Long-term effects of nutrient intervention on markers of bone remodeling and calciotropic hormones in late-postmenopausal women[1–4]

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ABSTRACT

Background: Adequate intakes of calcium and vitamin D reduce bone loss and fracture risk in the elderly. Other nutrients also affect bone health, and adequate intakes may influence bone turnover and balance.

Objective: We compared the long-term effects on bone turnover markers and calciotropic hormones of a multinutrient supplement, a calcium and vitamin D supplement, and dietary instruction aimed at increasing calcium intake through foods.

Design: Ninety-nine healthy postmenopausal women participated in a 3-y, randomized trial, receiving either *1*) supplemental calcium (1450 mg/d) and vitamin D [10 μg (400 IU)/d], *2*) calcium, vitamin D, and other nutrients (multinutrient supplement), or *3*) dietary instruction (dietary control group). Data are from 83 subjects who completed the trial.

Results: Increases over baseline in calcium intakes and serum 25-hydroxyvitamin D concentrations were sustained over 3 y in all treatment groups. Circulating parathyroid hormone concentrations were reduced at year 1 in all treatment groups but trended toward baseline thereafter. Bone turnover markers followed a similar pattern, and none of the changes in biochemical concentrations differed significantly between groups.

Conclusions: All 3 interventions offer long-term feasibility for increasing calcium intake and serum 25-hydroxyvitamin D concentrations. The dietary addition of micronutrients implicated in skeletal physiology confers no obvious bone-sparing effect in healthy postmenopausal women beyond that of calcium and vitamin D alone. The attenuation over time in suppression of parathyroid hormone and bone turnover might help explain why nutrient intervention tends to have less of a bone-sparing effect than do skeletally active medications such as estrogen or bisphosphonates. *Am J Clin Nutr* 2002;75:1114–20.

KEY WORDS Postmenopausal women, calcium, vitamin D, trace minerals, bone turnover, parathyroid hormone, 25-hydroxyvitamin D, insulin-like growth factor I

INTRODUCTION

Clinical trials in the elderly showed that supplemental calcium and vitamin D suppress bone turnover, slow bone loss, and reduce fracture risk (1–7), presumably by suppressing the secretion of parathyroid hormone (PTH; 8). Few trials have assessed the long-term response of PTH, vitamin D, and bone turnover to nutrient intervention or compared the effectiveness of different dietary and dietary supplement regimens in suppressing bone turnover.

Nutrients other than calcium and vitamin D affect bone health, and adequate intakes may influence bone turnover. Vitamins K and C, magnesium, zinc, manganese, molybdenum, and copper all influence the synthesis of bone matrix proteins (9–13). Boron may potentiate estrogen action in postmenopausal women (14), and zinc is postulated to stimulate the production of insulin-like growth factor I (IGF-I), the age-related decline of which may relate to postmenopausal bone loss (15). Therefore, it is possible that inadequate dietary intake and age-related changes in the absorption, utilization, or excretion of these nutrients may affect bone turnover in postmenopausal women.

We undertook a 3-y randomized controlled trial to compare the effects of a multiple vitamin and mineral supplement, a calcium and vitamin D supplement, and a program of dietary instruction aimed at increasing calcium intake through foods on markers of bone turnover, related calciotropic hormones, and bone mineral density (BMD) in healthy postmenopausal women.

SUBJECTS AND METHODS

The study protocol was approved by the Stanford University Administrative Panel on Human Subjects in Medical Research, and the University of California, Berkeley, Committee for the

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Protection of Human Subjects. All subjects provided written, informed consent.

Subjects

Women were recruited by newspaper advertisements and screened in a telephone interview. Participants were ≥59 y of age, postmenopausal (nonsurgically) for ≥5 y, and willing to comply with the study requirements. Exclusion criteria included current or recent use (in the past 6 mo) of hormone replacement medication, thiazide diuretics, or glucocorticoids and the presence of chronic illnesses such as diabetes, kidney disease, heart disease, cancer, or known parathyroid disease.

Treatments

The subjects were randomly assigned to 1 of 3 treatment groups, of which 1 was an active control. A true placebo was not used because of ethical considerations posed by the length of the trial and the potential for significant bone loss in women with inadequate dietary calcium and vitamin D intakes. The active control group (hereafter the dietary control group), received dietary instruction targeting the consumption of ≥800 mg Ca/d, with an ideal goal of 1450 mg/d.

