Vitamin D supplementation and bone mineral density in early postmenopausal women[1–3]

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ABSTRACT

Background: Increased vitamin D intake may preserve or increase bone mineral density (BMD) in older persons.

Objective: A 2-y double-blind study was undertaken to determine whether weekly administration of 10 000 units of vitamin D_2 maintained or increased BMD in younger postmenopausal women more efficiently than did calcium supplements alone.

Design: One hundred eighty-seven women who were ≥ 1 y postmenopausal were randomly assigned to take either 1000 mg Ca/d after the evening meal or 1000 mg Ca/d plus 10 000 U vitamin D_2/wk in a double-blind, placebo-controlled format. The BMD of the proximal forearm, lumbar spine, femoral neck, Ward's triangle, and femoral trochanter was measured at 6-mo intervals by osteodensitometry.

Results: During the 2-y period, there was no significant difference in the change in BMD at any site between the subjects taking calcium supplements and those taking calcium plus vitamin D_2. Both groups significantly ($P < 0.005$) gained BMD in Ward's triangle and the femoral trochanter but significantly ($P < 0.005$) lost bone in the proximal radius. There was no significant change in the lumbar spine or femoral neck BMD.

Conclusion: In younger postmenopausal women ($\bar{x}$ age: 56 y) whose average baseline serum 25-hydroxyvitamin D concentration was well within the normal range, the addition of 10 000 U vitamin D_2/wk to calcium supplementation at 1000 mg/d did not confer benefits on BMD beyond those achieved with calcium supplementation alone. *Am J Clin Nutr* 2003;77:1324–9.

KEY WORDS Menopause, vitamin D, bone density, postmenopausal women

INTRODUCTION

Bone mineral density (BMD) declines in women with the onset of menopause. There is both a reduction in the efficiency of absorption of calcium from the diet and an increased rate of bone resorption attributed to a decrease in serum estrogen, and the associated decrease in BMD may be accompanied by an increased risk of fracture due to minimal trauma. Studies have been reported in which additional calcium has been given by mouth in an attempt to overcome the negative calcium balance and reduce bone calcium loss (1–7). Variable results have been achieved with calcium supplementation, depending on concurrent dietary calcium intake, the number of years after menopause, the type of calcium used, and the bone site studied.

Serum parathyroid hormone (PTH) increases with age (8) and serum 25-hydroxyvitamin D [25(OH)D] declines with age (9, 10), and there is an inverse correlation between serum 25-hydroxyvitamin D_3 [25(OH)D_3] and serum PTH concentrations in older patients (11–15). Vitamin D deficiency is thought to contribute to bone loss in women (16, 17). In several studies, vitamin D has been given to postmenopausal women on the assumption that increases in concentrations of serum PTH may be suppressed and the rate of BMD loss slowed (9, 15, 18–20). Peacock et al (21) gave women ($\bar{x}$ age: 73.7 y) supplements containing 750 mg Ca/d, 15 μg 25(OH)D_3/d, or placebo. They found that supplemental calcium was more powerful than was 25(OH)D_3 in reducing the rate of BMD loss from the total hip, although the effect of calcium was greater when the serum 25(OH)D concentrations were lower. Hunter et al (22) gave 800 U of vitamin D_3/d for 2 y to postmenopausal monozygotic twins whose average age was 59 y. The change in the BMD of the spine and the neck of femur did not differ significantly between the placebo-treated group and the vitamin D_3–treated group. Vitamin D supplementation may increase BMD in older patients when the initial serum 25(OH)D concentration is low.

The present study examined the effects of vitamin D_2 supplementation on changes in BMD in younger ($\bar{x}$ age: 56 y) postmenopausal women who were also given 1000 mg Ca/d and compared those changes with the changes in BMD in women given 1000 mg Ca/d only. This study was undertaken and completed before the work of Hunter et al (22) and confirms that study's published findings.

