis of covariance to examine for differences in the slopes of serum 25OH vitamin D as a predictor of total hip BMD, serum calcium, and serum PTH. A similar analysis was performed by splitting at the median serum 25OH vitamin D level and using calcium intake as the predictor. The proportions of subjects who had vertebral or non-vertebral fractures were compared among groups using a χ^2 test.

Results

Baseline variables (Table 1)

Baseline variables were calculated for the 282 women and 111 men who had at least one BMD at femoral neck, total hip, spine, or total body measured after baseline. Compared to women, men in this study were taller, heavier, and older, had a higher dietary calcium intake, BMD, width of femur medulla and cortex, serum Cr, 24-h urine calcium, Cr clearance and lower serum calcium, PTH, $1,25(OH)_2$ vitamin D, bone turnover markers, and fasting urine calcium/Cr ratio. The percentage of subjects above the normal range for serum PTH was 8%, for serum osteocalcin 11%, and for fasting urine NTX/Cr 5%.

BMD changes (Tables 2 and 3 and Fig. 1)

There were no significant sex main-effects or sex-by-treatment interactions in any of the variables, and, hence,

TABLE 1. Baseline variables, mean (SD), in anthropometry, BMD, and biochemistry in men (M) and women (W) in relation to treatment groups (placebo: M = 37, F = 98; 25OH vitamin D_3: M = 37, F = 95; calcium: M = 37, F = 89) who had measurement of BMD at femoral neck, total hip, or spine and were studied at least one visit after baseline

Variable	Group	Placebo	25OH vitamin D_3	Calcium
Height (cm)	M[a]	173.0 (7.3)	174.7 (6.0)	172.0 (7.6)
	W	158.0 (6.6)	158.8 (7.0)	158.6 (6.5)
Weight (kg)	M[a]	80.0 (8.9)	80.8 (16.1)	79.7 (11.1)
	W	69.4 (14.7)	71.0 (19.9)	66.2 (14.5)
Age (yr)	M[b]	75.4 (7.6)	75.5 (7.2)	76.0 (7.7)
	W	72.3 (7.5)	74.1 (8.3)	73.9 (8.0)
Dietary calcium (mg/day)	M[a]	629 (249)	739 (335)	670 (325)
	W	586 (290)	572 (267)	564 (294)
Total hip BMD (g/cm^2)	M[a]	0.98 (0.14)	0.97 (0.14)	1.00 (0.16)
	W	0.81 (0.13)	0.78 (0.14)	0.79 (0.13)
Neck BMD (g/cm^2)	M[a]	0.88 (0.12)	0.88 (0.14)	0.90 (0.18)
	W	0.76 (0.13)	0.75 (0.13)	0.76 (0.13)
L2–L4 BMD (g/cm^2)	M[a]	1.21 (0.24)	1.23 (0.20)	1.23 (0.19)
	W	1.03 (0.18)	1.01 (0.19)	1.00 (0.19)
Total body BMD (g/cm^2)	M[a]	1.18 (0.08)	1.18 (0.10)	1.19 (0.11)
	W	1.03 (0.10)	1.00 (0.12)	1.02 (0.11)
Femur total width (mm)	M[a]	38.1 (2.5)	38.0 (3.1)	38.0 (2.7)
	W	33.0 (2.8)	33.7 (3.0)	32.8 (2.6)
Femur medulla width (mm)	M[a]	16.4 (2.8)	15.9 (2.7)	16.7 (2.4)
	W[c]	14.9 (2.4)	15.9 (3.0)	15.2 (2.5)
s calcium (mmol/L)	M[a]	2.25 (0.10)	2.28 (0.13)	2.25 (0.10)
	W	2.28 (0.10)	2.28 (0.10)	2.30 (0.10)
s PTH (pg/mL)	M[b]	37 (14)	37 (14)	35 (12)
	W	40 (19)	41 (18)	44 (20)
s 1,25 OH_2D (pmol/L)	M[a]	91 (24)	94 (29)	94 (24)
	W	106 (31)	106 (26)	101 (29)
s 25OH D (nmol/L)	M	65.0 (30)	65.0 (25)	67.5 (23)
	W	60.0 (30)	57.5 (33)	62.5 (25)
s creatinine (mmol/L)	M[a]	0.12 (0.02)	0.11 (0.02)	0.11 (0.02)
	W[d]	0.09 (0.01)	0.09 (0.02)	0.09 (0.02)
Osteocalcin (ng/mL)	M[e]	10.9 (5.8)	8.7 (3.7)	12.3 (7.6)
	W	11.8 (8.7)	13.1 (8.4)	11.7 (7.0)
24-h urine calcium (mmol)	M[b]	3.75 (2.1)	3.65 (2.3)	4.20 (2.1)
	W	3.10 (1.9)	3.50 (2.2)	3.28 (2.0)
f urine Ca/Cr (mmol ratio)	M[b]	0.25 (0.11)	0.25 (0.17)	0.31 (0.14)
	W	0.31 (0.17)	0.34 (0.20)	0.31 (0.14)
f urine NTX/Cr (nmol/mmol)	M[b]	33 (24)	34 (47)	33 (24)
	W	44 (35)	53 (40)	44 (51)
Cr clearance (mL/min)	M[a]	68 (23)	72 (31)	73 (23)
	W	61 (20)	56 (19)	57 (18)

[a] $P < 0.01$ men and women difference.
[b] $P < 0.05$ men and women difference.
[c] Placebo and vitamin D differences.
[d] Placebo and calcium difference.
[e] Calcium and vitamin D difference, Turkey-adjusted $P < 0.05$.