Women in the calcium–vitamin D group were instructed to take 6 tablets/d of a supplement, which provided 1450 mg Ca, 850 mg P, and 10 μg (400 IU) vitamin D. Women in the multinutrient group were instructed to take 6 tablets/d of a different supplement, which provided 1450 mg Ca, 850 mg P, 10 μg (400 IU) vitamin D, 600 mg Mg, 3 mg B, 15 mg Zn, 3.5 mg Mn, 2 mg Cu, 150 μg I, 130 μg Cr, 160 μg Mo, 15 μg Ni, 70 μg Se, 10 μg Sn, 2 μg V, 2 mg Si, 300 μg biotin, 400 μg folic acid, 20 mg niacin, 10 mg pantothenic acid, 1500 μg (5000 IU) vitamin A, 1.5 mg thiamine, 1.7 mg riboflavin, 2 mg vitamin B-6, 6 μg vitamin B-12, 120 mg vitamin C, 60 α-tocopherol equivalents (60 IU) vitamin E, and 80 μg vitamin K.

The calcium sources for the supplement groups consisted of a mixture of dicalcium phosphate, calcium citrate, calcium carbonate, calcium gluconate, and calcium lactate. Tablets used in the study were identical in shape and similar in color. Assignment to the supplement groups was double blind. Assignment to the dietary control group was not blinded because of the dietary changes required. All the subjects were instructed to discontinue consuming nonstudy dietary supplements 30 d before baseline testing, to maintain their usual physical activity patterns, and to report any changes in medications taken during the study. Those in the supplement groups were asked to maintain their dietary patterns during the study.

Study sample and randomization

Telephone screening yielded 147 eligible individuals who met the inclusion criteria, of whom 99 provided informed consent, underwent random assignment, and submitted to baseline measurements. Eighty-three participants completed the 3-y trial.

Measurements

At baseline and annually thereafter, the subjects met with the investigators to have their heights, weights, and BMDs measured, to provide fasting blood and urine samples, and to turn in 4-d food records. Height was measured with a wall-mounted stadiometer, and weight was measured on a balance scale. All blood samples were obtained in the morning after the subjects had fasted for 12 h. The subjects were instructed to empty their bladders on arising on the mornings of these visits and to record the time. At the Aging Study Unit 2 h later, they were again asked to void and to collect the total urine volume from the previous 2 h. The sera and 2-h fasting urine samples were stored at −70°C until analyzed. Hormones and bone turnover markers were analyzed in the laboratory of the senior investigator (RM) by methods that were validated and reported previously from that laboratory (16, 17). Serum 25-hydroxyvitamin D concentrations were measured following extraction of serum in reagent alcohol (90% ethanol, 5% methanol, 5% isopropanol) with a competitive protein-binding assay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). Serum intact PTH was measured with an enzyme-linked immunosorbent assay kit (Diagnostic Systems Laboratories, Inc, Webster, TX). Both serum IGF-I and IGF binding protein 3 (IGFBP-3) were measured by 2-site immunoradiometric assay (Diagnostic Systems Laboratories, Inc). Urinary creatinine was measured with a quantitative, colorimetric assay kit (Metra Biosystems, Inc, Mountain View, CA) Total pyridinium cross-links were measured with a competitive enzyme immunoassay kit (Metra Biosystems, Inc, Mountain View, CA). All determinations of 25-hydroxyvitamin D, PTH, IGF-I, IGFBP-3, and pyridinium cross-links were run on batched specimens in single-assay runs.

Human osteocalcin in serum was measured with a 2-site immunoradiometric assay kit (Diagnostic Systems Laboratories, Inc). Because of uncertainty regarding the conservation of osteocalcin in long-term storage, batched specimens from baseline and year 1 were thawed and measured together. Batched specimens from years 2 and 3 were similarly measured. The 4-d diet records were analyzed with the use of the NUTRITIONIST IV software program, versions 3.0 and 3.5.1 (First Databank, Inc, San Bruno, CA). Areal BMD (g/cm^2) was measured by dual-energy X-ray absorptiometry in the fan beam mode on a QDR-2000 X-ray bone densitometer (Hologic, Waltham, MA). The densitometer software (version 7.1) was not changed during the study. A phantom consisting of bone ash embedded in a 12-cm block was scanned every other week as a control, and the bone density of the phantom was stable throughout the study.