SUBJECTS AND METHODS

Subjects

Healthy white women who were postmenopausal by 1–10 y and who were not receiving hormone replacement therapy were recruited through media advertisements. All study participants

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were assessed by means of a medical history questionnaire. Subjects with malignant disease and those with a renal, hepatic, endocrine, or gastrointestinal disorder associated with abnormal calcium metabolism were excluded. Subjects who had used estrogen, progesterone, glucocorticoids, anticonvulsants, thiazide diuretics, vitamin D supplements, or other medications known to affect calcium or bone metabolism in the previous 12 mo were also excluded. Subjects with laboratory evidence of renal, hepatic, or endocrine disorder; a serum follicle-stimulating hormone concentration < 40 mIU/mL, or BMD at any site ± 2 SD from the mean for subjects matched for age were also excluded. One hundred eighty-seven women met all entry criteria and were enrolled in the study.

Study protocol

In this 2-y double blind, placebo-controlled study, all subjects received 1000 mg Ca/d and were randomly assigned to receive either placebo or 10 000 IU vitamin D_2 once a week. At the beginning of the study, subjects were assessed by physical examination. Medical, social, dietary, and exercise histories were recorded, and subjects were advised to report any significant variations to lifestyle during the study. Blood and urine were collected for the measurement of variables to assess bone metabolism, and BMD was measured at 6 sites to assess appendicular as well as axial changes to the skeleton. Subjects were seen 1 mo later to obtain blood for serum calcium and follicle-stimulating hormone measurement and then every 6 mo for the duration of the study. At each of the 6-mo visits, relevant medical problems were recorded and investigated as necessary, blood and urine were collected, BMD measurements were performed, and treatment compliance was assessed by tablet counts and diary review.

The Royal North Shore Hospital Ethics Committee approved the study protocol. All subjects provided written informed consent.

Supplements

Two calcium carbonate tablets (Cal-Sup; 3M Pharmaceutical, Sydney, Australia) each containing the equivalent of 500 mg elemental calcium were taken at bedtime. Vitamin D_2 (Ostelin; Boots Healthcare Pharmaceuticals, Sydney, Australia) was prepared in 2 batches; one was supplied at the beginning of the study and the other at the halfway point. The tablets were stored in lightproof containers at room temperature and underwent stability testing by the manufacturer immediately before supply. A certificate of analysis was issued with each batch. Placebo tablets consisted of lactose, microcrystalline cellulose, magnesium stearate, and coloring agents. The vitamin D_2 or placebo was taken every Sunday evening at bedtime.

Compliance and completion rate

Of the 187 women enrolled [94 receiving calcium (Ca group) and 93 receiving calcium and vitamin D (Ca+D group)], 153 completed the study—80 (85%) in the Ca group and 73 (78.5%) in the Ca+D group. Of those who withdrew, 12 did so for personal reasons (eg, family crisis, moving away, and lack of commitment), 6 developed unrelated intercurrent illness (eg, systemic lupus erythematosus or malignancy) and were thus disqualified on the basis of the study protocol, 2 had BMD measurements that fell > 2 SD below the mean for age during the course of the study, and 6 developed symptoms necessitating hormone replacement therapy. Nine subjects withdrew as a result of treatment-related side effects: 5 had constipation or abdominal discomfort, 2 were intolerant of the taste of the calcium supplement, 1 had a clinical diagnosis of renal calculus, and 1 developed hyperparathyroidism. Eight of these subjects were in the Ca+D group, which thus had significantly ($P = 0.02$) more treatment-related withdrawals. The mean (± SD) rate of compliance with treatment was 98.2 ± 6.1% for the Ca+D group and 97.7 ± 5.4% for the Ca group.