TABLE 2. Loss of BMD (g/cm^2) over 48 months at the total hip in 377 subjects using an intent-to-treat analysis

Group	n	Loss	*P*
Placebo	129	0.0144	0.0001
25OH Vitamin D_3	124	0.0095	0.002
Calcium	124	0.0023	0.45

The overall treatment effect was significant ($P = 0.017$); *P* values indicate significance of the bone loss from zero within each group.

men and women were combined in the analyses. Using an intent-to-treat analysis, the placebo group lost BMD at total hip; the calcium group did not lose BMD; and the loss of BMD in the 25OH vitamin D group was intermediate (Table 2). The overall treatment effect in the primary analysis was significant ($P = 0.017$). The results of repeated-measures analysis using all available data (Fig. 1) and analysis of those subjects who completed 48 months of study (Table 3) were very similar to those observed in the primary intent-to-treat analysis (Table 2). The placebo group lost BMD at the total hip at a rate of about 0.5% per year ($P < 0.001$) (Table 3 and Fig. 1). The change in BMD at total hip in the calcium group was significantly different from that in the placebo group ($P < 0.008$), but not from the 25OH vitamin D_3 group. The change in BMD in the 25OH vitamin D_3 group was intermediate between the calcium and the placebo group, but it was not significantly different from either. Although the change in BMD continued to diverge among groups up to 4 y (Fig. 1), a significant interaction was not detected. The changes at femoral neck, greater trochanter, and Wards triangle were similar to that at the total hip (data not shown). The effects at total body were similar to that at the hip (Fig. 1 and Table 3). However, at the spine all groups increased BMD during the study, with the change in the calcium group being

TABLE 3. Change from baseline in BMD and medullary width at the proximal femur in subjects who completed 48 months of study

	Placebo		25OH Vitamin D_3		Calcium	
	n	%	n	%	n	%
Men						
Total hip	22	−2.91	23	−1.37	19	0.33
Femoral neck	25	−2.55	24	−2.90	20	0.44
Spine	21	0.68	22	0.60	16	3.95
Total body	23	−1.32	23	−0.78	20	0.46
Medullary width	24	2.33	20	1.46	18	0.67
Women						
Total hip	50	−1.71	45	−2.27	51	0.12
Femoral neck	54	−3.00	47	−2.71	55	−1.04
Spine	51	0.30	42	1.00	51	2.42
Total body	48	−0.77	42	−1.38	52	−0.02
Medullary width	53	3.37	51	2.92	54	1.35

higher than the groups on placebo ($P < 0.02$) and 25OH vitamin D_3 ($P < 0.04$) (Fig. 1 and Table 3).

Femoral structure (Table 3 and Fig. 2)

The increase in medullary width in the placebo group was significantly greater than in the calcium ($P < 0.002$) and 25OH vitamin D ($P < 0.03$) groups. There was no significant treatment effect on total femur width. Thus, the overall effect was a decrease in cortical thickness of the femoral shaft over 4 yr in the placebo group ($P < 0.007$) (data not shown).

Calcium-regulating hormones (Fig. 3)

There was no difference in serum PTH between the calcium and the 25OH vitamin D_3 group, and both were significantly different from placebo ($P < 0.001$). Change in serum 1,25 $(OH)_2$ vitamin D in the calcium group was significantly greater than in the placebo ($P < 0.001$) and 25OH vitamin D_3 group ($P < 0.002$), and there was no difference between placebo and 25OH vitamin D_3 group. The 24-h urine calcium to Cr ratio increased in the calcium group more than the 25OH vitamin D_3 group ($P < 0.06$), which in turn increased more than the placebo ($P < 0.07$). The effects on the fasting urine calcium to Cr ratio were similar but less marked (data not shown). These results were similar whether groups were examined with those who remained in the study for the 4 yr or who dropped out over the 4-yr period.

Biomarkers (Fig. 4)

Serum osteocalcin was different between the calcium and both the 25OH vitamin D_3 ($P < 0.001$) and placebo groups ($P < 0.008$). Urine NTX/Cr in the calcium group remained lower than both the placebo ($P < 0.002$) and 25OH vitamin D_3 ($P < 0.02$) groups.

Calcium intake and vitamin D status interaction (Fig. 5)

In subjects with calcium intakes less than the median intake during the study of 716 mg/day, there was a positive relationship between serum 25OH vitamin D and the change in total hip BMD ($P < 0.06$) and serum calcium ($P < 0.03$) and a negative relationship with the change in serum PTH ($P < 0.001$). In subjects with calcium intakes greater than the median, these relationships were absent and significantly different for BMD ($P < 0.007$) and PTH ($P < 0.001$) from those on the lower calcium intake. Similarly, analysis of the relationship between calcium intake and bone loss at the hip above and below the median serum 25OH vitamin D concentration showed that at the lower vitamin D status the