Adherence and masking

The subjects met every 3 mo with the research staff, and supplement adherence was measured by a tablet count procedure. The subjects were assigned an adherence score of 1 (excellent), 2 (good), or 3 (fair) based on the number of tablets remaining in the supplement containers returned at each visit. The adequacy of treatment masking in the supplement groups was assessed by a poststudy telephone survey.

Statistical analyses

A per-protocol analysis was performed on the 83 participants who completed the 3-y trial. Differences in baseline characteristics between treatment groups were compared by one-way analysis of variance (ANOVA) for continuous variables, by chi-square tests for categorical variables, and by the Kruskal-Wallis nonparametric one-way ANOVA method (18) for ordinal variables. Differences between groups at each year of follow-up were compared by repeated-measures analysis of covariance with the baseline value as the covariate and time as the repeated measure. Within-group differences were compared by repeated-measures ANOVA with time as the repeated measure. A Bonferroni adjustment (19) was made to account for multiple comparisons within

TABLE 1
Baseline characteristics by treatment group[1]

Variable	Dietary control group (n = 25)	Calcium–vitamin D group (n = 26)	Multinutrient group (n = 32)
Age (y)	66 ± 5 (67)	65 ± 4 (64)	66 ± 5 (65)
Time since menopause (y)	13.8 ± 6.4 (13.0)	13.8 ± 4.5 (14.0)	16.5 ± 6.8 (15.5)
BMI (kg/m^2)	25.9 ± 4.5 (25.0)	25.4 ± 3.4 (24.4)	25.1 ± 3.5 (25.2)
Current smokers (%)	0 (0)	3 (11)	1 (3)
Physical activity score[2]	5.6 ± 1.6 (6.0)	5.3 ± 2.3 (5.0)	5.1 ± 2.2 (4.5)
Bone mineral density (g/cm^2)			
Lumbar spine	0.938 ± 0.161 (0.907)	0.867 ± 0.118 (0.843)	0.854 ± 0.121 (0.855)
Femoral neck	0.700 ± 0.134 (0.666)	0.655 ± 0.100 (0.634)	0.663 ± 0.093 (0.660)
Total hip	0.824 ± 0.148 (0.796)	0.780 ± 0.121 (0.753)	0.772 ± 0.122 (0.777)
Whole body	0.995 ± 0.134 (0.957)	0.958 ± 0.082 (0.941)	0.958 ± 0.096 (0.962)
25-Hydroxyvitamin D (nmol/L)	41.9 ± 17.5 (39.7)	41.4 ± 24.2 (34.9)	40.2 ± 18.5 (43.2)
Parathyroid hormone (pmol/L)	5.93 ± 6.90 (3.78)	3.53 ± 2.34 (2.60)	3.52 ± 2.21 (3.28)
Osteocalcin (nmol/L)	1.94 ± 1.55 (1.79)	2.29 ± 1.60 (2.43)	2.40 ± 1.51 (2.21)
Pyridinium cross-links (nmol/mmol creatinine)	34.7 ± 10.6 (32.4)	40.1 ± 14.3 (36.9)	41.2 ± 12.6 (37.7)
IGF-I (nmol/L)	24.26 ± 8.44[3] (23.36)	19.21 ± 6.32 (19.14)	19.24 ± 6.33 (18.67)
IGFBP-3 (nmol/L)	118.1 ± 19.3 (117.9)	120.4 ± 14.6 (118.7)	114.6 ± 12.7 (113.2)

[1] $\bar{x}$ ± SD; median in parentheses. IGF-I, insulin-like growth factor I; IGFB-3, IGF binding protein 3.
[2] Range: 1–10.
[3] Significantly different from the supplement groups, P = 0.029 (Kruskal-Wallis ANOVA).

or among treatments with an experiment-wise α of 0.05. Model assumptions of independent sampling, equal variances, and normally distributed data were met. SAS software (version 6.12; SAS Institute Inc, Cary, NC) was used in the data analyses.