Bone density measurements

BMD was measured at 6-mo intervals at the lumbar spine, neck of femur, trochanter, Ward's triangle, proximal radius and ulna, and proximal radius with the use of a dual-energy X-ray absorptiometer (XR26; Norland Corp, Fort Atkinson, WI). External and internal calibrations were performed daily with the use of a hydroxyapatite phantom embedded in perspex (Norland Corp). The CVs were 1.0% for lumbar spine, 1.2% for neck of femur, 1.8% for trochanter, 5.3% for Ward's triangle, 0.8% for proximal radius and ulna, and 0.9% for proximal radius. There was no long-term drift in the phantom measurements.

Biochemistry

Blood and urine were collected at baseline and then at 6-mo intervals throughout the study, with an additional blood collection at 1 mo, as mentioned previously. Serum 25(OH)D, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], and osteocalcin concentrations were measured by radioimmunoassay (Incstar Corp, Stillwater, MN). 25$(OH)D_2$ is measured with the same efficiency as 25$(OH)D_3$, and therefore, the value obtained will be the sum of the circulating 25$(OH)D_2$ and 25$(OH)D_3$ concentrations. Serum PTH was measured by in-house radioimmunoassay using polyclonal antibodies against the intact molecule (23). Urinary deoxypyridinoline crosslinks were performed with the use of a competitive enzyme-linked immunoassay (Metra Biosystems Inc, Mountain View, CA) on fasting second-morning voids. The intraassay and interassay CVs were < 15.0%. At each 6-mo visit, 24-h urinary samples were collected for calcium and creatinine measurements. Serum analytes were measured on a biochemical analyzer (Boehringer Mannheim 747; Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's recommended methods. Urinary analytes were measured on a Beckman CX-7 Biochemical Analyzer (Beckman Instruments, Fullerton, CA) in accordance with the manufacturer's protocol. Serum and urine analyses were carried out in the Department of Biochemistry, Royal North Shore Hospital.

The height and weight of each participant were measured at each 6-mo visit. Dietary calcium intake was assessed at the outset of the study and again at 1 y by means of a food-frequency questionnaire (24) and subsequent calculation of daily intake. To ensure adequate randomization, sun exposure was calculated with the use of a questionnaire designed to assess time outdoors, activity undertaken, usage of sunscreen and frequency of application, and cloud cover.

Statistical analysis

A power analysis was undertaken before the study so that a change of 2% in BMD over 2 y could be detected with 80% power, with the use of a $P < 0.05$ significance level (two-sided test), provided that 74 persons were included in each arm of the study. Each patient's 6-mo data were used to construct a regression coefficient measuring the annual rate of change for that

TABLE 1
Baseline characteristics of the postmenopausal women supplemented with either calcium (Ca group) or calcium and vitamin D (Ca+D group) for 2 y[1]

	Ca group (n = 94)	Ca+D group (n = 93)
Age (y)	56.1 ± 4.7[2]	56.5 ± 4.2
Years after menopause	5.4 ± 3.0	6.1 ± 2.8
Weight (kg)	67 ± 12	67 ± 11.9
Height (cm)	162.4 ± 5.8	162.4 ± 5.8
Dietary calcium (mg/d)[3]	811.2 ± 324.8	754.4 ± 288.3
Ethanol (g/d)	5.3 ± 9.6	6.4 ± 10
Sun exposure (min/d)	115.8 ± 80	113.7 ± 91.7
Smokers (n)	6	7

[1] There were no significant differences between the groups.
[2] $\bar{x}$ ± SD.
[3] Dietary calcium was reassessed at 12 mo. The mean (±SD) dietary calcium in the Ca group was 825.7 ± 358.5 mg/d and that in the Ca+D group was 836.2 ± 393.9 mg/d.