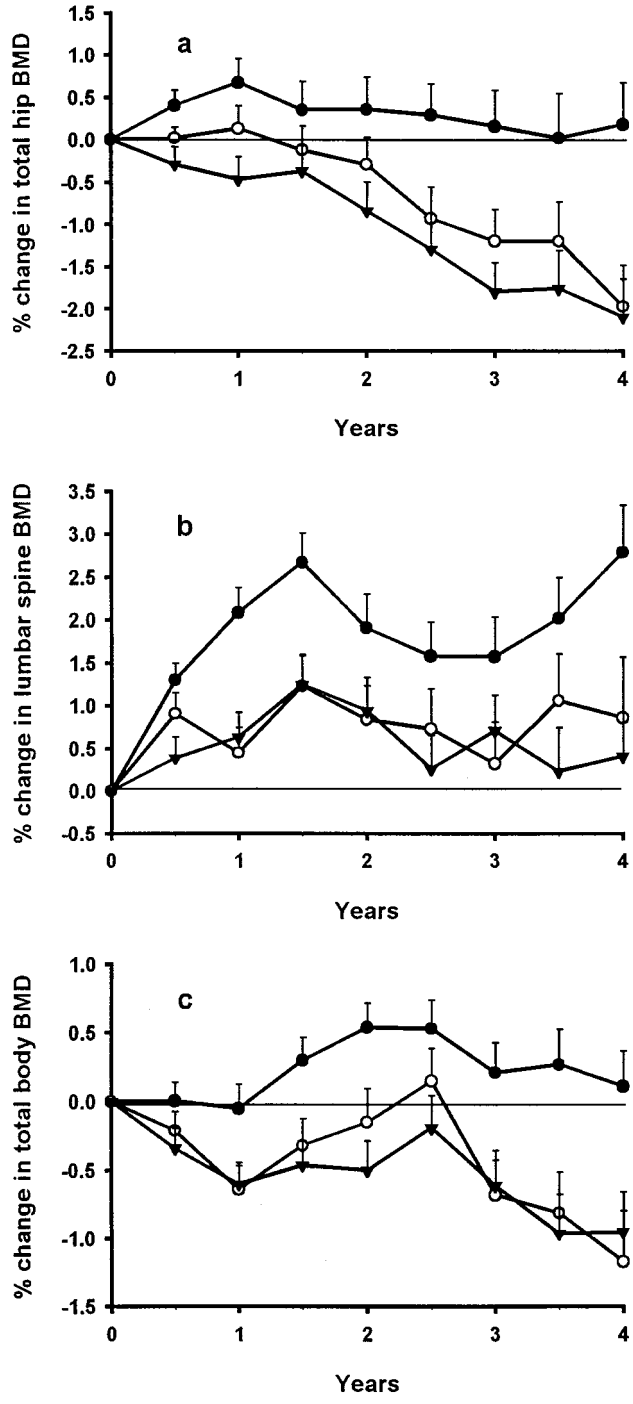

FIG. 1. The effect of calcium (●), 25OH vitamin D_3 (○), and placebo (▲) on the change in BMD at the total hip (a), lumbar spine (b), and total body (c) with years on supplement (mean and 1 SE). Calcium was different from placebo at the hip ($P < 0.008$), the spine ($P < 0.02$), and at the total body ($P < 0.08$). 25OH vitamin D_3 and placebo did not differ.

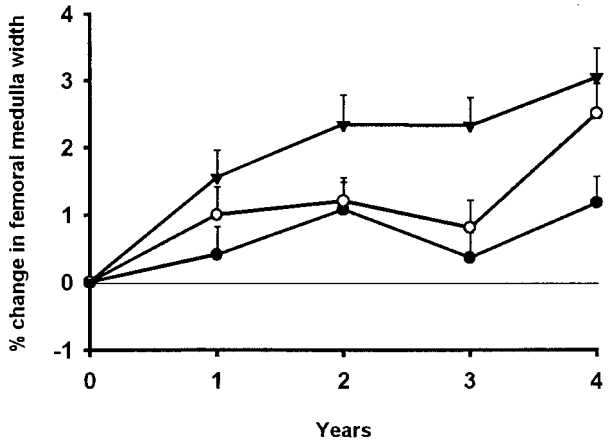

FIG. 2. The effect of calcium (●), 25OH vitamin D_3 (○), and placebo (▲) on the change in medullary width at the mid shaft of the femur with years on supplement (mean and 1 SE). The increase in medullary width on placebo was greater than with calcium ($P < 0.002$) and 25OH vitamin D_3 ($P < 0.04$). There was no difference between calcium and 25OH vitamin D_3.

slope between calcium and BMD at the hip was more positive than at the higher vitamin D status ($P < 0.06$).

Fractures (Table 4)

The number of nonvertebral and vertebral fractures occurring in the calcium and 25OH vitamin D_3 supplement groups and in the placebo group did not significantly differ ($P = 0.680$).

Supplementation and dropout

The 25OH vitamin D_3, supplement increased the mean serum 25OH vitamin D from 60.5 nmol/L to 118.8 nmol/L at 12 months, which remained close to this value throughout the remainder of the study. Only 10 subjects had serum 25OH vitamin D less than 97.5 nmol/L, and none had values higher than 250 nmol/L. The median dietary calcium intake at baseline was 546 mg/day. In an individual dietary calcium intake remained fairly constant (within person SD = 175 mg/day), and the median intake for each group did not change significantly over the study period. Compliance was 80% (SD = 20%) for the calcium supplement, 89% (SD = 16%) for the 25OH vitamin D_3 supplement, and 85% (SD = 19%) for the placebo. The number of subjects who failed to complete the 4-yr study was not significantly different in the three groups, but was lowest in the placebo. Of the 437 subjects randomized to the study, 352 completed 1 yr, 297 completed 2 yr, 271 completed 3 yr, and 236 completed 4 yr. 66% of the men and 59% of the women completed the 4 yr. The number of subjects who dropped out in the calcium group (n = 71) and the 25OH vitamin D group (n = 69) were higher, but not significantly so ($P = 0.48$) than in the placebo group (n = 61). Early withdrawal due to death (n = 20), fracture (n = 9), concurrent illness (n = 47), subject request (n = 119), and increasing serum Cr (n = 6) were not different in the three groups. Gastrointestinal symptoms, mainly constipation, caused 12 subjects to drop out, and 10 of these were taking the calcium supplement. One subject taking calcium developed a kidney stone, one developed primary hyperparathyroidism, and one on placebo developed hypercalcemia. All patients who dropped out were encouraged to return for follow-up visits.