RESULTS

Withdrawals and reported side effects

Sixteen of the 99 volunteers withdrew from the study. Eight withdrawals occurred in each of the first 2 y. Eight withdrawals were for reasons unrelated to the study. Two subjects withdrew from the dietary control group because of difficulty maintaining the diet. Of the 10 subjects who withdrew from the calcium–vitamin D group, 4 complained of side effects apparently related to the supplements (constipation was reported by 2 individuals, indigestion by 1, and a combination of night sweats and hot flashes by 1), and 1 had difficulty swallowing tablets. Four subjects withdrew from the multinutrient group, and one cited supplement intolerance as the reason.

Protocol adherence and blinding

The mean (±SD) adherence score was 1.3 ± 0.3 in the calcium–vitamin D group and 1.3 ± 0.5 in the multinutrient group. Mean body weights fluctuated slightly during the trial, but did not differ significantly between treatment groups at any time point. In the poststudy test of the success of masking the treatment assignments in the 2 supplement groups, the proportions of subjects who believed they had taken the multinutrient or were unsure about the supplement composition were equally distributed between the 2 groups.

Baseline characteristics

The 83 participants who completed the trial had an average age of 66 ± 5 y (range: 59–79 y), time since menopause of 15 ± 6 y, body mass index (in kg/m^2) of 25.4 ± 3.7, and daily calcium consumption of 793 ± 280 mg (median: 742 mg). No significant differences between groups were found at baseline for age, years since menopause, body mass index, percentage body fat, self-reported physical activity score (on a scale of 1 to 10), bone mineral density, serum 25-hydroxyvitamin D and PTH concentrations, or bone turnover markers (**Table 1**). The treatment groups differed significantly at baseline in dietary calcium intake (**Table 2**) and serum IGF-I concentration (Table 1).

Fifteen subjects had baseline PTH concentrations (120 ± 77 ng/L) >3 SDs above the nominal upper limit of the normal range [6.5 pmol/L (65 pg/mL); mean ± SD = 12 ± 7.7 pmol/L (120 ± 77 pg/mL)]. None of these women had hypercalcemia. To avoid the confounding effect of secondary hyperparathyroidism on the interpretation of results, all reported analyses are confined to the 68 women whose baseline PTH concentrations were within the normal range. They were distributed among treatment assigments as follows: dietary control group, 21; calcium–vitamin D group, 22; multinutrient group, 25.

Dietary calcium intake and serum 25-hydroxyvitamin D concentration

At year 1 of follow-up, mean daily calcium intake had increased significantly over baseline (43%) in the dietary control group, and this increase persisted throughout the trial (Table 2). In contrast, dietary calcium consumption for the calcium–vitamin D and multinutrient groups (not including supplementary calcium) did not change significantly over the course of the trial. The mean total daily calcium intake in the dietary control group ranged from 1122 to 1242 mg/d during the 3-y trial; mean total calcium intake (from diet and supplements) in the calcium–vitamin D and multinutrient groups ranged from 2122 to 2178 mg/d and from 2246 to 2345 mg/d, respectively.

Changes in circulating 25-hydroxyvitamin D showed a highly significant group × time interaction (**Table 3**). Mean serum concentrations increased 45% in the dietary control group from baseline to the end of the study. The mean serum concentration in the calcium–vitamin D group increased 84% from baseline to the end of the study, with the peak at follow-up year 2, which

TABLE 2
Daily dietary nutrient intake during the study[1]