TABLE 2
Baseline values for biochemical variables in the postmenopausal women supplemented with either calcium (Ca group) or calcium and vitamin D (Ca+D group) for 2 y[1]

	Ca group (n = 94)	Ca+D group (n = 93)
Serum		
Calcium (mmol/L)	2.40 ± 0.10	2.40 ± 0.10
25(OH)D (nmol/L)	82.6 ± 27.0	81.6 ± 24.4
$1,25(OH)_2D$ (pmol/L)	93.4 ± 29.7	93.5 ± 31.1
Phosphate (mmol/L)	1.17 ± 0.12	1.17 ± 0.11
PTH (ng/mL)	0.2 ± 0.1	0.2 ± 0.2
ALP (U/L)	89.1 ± 26.3	85.4 ± 18.8
Osteocalcin (ng/mL)	4.3 ± 1.7	4.6 ± 2.4
Urine		
DPYR (nmol/mmol creatinine)	4.7 ± 3.4	4.4 ± 1.7
Ca (mmol/d)	4.4 ± 2.0	4.0 ± 2.0

[1] $\bar{x}$ ± SD. 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; ALP, alkaline phosphatase; DPYR, deoxypyridinoline.

person. The within-patient changes were compared between groups with the use of two-factor repeated-measures analysis of variance with interaction. The P values of the main effects of time and treatment and time-and-treatment interaction were obtained. Bonferroni's correction was used where multiple comparisons were made.

RESULTS

The baseline characteristics are shown in **Tables 1** and **2**. There were no significant differences between the 2 groups. The mean BMD at each site examined in the 2 treatment groups at 5 time points is shown in **Table 3**.

Bone mineral density

When studied through the 2-y studied period, the change in BMD at any of the sites studied did not differ significantly between subjects taking calcium supplements (Ca group) and subjects taking calcium and vitamin D_2 supplements (Ca+D group) (**Table 4**). There were no statistically significant interactions between the effects of time and treatment on the annual percentage changes from baseline over 2 y. The lumbar spine exhibited no significant percentage changes from baseline over 2 y. The trochanter and Ward's triangle exhibited significantly ($P < 0.005$) positive percentage changes from baseline. The proximal radius and the proximal radius and ulna showed significantly ($P < 0.005$) negative percentage changes over 2 y. The neck of the femur and the proximal radius had significantly ($P < 0.001$ and $P = 0.007$, respectively) different percentage changes from baseline in each year of the study (**Table 5**).

Biochemistry

The baseline indexes did not differ significantly between the 2 groups (Table 2). The analysis of the changes in the biochemical variables is shown in **Table 6**. Only 25(OH)D showed a significant ($P < 0.001$) interaction between the effects of time and treatment on the annual change. The concentrations of 25(OH)D changed significantly ($P < 0.05$, Bonferroni's adjustment for multiple comparisons) in the Ca+D group in both years of the study: in the first year, the mean (± SD) concentrations increased by

TABLE 3
Bone mineral density measurements at 5 time points in the postmenopausal women supplemented with either calcium (Ca group) or calcium and vitamin D (Ca+D group) for 2 y[1]

	Baseline	6 mo	12 mo	18 mo	24 mo
			g/cm²		
Ca group					
Lumbar spine (L2–L4)	0.965 ± 0.160	0.979 ± 0.156	0.974 ± 0.155	0.976 ± 0.163	0.981 ± 0.153
Neck of femur	0.813 ± 0.128	0.819 ± 0.113	0.823 ± 0.114	0.818 ± 0.109	0.840 ± 0.122
Trochanter	0.666 ± 0.099	0.676 ± 0.099	0.683 ± 0.101	0.694 ± 0.100	0.682 ± 0.101
Proximal radius and ulna	0.669 ± 0.078	0.675 ± 0.080	0.670 ± 0.083	0.683 ± 0.080	0.673 ± 0.080
Proximal radius	0.668 ± 0.075	0.669 ± 0.078	0.662 ± 0.081	0.674 ± 0.075	0.662 ± 0.078
Ca+D group					
Lumbar spine (L2–L4)	0.953 ± 0.148	0.966 ± 0.158	0.952± 0.148	0.962 ± 0.144	0.955 ± 0.146
Neck of femur	0.808 ± 0.123	0.810 ± 0.118	0.793 ± 0.110	0.810 ± 0.114	0.815 ± 0.108
Trochanter	0.647 ± 0.106	0.659 ± 0.103	0.654 ± 0.105	0.673 ± 0.100	0.658 ± 0.100
Proximal radius and ulna	0.672 ± 0.081	0.676 ± 0.087	0.670 ± 0.087	0.663 ± 0.096	0.670 ± 0.080
Proximal radius	0.671 ± 0.081	0.668 ± 0.085	0.664 ± 0.086	0.661 ± 0.083	0.663 ± 0.080