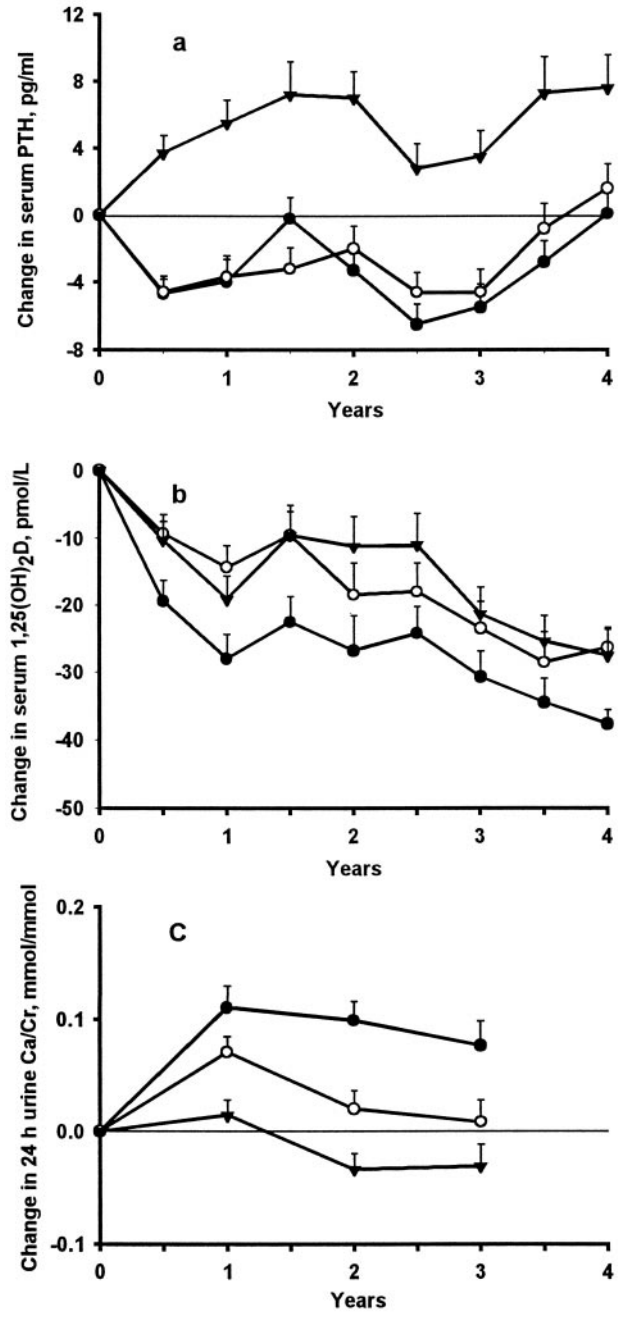

FIG. 3. The effect of calcium (●), 25OH vitamin D_3 (○), and placebo (▲) on the change in serum PTH (a), $1,25(OH)_2$ vitamin D (b), and 24-h urine calcium/Cr (c) with years on supplement (mean and 1 SE). PTH changes on calcium and 25OH vitamin D_3 were different from placebo ($P < 0.001$), but not from each other. $1,25(OH)_2$ vitamin D decreased on calcium more than on 25OH vitamin D_3 ($P < 0.002$) and placebo ($P < 0.001$). Twenty-four-h urine calcium on calcium was higher than on 25OH vitamin D_3 ($P < 0.06$) and on placebo ($P < 0.001$) and 25OH vitamin D_3 was different from placebo ($P < 0.07$). A 24-h urine was not collected on study at the fourth year visit.