Nutrient	Dietary control group (n = 21)	Calcium–vitamin D group (n = 22)	Multinutrient group (n = 25)
Protein (g)			
Baseline	74 ± 16	70 ± 16	71 ± 20
Year 1	83 ± 20	63 ± 14	68 ± 16
Year 2	77 ± 16	66 ± 15	69 ± 18
Year 3	85 ± 18	64 ± 15	68 ± 15
Calcium			
Baseline	871 ± 373	672 ± 158[2]	831 ± 246
Year 1	1242 ± 482[3]	690 ± 250	895 ± 370
Year 2	1122 ± 482[3]	705 ± 222	796 ± 257
Year 3	1241 ± 513[3]	728 ± 287	836 ± 305
Vitamin D [μg (IU)]			
Baseline	3.45 ± 3.90 (138 ± 152)	4.45 ± 8.05 (178 ± 322)	3.95 ± 7.3 (158 ± 292)
Year 1	6.03 ± 6.15 (241 ± 246)	6.83 ± 15.1 (273 ± 604)	8.90 ± 14.5 (356 ± 581)
Year 2	4.33 ± 3.45 (173 ± 138)	9.83 ± 29.0 (393 ± 1160)	4.98 ± 5.58 (199 ± 223)
Year 3	5.73 ± 5.78 (299 ± 231)	2.85 ± 1.95 (114 ± 78)	3.3 ± 2.63 (133 ± 105)
Zinc (mg)			
Baseline	8.6 ± 2.2	9.7 ± 2.4	8.6 ± 3.4
Year 1	10.3 ± 3.1	7.2 ± 2.3	8.8 ± 2.9
Year 2	9.10 ± 2.8	8.2 ± 2.7	9.8 ± 5.1
Year 3	9.70 ± 2.8	7.8 ± 2.7	8.3 ± 1.8

[1] $\bar{x}$ ± SD daily intakes derived from 4-d food records, not including supplements.
[2] Significantly different from the other groups, $P = 0.03$ (one-way ANOVA).
[3] Significantly different from baseline, $P < 0.01$ (repeated-measures ANOVA).

was a 92% increase from baseline. Mean serum values in the multinutrient group increased 117% from baseline to the end of the study. Mean concentrations were significantly higher in each of the supplement groups at follow-up years 2 and 3 than in the dietary control group and in the multinutrient group at year 1 than in the dietary control group. Only 7 subjects (8.4%) had a baseline serum 25-hydroxyvitamin D concentration >75 nmol/L (30 ng/mL). At the end of the trial, this number increased to 39 of 83 subjects (47.0%).

PTH and bone turnover markers

The time course of changes in serum PTH concentration and in markers of bone turnover within treatment groups was examined in the 68 subjects for whom complete marker data were available. The overall ANOVA showed a significant treatment × time interaction. Mean serum PTH concentrations did not change significantly in the dietary control group but decreased from baseline in both supplement groups in the first year of follow-up, followed by an increasing trend toward baseline thereafter (**Table 4**). By years 2 and 3 of follow-up, PTH concentrations did not differ significantly from baseline and did not differ significantly between groups at any time point.

Mean values for serum osteocalcin concentration, a measure of bone formation activity, showed a nonsignificant trend to be reduced from baseline at year 1 of follow-up for each treatment group, with another nonsignificant trend upward at follow-up years 2 and 3. No significant differences between groups were found at any time point. For the pyridinoline marker of bone resorption, a significant treatment × time interaction was observed. Mean pyridinium cross-link excretion, an index of bone resorption activity, decreased from baseline in the first 2 y of follow-up in the multinutrient group and then rose toward or above baseline values in the final follow-up year. Mean values at year 3 were significantly higher in the multinutrient group than in the calcium–vitamin D group (Table 4).

Serum IGF-I and IGFBP-3 response

Mean serum IGF-I and IGFBP-3 concentrations at annual follow-up visits did not differ significantly between groups, nor did they differ significantly from baseline for any treatment group (**Table 5**).

Bone mineral density

The percentage changes in BMD are shown in **Figure 1** for the lumbar spine, proximal femur, and whole body. Changes did not differ significantly between groups at any time point, and adjustment for baseline differences in calcium intake and serum IGF-I concentration did not affect the results.

DISCUSSION

This study compared 3 methods of nutrient intervention in postmenopausal women. All 3 interventions resulted in increased calcium intakes and serum 25-hydroxyvitamin D concentrations over 3 y, but the addition of assorted micronutrients conferred no obvious skeletal effect beyond that of calcium and vitamin D alone. This interpretation is tempered by our relatively small number of subjects. We considered a 3% difference in BMD response between treatment groups to be clinically meaningful and designed the study with adequate power to make such a distinction. Although the results do not show even a trend toward an advantage for multinutrient supplementation, it is possible that differences smaller than 3% were missed.

The only comparable study of which we are aware that compared the skeletal effects of calcium with or without trace minerals in postmenopausal women suggested that a multinutrient approach might be superior to that of calcium alone (20). However, inferences from that study were limited by small sample size, high withdrawal rate, restriction of BMD data to the spine, and lack of corroborating bone turnover data.