[1] $\bar{x}$ ± SD. The number of subjects in the Ca and Ca+D groups, respectively, at the 5 time points were as follows: baseline, 94 and 93; 6 mo, 89 and 80; 12 mo, 84 and 74; 18 mo, 81 and 73; and 24 mo, 80 and 73.

TABLE 4
Annual rate of change in bone mineral density in the postmenopausal women supplemented with either calcium (Ca group) or calcium and vitamin D (Ca+D group) for 2 y[1]

	Ca group (n = 80)	Ca+D group (n = 73)
	%	
Lumbar spine (L2–L4)	0.24 ± 2.35	0.15 ± 2.49
Neck of femur	0.59 ± 2.06	0.26 ± 2.14
Trochanter	1.11 ± 2.41[2]	1.26 ± 2.99[2]
Ward's triangle	1.40 ± 4.18[2]	1.40 ± 4.20[2]
Proximal radius and ulna	−0.13 ± 4.06[3]	−0.67 ± 2.64[3]
Proximal radius	−0.73 ± 1.81[3]	−0.86 ± 1.60[3]

[1] $\bar{x}$ ± SD. There were no statistically significant differences between annual percentage changes from baseline between the Ca and Ca+D groups in the 2-y study.
[2] Significant changes from baseline, $P < 0.005$.
[3] Significantly negative changes from baseline over 2 y, $P < 0.005$.

5.3 ± 18.1 nmol/L, and in the second year, they decreased by 6.4 ± 5.6 nmol/L. In contrast, the Ca group showed a steady decrease over the 2 y at a significant ($P < 0.05$, Bonferroni's adjustment for multiple comparisons) average annual rate of −6.7 ± 0.7 nmol/L. These data are shown graphically in **Figure 1**. Of the remaining variables, PTH, alkaline phosphatase (ALP), osteocalcin, urinary calcium (UCa), and serum calcium showed no statistically significant difference between the changes observed in year 1 and year 2. ALP, UCa, and serum calcium decreased significantly with time. There was no significant difference in the concentrations of PTH, ALP, osteocalcin, UCa, or serum calcium between the treatment groups.

There was no significant correlation between the starting serum 25(OH)D concentration and the subsequent change in BMD at any site in either group or any significant correlation between the percentage change in serum 25(OH)D or PTH and the change in BMD at any site in either group.