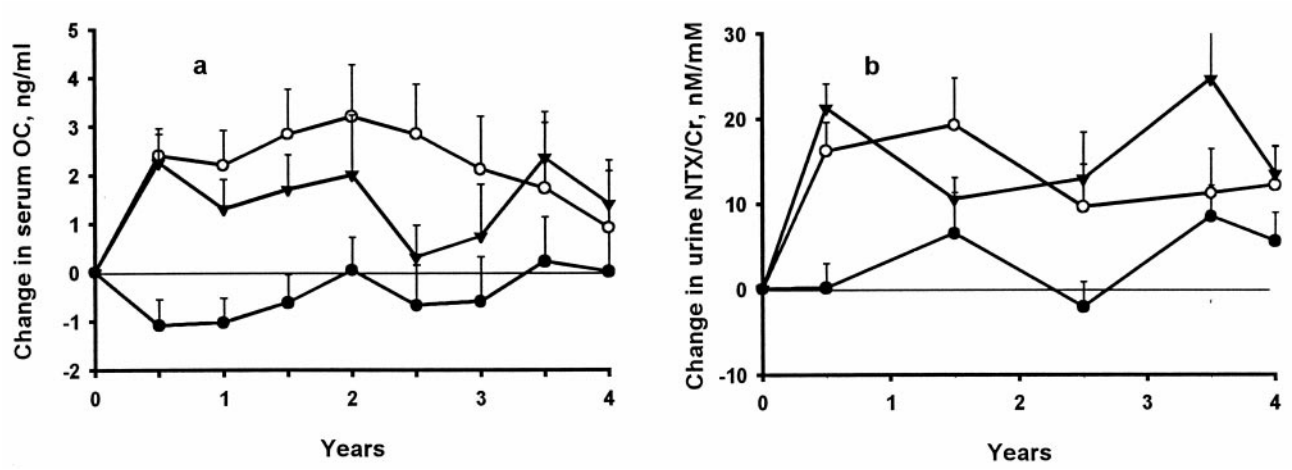

FIG. 4. The effect of calcium (●), 25OH vitamin D_3 (○), and placebo (▲) on the change in osteocalcin (a) and urine NTX/Cr (b) with years on supplement (mean and 1 SE). Osteocalcin change on calcium differed from 25OH vitamin D_3 ($P < 0.01$) and placebo ($P < 0.05$). NTX/Cr change on calcium was lower than 25OH vitamin D_3 ($P < 0.02$) and placebo ($P < 0.002$). 25OH vitamin D_3 and placebo did not differ.

Safety biochemistry

The change in serum Cr concentration was not significantly different among placebo, calcium and 25OH vitamin D_3 groups over the 4-yr study. Urinary calcium increased in the calcium supplement group ($P < 0.001$) and the 25OH vitamin D_3 group ($P < 0.05$). There was no episode of consistent (*i.e.* remaining high on a repeated collection) hypercalciuria defined by a fasting urine Ca/Cr above 0.99 (mM/mM) and a 24-h urine calcium above 7.5 mmol in women and 8.7 mmol in men.

Discussion

The beneficial effects of calcium supplementation in preventing loss of BMD at the proximal femur in men and women over the age of 60 yr in this study were corroborated by positive effects on BMD at total body and lumbar spine. Importantly, beneficial effects also occurred in the structure of the femur. The age-related expansion of the femoral medullary cavity was slowed, resulting in a reduction in the loss of cortical bone. Thus, two important components of bone strength at the hip, BMD, and cortical thickness were preserved with calcium supplementation. These beneficial effects on the proximal femur support the results of several intervention trials in elderly women using calcium supplements alone. Dawson-Hughes *et al.* (29) found that a 500-mg/day calcium supplement reduced the rate of bone loss at the hip in women whose dietary calcium intake was less than 400 mg/day; Reid *et al.* (30) found that a 1000-mg/day calcium supplement had a significant treatment effect at the hip; and Riggs *et al.* (31) found that a calcium supplement of 1600 mg/day had a beneficial effect on hip BMD. In our study, as in the trial of Riggs *et al.* (31), the beneficial effects on bone were associated with a reduction in serum PTH and in biomarkers of bone turnover, strongly suggesting that subjects were calcium insufficient at base line (32). Half of our study population had a daily dietary intake less than 540 mg/day, which is typical of this age group (7). The amount of calcium supplement provided in this study was 750 mg/day, an amount that increased the calcium intake of our subjects above the median of 540 mg/day without increasing it beyond the upper end of the normal range. In general, the calcium supplement was well tolerated, with gastrointestinal upset being the only common complaint.

Supplementation with oral 25OH vitamin D_3 had only a marginal benefit on bone loss. The 25OH vitamin D_3 supplement decreased serum PTH to about the same degree as the calcium supplement, but, in contrast, it had little effect on bone turnover. About 50% of our subjects were in the vitamin D insufficiency range at baseline, with the median serum 25OH vitamin D being 59 nmol/L (32). Oral 25 hydroxy vitamin D_3, 15 mcg/day, increased serum 25OH vitamin D concentration into the upper half of the elderly normal range. The mean increase was 58 nmol/L, an increase expected with a supplement of oral vitamin D_3 of about 800 IU/day (33, 34). A vitamin D supplement given without calcium supplementation has been shown in the elderly to have beneficial effects on bone mass in some studies. Nordin *et al.* (35) found that 15,000 IU vitamin D_2/week reduced the loss of metacarpal cortical thickness in elderly women; in women supplemented with 400 IU vitamin D_3/day, Dawson-Hughes *et al.* (33) found that seasonal changes in spine BMD were less than women on placebo, which resulted in overall benefit to the skeleton; and Ooms *et al.* (34) found that 400 IU vitamin D_3/day over a 2-yr period in women prevented bone loss at the femoral neck. In the latter two studies, the vitamin D supplement reduced serum PTH concentration, but as in our study, had no effect on bone turnover markers.

In subjects on placebo, BMD decreased, cortical bone thinned, secondary hyperparathyroidism progressed, and high bone turnover increased. The rate of bone loss at the hip was over 0.5%/yr, a rate similar to those found in population-based studies (13–15). BMD at total body also decreased, but at the spine it increased by about 0.1%/yr. The increase at spine in subjects over the age of 60 is generally considered to be due to a rise in the incidence and severity of spinal osteoarthritis and not to an increase in BMD of vertebral bone (36, 37). Consistent with the observation that the skeleton expands with age (38), femoral width increased. However,