TABLE 3
Serum 25-hydroxyvitamin D concentrations by treatment group[1]

	Dietary control group (n = 21)	Calcium–vitamin D group (n = 22)	Multinutrient group (n = 25)
		nmol/L (%)	
Baseline	39.2 ± 15.3	42.3 ± 24.4	38.7 ± 16.8
Year 1	33.5 ± 15.1[a,2] (−12.6)	50.3 ± 19.1[b,3] (42.3)	54.3 ± 22.2[a,b,4] (62.6)
Year 2	48.2 ± 14.4[a,2] (35.3)	80.4 ± 21.6[b,5] (138.0)	76.9 ± 22.1[b,5] (132.8)
Year 3	58.4 ± 32.5[a,2] (60.3)	76.6 ± 22.1[b,5] (142.7)	87.7 ± 30.5[b,5] (168.2)

[1] $\bar{x}$ ± SD; percentage change from baseline in parentheses. Group × time interaction, $P = 0.0004$. Values in a row with different superscript letters are significantly different, $P < 0.05$.
[2–5] Significantly different from baseline (repeated-measures ANOVA): [2] $P < 0.01$, [3] $P < 0.05$, [4] $P < 0.001$, [5] $P < 0.0001$.

TABLE 4
Serum parathyroid hormone, osteocalcin, and urinary pyridinium cross-links among subjects with complete biochemical data

	Dietary control group (n = 21)	Calcium–vitamin D group (n = 22)	Multinutrient group (n = 25)
Parathyroid hormone (pmol/L)			
Baseline	3.26 ± 0.95	3.10 ± 1.47	3.37 ± 1.41
Year 1	2.91 ± 1.17	2.49 ± 1.32[2]	2.76 ± 1.16[3]
Year 2	3.19 ± 2.06	2.92 ± 1.41	3.32 ± 1.40
Year 3	2.99 ± 1.25	2.96 ± 1.20	3.26 ± 1.09
Osteocalcin (nmol/L)			
Baseline	2.14 ± 1.56	2.30 ± 1.60	2.53 ± 1.60
Year 1	1.97 ± 1.60	1.88 ± 1.20	2.18 ± 1.18
Year 2	2.99 ± 1.19	2.54 ± 1.39	3.30 ± 1.23
Year 3	2.46 ± 1.39	2.46 ± 1.08	3.20 ± 1.00
Pyridinium cross-links (nmol/mmol creatinine)			
Baseline	36.8 ± 13.0	41.5 ± 15.8	40.2 ± 12.6
Year 1	34.5 ± 11.5	31.6 ± 12.1[3]	32.8 ± 8.7[2]
Year 2	34.5 ± 10.6	34.15 ± 15.9	32.5 ± 9.5[2]
Year 3	37.5 ± 7.6	36.8 ± 9.8	46.4 ± 13.0[2,4]

[1] $\bar{x}$ ± SD. Significant group × time interaction for parathyroid hormone, $P = 0.04$ (ANOVA); for pyridinium cross-link, $P = 0.049$ (ANOVA).
[2,3] Significantly different from baseline (repeated-measures ANOVA): [2] $P < 0.05$, [3] $P < 0.001$.
[4] Significantly different from the calcium–vitamin D group, $P < 0.05$.

We observed no obvious treatment-related differences in bone response, but the secular changes in biochemical endpoints warrant comment. The first-year reductions in circulating PTH and bone turnover markers indicate suppression of bone remodeling in response to mineral supplementation. However, PTH and bone turnover markers reverted toward baseline values in subsequent years despite adherence to protocol. This increase coincided with a trend toward greater bone loss from the proximal femur and whole body in the final year of the trial. The consistency of the biochemical and BMD findings support the conclusion that bone remodeling increased during year 3.

Published short-term nutrient intervention trials consistently show initial suppression of PTH, remodeling activity, or both (2, 6, 21–24). However, there are fewer reports from long-term trials. Data from other long-term nutrient intervention trials using placebos rather than active dietary controls indicate that PTH concentrations may remain suppressed for up to 4 y (5, 8). Nonetheless, loss of suppression over time, were it generally to occur, might partly explain why nutrient intervention appears to offer less-sustained skeletal protection than do other skeletally active medications such as estrogen or bisphosphonates.