DISCUSSION

This study was designed to examine the efficacy of vitamin D supplementation, given as vitamin D_2 in a single dose of 10 000 U/wk, in maintaining BMD in the early postmenopausal period. The study found that there was no significant additional benefit to BMD in either the axial or appendicular skeleton of early postmenopausal women when vitamin D_2 in addition to calcium was given, rather than calcium alone. A trial by Komulainen et al (25) examined healthy, early postmenopausal ($\bar{x}$ 1.2 y after menopause) women and failed to detect any significant benefit to BMD of daily supplementation with small amounts (300 IU) of vitamin D. In contrast, studies in elderly populations have shown supplements of vitamin D to be beneficial to BMD. Chapuy et al (18) gave 800 IU vitamin D/d and 1000 mg Ca/d to elderly women whose average serum 25(OH)D concentration before treatment was 40 nmol/L, and they found a significant increase in femoral neck BMD and a reduction in the rate of fracture. It is not clear in this study whether the beneficial effect is from the calcium or from the vitamin D supplementation. Ooms et al (19) gave 400 IU vitamin D_3/d to women with an average age of 80.1 y. The serum 25(OH)D concentration increased from 27 nmol/L to 62 nmol/L, and the femoral neck BMD increased significantly (1.8%) in the first year and by an additional 0.2% in the second year. The effects were independent of the serum 25(OH)D concentration at baseline. Dawson-Hughes et al (20) gave 70-y-old women 700 U vitamin D and calcium for 3 y. After one year of treatment, there was significantly less loss of total BMD and of spinal BMD but no change in femoral neck BMD. The baseline serum 25(OH)D concentration in the women in that study was 70.3 nmol/L, which is not very different from the baseline serum 25(OH)D concentration in the present study, but it increased to 109.7 nmol/L with 700 U vitamin D_3/d supplementation. As in the study by Ooms et al (19), most of the effects in the study by Dawson-Hughes et al (20) were seen within the first year of treatment. Adams et al (15) studied 12 women ($\bar{x}$ age: 60 y) with a baseline serum 25(OH)D concentration of 25.1 nmol/L who received 500 000 U vitamin D_2 over a 5-wk period, which is the same dose that our patients received over a 12-mo period. In that study, the serum PTH concentration was reduced by 32.9 pg/mL, and the spine and femoral neck BMD increased by 4–5% per year; these findings support the concept that vitamin D administration will increase BMD in persons whose initial serum 25(OH)D concentrations are much lower than those found in our patient group. In a randomized double-blind protocol, Peacock et al (21) gave women ($\bar{x}$ age: 73.7 y) either 750 mg Ca/d, 15 μg 25(OH)D_3/d, or placebo. The mean baseline serum 25(OH)D concentration was 65 nmol/L, and the concentration increased to 118 nmol/L with the 25(OH)D treatment. The effect of calcium supplements on reducing the loss of BMD from the total hip was greater than that seen when 25(OH)D_3 was given, and the effect of supplemental calcium was greater when the initial serum 25(OH)D_3

TABLE 5
Annual rate of change in bone mineral density in the postmenopausal women during years 1 and 2 of supplementation with either calcium (Ca group) or calcium and vitamin D (Ca+D group)[1]

	Year 1		Year 2		
	Ca group (n = 84)	Ca+D group (n = 74)	Ca group (n = 80)	Ca+D group (n = 73)	P[2]
	%		%		
Lumbar spine (L2–L4)	0.44 ± 4.20	−0.19 ± 4.13	0.59 ± 3.58	0.75 ± 3.73	0.6
Neck of femur	−0.40 ± 3.72	−1.81 ± 3.90[3]	2.15 ± 4.47	3.09 ± 4.90	<0.001
Trochanter	1.53 ± 4.09	1.31 ± 5.03	-0.02 ± 4.15	0.92 ± 5.17	0.5
Ward's triangle	2.72 ± 9.32	1.17 ± 7.58	1.89 ± 10.37	2.23 ± 7.86	0.6
Proximal radius and ulna	0.94 ± 10.88	−0.64 ± 5.60	−0.19 ± 2.51	0.25 ± 2.26	0.8
Proximal radius	−0.69 ± 3.60	−1.69 ± 2.53	−0.34 ± 2.20	0.01 ± 2.43	0.007

[1] $\bar{x}$ ± SD. There were no significant interactions between the effects of time and treatment during 2 y.
[2] Comparison of year 1 with year 2 (ANOVA).
[3] Significantly different from Ca group, $P = 0.02$.