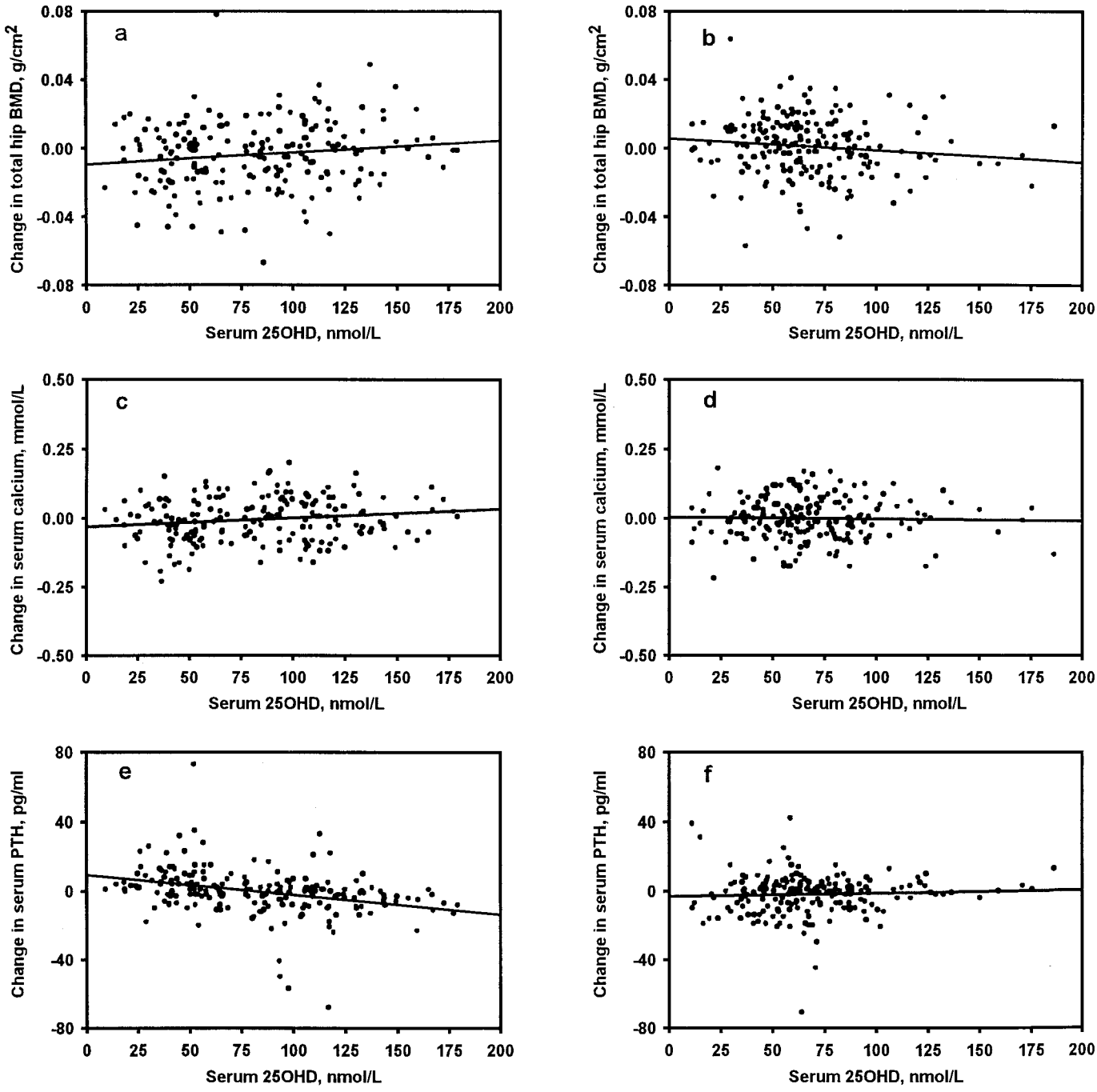

FIG. 5. The relationship between serum 25OH vitamin D and the change at 6 months and at 1 yr in subjects taking less than the median daily calcium intake of 716 mg at total hip BMD (a) ($r = 0.13$, $P < 0.06$), serum calcium (c) ($r = 0.16$, $P < 0.03$), and serum PTH (e) ($r = -0.32$, $P < 0.001$) and in subjects taking more than the median daily calcium intake of 716 mg at total hip BMD (b) ($r = -0.11$, $P = 0.11$), serum calcium (d) ($r = -0.02$, $P = 0.73$), and serum PTH (f) ($r = 0.05$, $P = 0.49$).

the medullary cavity expanded more rapidly at over 0.5%/yr, which resulted in an overall decrease in cortical thickness.

At baseline, there was secondary hyperparathyroidism and increased turnover. Both calcium and 25OH vitamin D_3 reversed the secondary hyperparathyroidism. However, calcium had a much greater effect on bone turnover and calcium excretion, suggesting that the beneficial effects of calcium are due not only to suppression of secondary hyperparathyroidism but also to the increased supply of calcium to bone.

A number of trials of calcium combined with vitamin D supplementation on bone mass (21, 22, 39) and on fracture (22, 23) have been reported. From their study design, however, it is difficult to elucidate whether a beneficial effect was due to calcium, to vitamin D, or to the combination. In our

TABLE 4. Fractures: the number of nonvertebral and vertebral fractures that occurred during the study in placebo, vitamin D, and calcium groups

Group	Nonvertebral fractures		Vertebral fractures	
	Men	Women	Men	Women
Placebo	1	9	3	10
Vitamin D	4	10	4	15
Calcium	2	9	2	5

study, subjects not on calcium supplements had vitamin D insufficiency (32, 40) as evidenced by the negative relationship between serum 25OH vitamin D and serum PTH and urine NTX/Cr. However, in the presence of calcium insufficiency, the concentration of serum 25OH vitamin D that separates vitamin D insufficiency from sufficiency is problematic as shown in Fig. 5. These subjects showed a positive effect of vitamin D status (serum 25OH Vitamin D concentration) on bone mass, indicating that vitamin D insufficiency produces a state of calcium insufficiency. In contrast, a high vitamin D status in calcium-supplemented subjects provided no additional benefit. Indeed, the effect of calcium on bone loss was blunted in subjects with the highest levels of serum 25OH vitamin D. Whether this represents an effect of high serum 25OH vitamin D to increase bone resorption (41) is not clear, but it perhaps points to the danger of over-supplementation of the elderly with vitamin D if they are on an adequate calcium intake.