The serum concentrations of 25-hydroxyvitamin D proposed as adequate to prevent compensatory hypersecretion of PTH vary from ≈62 to 100 nmol/L (25–40 ng/mL; 25). The median baseline 25-hydroxyvitamin D concentration in this study was 38.9 nmol/L (15.6 ng/mL), with only 7 subjects having values >75 nmol/L (30 ng/mL). These were associated with mean and median vitamin D dietary intakes of 3.53 ± 5.13 and 2.15 μg (141 ± 205 and 86 IU)/d. The current daily dietary vitamin D intake deemed adequate for women aged 50–70 y is 10 pg [400 IU]); for women aged >70 y it is 15 pg (600 IU; 26). In the present study, intake of 10 μg (400 IU)/d vitamin D in the supplement groups did result in average serum 25-hydroxyvitamin D concentrations >75 nmol/L (30 ng/mL). Of 39 participants whose 25-hydroxyvitamin D values exceeded this value at the end of the trial, 82% were from the supplement groups. Thus, the vitamin D intake resulting from daily consumption of ≈1200 mg Ca in dairy foods did not generally achieve desirable vitamin D nutriture, whereas a daily vitamin D supplement of 10.0 μg (400 IU) achieved vitamin D nutritional adequacy in approximately one-half of the recipients. Therefore, recent proposals to raise substantially the requirement for vitamin D intake in postmenopausal women appear sensible.

Interest has been expressed in the relation of skeletal maintenance to age-related decreases in IGF-I concentrations (27). Recombinant IGF-I increases bone formation activity in postmenopausal women (17, 28, 29), suggesting that increasing IGF-I concentrations may help restore bone mass. IGF-I production is markedly affected by nutrient intake (27). Restricted intakes of total energy, protein, zinc, and magnesium impair IGF-I status. In a 2-y clinical trial with 119 healthy postmenopausal women, zinc intake was the only significant determinant of IGF-I status found in a multivariate analysis, and the authors proposed that supplemental zinc may correct age-related declines in IGF-I (15). As in that study, mean zinc intakes for each treatment group in the present study were below the 12 mg/d recommended allowance, despite adequate protein intakes (Table 2). The

TABLE 5
Serum insulin-like growth factor I (IGF-I) and IGF binding protein 3 (IGFBP-3) concentrations among subjects with complete biochemical data[1]

Visit	Dietary control group (n = 15)	Calcium–vitamin D group (n = 19)	Multinutrient group (n = 25)
IGF-I (nmol/L)			
Baseline	25.7 ± 9.2	19.5 ± 5.6	19.7 ± 6.9
Year 1	23.4 ± 9.4	20.7 ± 5.3	20.3 ± 7.0
Year 2	25.7 ± 9.1	21.4 ± 4.8	19.9 ± 6.5
Year 3	26.1 ± 9.6	21.1 ± 4.5	20.3 ± 7.8
IGFBP-3 (nmol/L)			
Baseline	120.3 ± 20.3	120.2 ± 14.0	114.4 ± 13.3
Year 1	120.3 ± 14.3	123.8 ± 16.6	113.0 ± 15.9
Year 2	121.3 ± 13.0	119.4 ± 13.8	116.4 ± 13.1
Year 3	122.9 ± 15.0	126.2 ± 15.6	116.9 ± 14.2

[1] $\bar{x}$ ± SD. There were no significant within- or between-group differences (repeated-measures ANOVA and analysis of covariance with the baseline value as the covariate).