TABLE 6
Changes in the serum and urine variables relevant to calcium metabolism in the postmenopausal women supplemented with either calcium (Ca group) or calcium and vitamin D (Ca+D group) for 2 y[1]

	Ca group (n = 80)	Ca+D group (n = 73)	P	
			Treatment	Time × treatment
Serum				
Calcium (mmol/L)	−0.02 ± 0.10	−0.04 ± 0.10	0.6	0.7
25(OH)D (nmol/L)	−13.4 ± 23.70	−1.10 ± 21.30	0.02	<0.001
$1,25(OH)_2D$ (pmol/L)	−1.10 ± 41.80	−4.50 ± 43.80	0.6	0.4
Phosphate (mmol/L)	−0.04 ± 0.12	−0.04 ± 0.14	0.5	0.2
PTH (ng/mL)	−0.004 ± 0.18	−0.02 ± 0.23	0.3	0.4
ALP (U/L)	−4.50 ± 13.20	−7.80 ± 13.10	0.4	0.2
Osteocalcin (ng/mL)	0.50 ± 2.10	0.02 ± 2.60	0.8	0.5
Urine				
DPYR (nmol/mmol creatinine)	−0.24 ± 3.50	0.10 ± 2.10	0.03	0.5
Calcium (mmol/d)	−0.60 ± 1.60	−0.50 ± 1.70	0.2	0.8

[1] $\bar{x}$ ± SD. 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; ALP, alkaline phosphatase; DPYR, deoxypyridinoline.

concentration was low, presumably because a greater decrease in serum PTH occurred in this situation.

In a study by Lips et al (13) of elderly (aged 81–84 y), the initial serum 25(OH)D concentration was inversely correlated with the serum PTH concentration, and there was an inverse relation between the change in serum 1,25(OH)D and the pretreatment serum 25(OH)D after supplementation with vitamin D_3 (13). In the older women in that study, vitamin D_3 supplements decreased the serum PTH, whereas in our study, the serum PTH concentration actually increased during the first year of treatment with vitamin D_2, a change in our study that is not explained. Thomas et al (26) also found that, in hospitalized patients, the serum PTH concentration was significantly higher when the serum 25(OH)D concentration was < 37.5 nmol/L. Malabanan et al (27) found that, when the baseline serum 25(OH)D concentration was < 50 nmol/L, the serum PTH concentration increased and then fell significantly when 50 000 U vitamin D_2 was given weekly for 8 wk. It is interesting that the serum PTH did not decrease if the baseline serum 25(OH)D had been > 50 nmol/L.

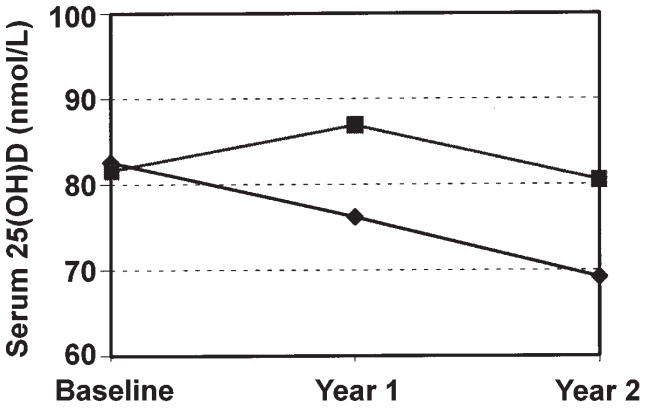

FIGURE 1. Serum 25-hydroxyvitamin D [25(OH)D] concentrations in the postmenopausal women at baseline (year 0) and during years 1 and 2 of supplementation with either calcium (Ca group; ◆) or calcium and vitamin D (Ca+D group; ■). In the Ca+D group, concentrations increased 5.3 ± 18.1 nmol/L ($\bar{x}$± SD; $P < 0.05$) in the first year and then decreased 6.4 ± 15.6 nmol/L ($P < 0.05$) in the second year. In contrast, concentrations in the Ca group decreased significantly ($P < 0.05$, Bonferroni's adjustment for multiple comparisons) during the 2-y study at an average annual rate of 6.7 ± 20.7 nmol/L. SEs for the Ca group were 3.039 for year 0 (n = 94), 2.858 for year 1 (n = 84), and 2.849 for year 2 (n = 79); those for the Ca+D group were 3.192 for year 0 (n = 93), 3.002 for year 1 (n = 75), and 2.164 for year 2 (n = 72). There was a significant ($P < 0.001$) interaction between the effects of time and treatment.