There are several weaknesses in this study. Firstly, the sample of men and women recruited may not reflect the general elderly population. However, their rates of bone loss and biochemical abnormalities are similar to those found in studies of large populations. Secondly, the study contained no minorities and the results can only be applied to white men and women over the age of 60. Thirdly, the dropout rate was about 11% per year. This largely reflected the fact that inclusion and exclusion criteria were set to include subjects at risk of calcium and vitamin D insufficiency and not just the healthy; that the study was designed to last 4 yr; and that the age range of the subjects was not restricted, ranging from 60–97. On the other hand, the subjects were free living and not a particularly disadvantaged group such as would be found with non-free living nursing home residents. Nevertheless, whether the data were analyzed using an intent-to-treat analysis or a repeated measure analysis, the results were the same.

Supplementation was safe with no troublesome hypercalciuria or hypercalcemia. The main adverse event was in the calcium supplemented subjects who had more gastrointestinal symptoms than the vitamin D or placebo group. There was no significant effect on fracture as expected from our sample size. However, the calcium-supplemented group had the lowest fracture rate and the vitamin D supplement the highest.

In conclusion, the elderly continuously lose bone mass at the hip, and a substantial proportion of them are calcium and vitamin D insufficient. A calcium supplement that increases the calcium intake close to the Adequate Intake of 1200 mg/day prevents bone loss at the hip and at other skeletal sites and has beneficial effects on bone structure at the upper femur. The supplement is safe, well tolerated and, is applicable to the general population. The mechanism of action is probably through the reversal of a state of calcium insufficiency, resulting in an increased supply of calcium to the skeleton and a reduction in secondary hyperparathyroidism and high bone turnover. The effect of a vitamin D supplement is less marked and is most beneficial in subjects who are vitamin D and calcium insufficient. A concerted effort is required to educate the elderly to take 1.2 g/day of calcium in the diet, to maintain their serum 25OH vitamin D above the vitamin D insufficiency level of 75 nmol/L with the expectation that this will reduce the incidence of fractures, particularly hip fracture.

Acknowledgments

We are grateful to Procter and Gamble for supplying the calcium citrate malate tablets and the placebo and for monitoring the study; to Bone Care for supplying the 25OH vitamin D_3 capsules and the placebo; to the Franklin Masonic Home for accommodation to perform the study; and to Marcia Ryker and Cindy McClintock for the measurements of BMD.

References

1. **Cooper C, Campion G, Melton III LJ.** 1992 Hip fractures in the elderly: a world-wide projection. Osteoporosis Int. 2:285–289.
2. **Horsman A, Marshall DH, Peacock M.** 1985 A stochastic model of age-related bone loss and fractures. Clin Orthop. 195:207–215.
3. **Grisso JA, Kelsey JL, Strom BL, et al. and Northeast Hip Fracture Study Group.** 1991 Risk factors for falls as a cause of hip fracture in women. N Engl J Med. 324:1326–1331.
4. **Dawson-Hughes B.** 1991 Calcium supplementation and bone loss: a review of controlled clinical trials. Am J Clin Nutr. Suppl 54:274S–280S.
5. **Nordin BEC.** 1997 Calcium and osteoporosis. Nutrition. 13:664–686.
6. **Peacock M, Hordon L.** 1989 Femoral fracture: the role of vitamin D. In: Kleerekoper M, Krane SM, eds. Clinical disorders of bone and mineral metabolism. New York: Mary Ann Liebert; 265–271.
7. **Alaimo K, McDowell MA, Briefel RR, et al.** 1994 Dietary intake of vitamins, minerals, and fiber of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, Phase 1, 1988–91. Hyattsville, MD: National Center for Health Statistics, U.S. Department of Health and Human Services.
8. **National Research Council** 1989 Recommended dietary allowances. Washington, DC: National Academy Press.
9. **Baker MR, Peacock M, Nordin BEC.** 1980 The decline of vitamin D status with age. Age Aging. 9:249–252.
10. **Food and Nutrition Board, Institute of Medicine.** 1997 Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy Press.
11. **Pearson J, Dequeker J, Reeve J, et al.** 1995 Dual x-ray absorptiometry of the proximal femur: normal European values standardized with the European spine phantom. J Bone Min Res. 10:315–324.
12. **Looker AC, Wahner HW, Dunn WL, et al.** 1995 Proximal femur bone mineral levels of U.S. adults. Osteoporosis Int. 5:389–409.
13. **Jones G, Nguyen T, Sambrook P, Kelly PJ, Eisman JA.** 1994 Progressive loss of bone in the femoral neck in elderly people: longitudinal findings from the Dubbo osteoporosis epidemiology study. Br Med J. 309:691–695.
14. **Burger H, De Laet CE, Van Daele PL, et al.** 1998 Risk factors for increased bone loss in an elderly population—The Rotterdam Study. Am J Epidemiol. 147:871–879.
15. **Ensrud KE, Palermo L, Black DM, et al.** 1995 Hip and calcaneal bone loss increase with advancing age: longitudinal results from the study of osteoporotic fractures. J Bone Miner Res. 10:1778–1787.
16. **Gluer CC, Cummings SR, Pressman A, et al.** 1994 Prediction of hip fractures from pelvic radiographs: the study of osteoporotic fractures. J Bone Min Res. 9:671–677.
17. **Peacock M, Liu G, Carey M, et al.** 1998 Bone mass and structure at the hip in men and women over the age of 60. Osteoporosis Int. 8:231–239.
18. **Melton III LJ, Khosla S, Atkinson EJ, O'Fallon WM, Riggs BL.** 1997 Relationship of bone turnover to bone density and fractures. J Bone Min Res. 12:1083–1091.
19. **Riggs BL, Melton LJI.** 1990 Clinical heterogeneity of involutional osteoporosis: implications for preventive therapy. J Clin Endocrinol Metab. 70:1229–1232.
20. **Delmas PD, Stenner D, Wahner HW, Mann KG, Riggs BL.** 1983 Increase in serum bone gamma-carboxyglutamic acid protein with aging in women.