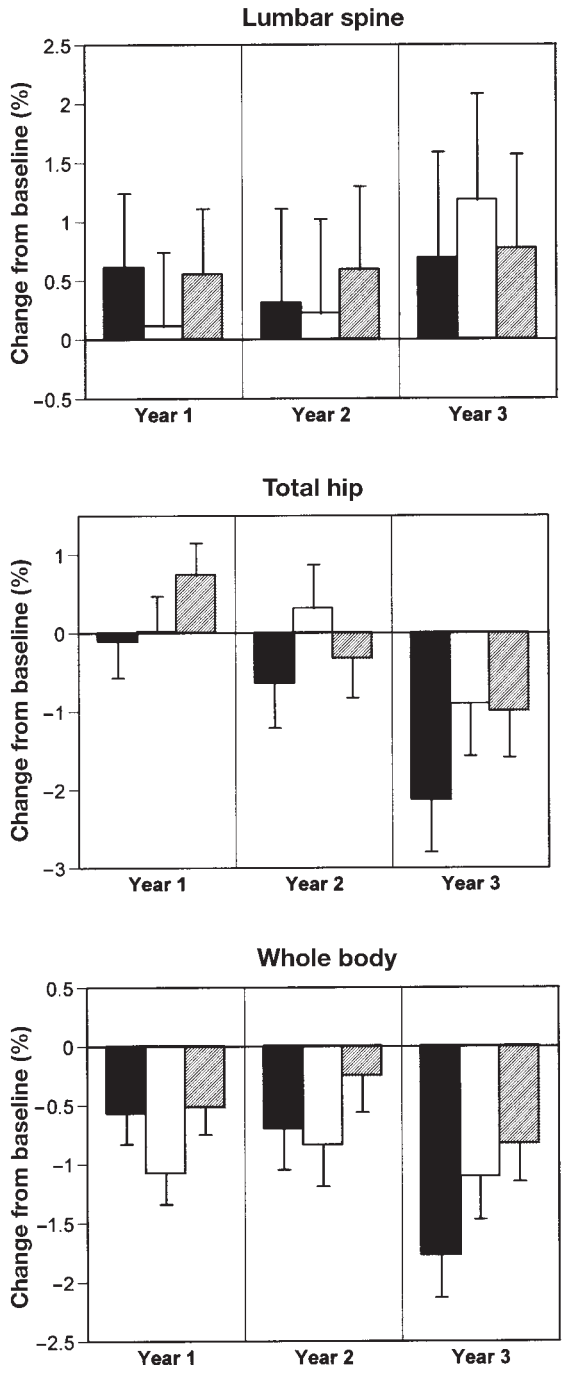

FIGURE 1. Mean (±SEM) percentage change in bone mineral density (BMD) at the lumbar spine, total hip, and whole body at years 1, 2, and 3 of intervention. ■, Dietary control group (n = 21); □, calcium–vitamin D group (n = 22); ▩, multinutrient supplement group (n = 25).

multinutrient group received 15 mg Zn/d as part of their study supplement, but the calcium–vitamin D and dietary control groups received no supplemental zinc. However, no significant differences in IGF-I or IGFBP-3 concentrations were found at any time (Table 5). In addition, baseline zinc intake did not correlate significantly with IGF-I concentration at any time. These findings indicate that 15 mg Zn/d, provided for 3 y in a multinutrient supplement to healthy older women with marginal zinc status, did not influence the circulating concentrations of IGF-I or of its major circulating binding protein, IGFBP-3.

Early, relatively short-term studies of calcium–vitamin D supplementation showed a bone-sparing effect attributable to a reduced bone remodeling space (9). This transient effect occurs when activation of new bone remodeling units is suppressed while deployed units complete their programmed cycle. A temporary imbalance between the number of new and existing units going toward completion results in a net increase in bone until a new steady state is reached. Of the 5 published nutrient intervention studies that were >2 y in duration, 3 presented early rates of bone loss separately from subsequent steady state values (5, 8, 30). The others presented only the overall percentage changes in BMD (7, 31). Most studies are inadequately powered to detect significant treatment effect differences at steady state. Nonetheless, steady state rates of bone loss with active treatment generally parallel those in placebo control subjects. Partial return of PTH concentrations toward baseline with continued intervention may help explain this parallel rate of steady state bone decline.

That the beneficial effect of nutrient intervention primarily represents a remodeling transient does not discount its value in preventing osteoporotic fracture, nor does it imply that intervention should be discontinued after 1 or 2 y. In fact, short-term intervention trials with calcium, vitamin D, or both have shown reductions in osteoporotic fracture (6, 32, 33). Moreover, most of the bone gains accrued during the remodeling transient are lost if the intervention stops (34–38). Thus, for nutrient intervention to achieve its full public health potential in reducing fracture risk, a permanent change in lifestyle would be required. This study indicates that such lifestyle changes are possible through dietary and supplementation approaches.

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