In the design of the present study, it was thought that a weekly dose of 10 000 U vitamin D_2 given for 2 y would provide a reasonable supplement of vitamin D without causing adverse effects and would allow a conclusion as to whether supplementation of younger postmenopausal women with vitamin D could be associated with a preservation of or increase in BMD. An unexpected finding was the small increase in the serum 25(OH)D concentration at the end of the first year after weekly 10 000 U vitamin D_2 supplementation. At that time, the difference in serum 25(OH)D concentration between the Ca and the Ca+D groups was 12.0 nmol/L, and, at 2 y, it was 12.3 nmol/L. Lips et al (13), however, found that in older women (aged 81–84 y) supplemented with vitamin D_3, the increment in the serum 25(OH)D concentration was no greater with a daily supplement of 800 U vitamin D_3 than with that of 400 U vitamin D_3. In a subsequent study (14), the increase in serum 25(OH)D after 400–600 U vitamin D was given daily depended on the initial serum 25(OH)D concentration. When the baseline serum 25(OH)D concentration was < 25, 25–50, and > 50 nmol/L, the increments in serum 25(OH)D were 58.4, 39.4, and 12.5 nmol/L, respectively.

In the present study, the initial serum 25(OH)D concentration was 81 nmol/L and the increment with weekly supplementation with 10 000 U vitamin D_2 in the first year was 5.3 nmol/L, which implies that there are mechanisms that accelerate the metabolic clearance of vitamin D when concentrations of serum 25(OH)D in the blood begin to rise. This mechanism may accelerate with time because, during the second year of supplementation, the serum 25(OH)D concentration actually decreased toward baseline again. In the study by Hunter et al (22), the serum 25(OH)D concentration decreased in the supplemented patients between 6 mo and 24 mo of treatment.

These observations led to further consideration of the controversial concept of what constitutes the vitamin D–replete state, which has been defined in terms of simultaneous serum PTH concentrations. For example, Thomas et al (26) found that, in an inpatient population study, the serum PTH began to rise when the serum 25(OH)D was < 37.5 nmol/L. Gloth et al (12) found that the serum PTH was below the upper limit of normal if the serum 25(OH)D was > 40 nmol/L. However, Dawson-Hughes (20) found that a nadir of suppression of serum PTH occurred when the serum 25(OH)D was 110 nmol/L, and the decrease in the serum PTH (remaining within the normal range) was achieved by giving small amounts of vitamin D to postmenopausal women in winter which reduced bone loss (16).

In any case, in our study, increments in the serum 25(OH)D concentration to > 80 nmol/L did not lead to better preservation of BMD than did calcium supplements alone. It is possible that greater effects on BMD would have been achieved if the serum 25(OH)D concentration had been sustained at a higher value, but this would seem unlikely because, in the study of Peacock et al (21), the achievement of serum concentrations of 25(OH)D of 118 nmol/L caused a smaller reduction in BMD loss than was seen when calcium alone was given.

In summary, the present study failed to show any additional benefit in preservation of BMD in postmenopausal women ($\bar{x}$ age: 56.1 y) when 10 000 U vitamin D_2/wk was added to daily calcium supplementation of 1000 mg. The use of calcium alone over a 2-y period was associated with no significant loss in BMD in the spine or femoral neck, a significant gain in BMD in the femoral trochanter and Ward's triangle, but a significant loss in BMD in the proximal radius and ulna.

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LC was responsible for the study design, data collection, data analysis, and the writing of the manuscript. PBC-B was responsible for the study design, data analysis, and the writing of the manuscript. MLN was responsible for data collection, data analysis, and recruitment of patients. GF, ST, and EH were responsible for data collection. BGR was responsible for the study design, data analysis, and the writing of the manuscript. The authors had no financial or personal interest in any of the pharmaceutical companies involved in the study, nor were they on any advisory board that had an interest in this study.

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