Implications for the mechanism of age-related bone loss. J Clin Invest. 71:1316–1321.
21. **Chevalley T, Rizzoli R, Nydegger V, et al.** 1994 Effects of calcium supplements on femoral bone mineral density and vertebral fracture rate in vitamin-D-replete elderly patients. Osteoporosis Int. 4:245–252.
22. **Dawson-Hughes B, Harris SS, Krall EA, Dallal GE.** 1997 Effect of calcium and vitamin D supplementation on bone, density in men and women 65 years of age or older. N Engl J Med. 337:670–676.
23. **Chapuy MC, Arlot ME, Duboeuf F, et al.** 1992 vitamin D_3 and calcium to prevent hip fractures in elderly women. N Engl J Med. 327:1637–1642.
24. **Epstein S, Poser J, McClintock R, Johnston Jr CC, Bryce G, Hui S.** 1984 Differences in serum bone GLA protein with age and sex. Lancet. 1:307–310.
25. **Melton III LJ, Kan SK, Frye MA, Wahner HW, O'Fallon WM, Riggs BL.** 1989 Epidemiology of vertebral fractures in women. Am J Epidemiol. 129:1000–1011.
26. **Block G, Coyle LM, Hartman AM, Scoppa SM.** 1994 Revision of dietary analysis software for the Health Habits and History Questionnaire. Am J Epidemiol. 139:1190–1196.
27. **Diggle PJ, Kenward MG.** 1994 Informative drop-out in longitudinal data analysis. Appl Statist. 43:49–93.
28. **Smith DM, Robertson WH, Diggle PJ.** 1996 Oswald: object-oriented software for the analysis of longitudinal data in S'. Technical Report MA 96/192. Lancaster, UK: Department of Mathematics and Statistics, University of Lancaster.
29. **Dawson-Hughes B, Dallal GE, Krall EA, Sadowski L, Sahyoun N, Tannenbaum S.** 1990 A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. N Engl J Med. 323:878–883.
30. **Reid IR, Ames RW, Evans MC, Gamble GD, Sharpe SJ.** 1995 Long-term effects of calcium supplementation on bone loss and fractures in postmenopausal women: a randomized controlled trial. Am J Med. 98:331–335.
31. **Riggs BL, O'Fallon WM, Muhs J, O'Connor MK, Kumar R, Melton III LJ.** 1998 Long-term effects of calcium supplementation on serum parathyroid hormone level, bone turnover, and bone loss in elderly women. J Bone Min Res. 13:168–174.
32. **Peacock M.** 1998 Effects of calcium and vitamin D insufficiency on the skeleton. Osteoporosis Int. 8(Suppl):S45–S51.
33. **Dawson-Hughes B, Dallal GE, Krall EA, Harris S, Sokoll LJ, Falconer G.** 1991 Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. Ann Intern Med. 115:505–512.
34. **Ooms ME, Roos JC, Bezemer PD, VanDerVijgh JF, Bouter LM, Lips P.** 1995 Prevention of bone loss by vitamin D supplementation in elderly women: a randomized double-blind trial. J Clin Endocrinol Metab. 80:1052–1058.
35. **Nordin BEC, Baker MR, Horsman A, Peacock M.** 1985 A prospective trial of the effect of vitamin D supplementation on metacarpal bone loss in elderly women. Am J Clin Nutr. 42:470–474.
36. **Liu G, Peacock M, Eilam O, Dorulla G, Braunstein E, Johnston CC.** 1997 Effect of osteoarthritis in the lumbar spine and hip on bone mineral density and diagnosis of osteoporosis in elderly men and women. Osteoporosis Int. 7:546–569.
37. **Jones G, Nguyen T, Sambrook PN, Kelly PJ, Eisman JA.** 1995 A longitudinal study of the effect of spinal degenerative disease on bone density in the elderly. J Rheumatol. 22:932–936.
38. **Garn SM, Wagner B, Rohman CG, Ascoli W.** 1968 Further evidence for continuing bone expansion. Am J Physiol Anthrop. 28:219–221.
39. **Orwoll ES, Oviatt SK, McClung MR, Deftos LJ, Sexton G.** 1990 The rate of bone mineral loss in normal men and the effects of calcium and cholecalciferol supplementation. Ann Intern Med. 112:29–34.
40. **McKenna MJ, Freaney R.** 1999 Secondary hyperparathyroidism in the elderly: means to defining hypovitaminosis D. Osteoporosis Int. 8(Suppl):S3–S6.
41. **Adams JS, Lee G.** 1997 Gains in bone mineral density with resolution of vitamin D intoxication. Ann Intern Med. 127:203–206.