Osteoporos Int (1998) 8:255–260

Osteoporosis International

Original Article

Calcium and Vitamin D Supplementation Increases Spinal BMD in Healthy, Postmenopausal Women

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Abstract. We undertook a double-masked, randomized, placebo-controlled trial to evaluate the effect of a calcium and vitamin D supplement and a calcium supplement plus multivitamins on bone loss at the hip, spine and forearm. The study was performed in 240 healthy women, 58–67 years of age. Duration of treatment was 2 years. Bone mineral density (BMD) was measured at the lumbar spine, hip and forearm. A dietary questionnaire was administered twice during the study and revealed a fairly good calcium and vitamin D intake (919 mg calcium/day; 3.8 μg vitamin D/day). An increase in lumbar spine BMD of 1.6% was observed in the treatment group after 2 years ($p < 0.002$). In the placebo group no significant changes were observed during the 2 years. Lumbar spine BMD was significantly higher in the treatment group at both 1 ($p < 0.01$) and 2 years ($p < 0.05$) compared with the placebo group. Though not significant, the same trend was seen at the hip. No significant changes from baseline values were observed at the distal forearm in either the treatment or the placebo group. In conclusion, we found a significant increase in urinary calcium excretion in the treatment group compared with the placebo group. Together with significant changes in serum calcium and serum parathyroid hormone, this indicates that a long-term calcium and vitamin supplement of 1 g elementary calcium (calcium carbonate) and 14 μg vitamin D_3 increases intestinal calcium absorption. A positive effect on BMD was demonstrated, even in a group of early postmenopausal age, with a fairly good initial calcium and vitamin D status.

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Keywords: Calcium intake; Osteoporosis; Prevention; Vitamin D intake

Introduction

During the last decade there has been lack of consensus regarding the role of calcium and vitamin D supplementation in the primary or secondary prevention of postmenopausal osteoporosis [1–3]. Several studies have shown an effect of calcium supplementation on bone loss and fracture risk in the postmenopausal years [4–6]. The positive effect has been seen in the late postmenopausal period, while there seems to be no effect of a calcium supplement in the initial 3–5 years after menopause [5,7]. Elders et al. [8] found no role for calcium supplementation in modulating the rate of bone loss from the lumbar spine in the years immediately after the menopause.

Several studies have proved an effect of vitamin D supplementation on the rate of bone loss and incidence of fractures [9–11]. With increasing age serum 25-OH-vitamin D_3 is seen to decline due to decreased sunlight exposure and decreased capacity of the skin to produce provitamin cholecalciferol combined with decreased dietary intake of vitamin D_3. Simultaneously, an increase in serum parathyroid hormone (PTH) is observed. In a study by Khaw et al. [12], a single-dose treatment with vitamin D_3 was found to reverse these changes in the elderly and to produce a significant decrease in PTH and increase in serum 25-OH-vitamin D_3. The reduction in PTH is thought to increase bone density by reducing bone resorption. A significant reduction in the incidence of fractures was observed in a large study of combined calcium and vitamin D supplementation [13]. Elderly

women are likely to benefit more than younger women, who are expected to have a better calcium and vitamin D status, but even in the perimenopausal years vitamin D_3 has been seen to reduce bone loss [14]. An effect has been seen with a daily vitamin D intake reaching the recommended value of 5.0 μg [11], but higher intakes (up to 20 μg/day) seem safe and effective [10,11].

Uncertainty remains regarding the optimal calcium intake. US recommendations [15], suggesting 1000–1500 mg/day for postmenopausal women, are somewhat higher than the Nordic guidelines [16]. In postmenopausal Danish women, the mean dietary intakes of both calcium and vitamin D are found to be quite high (887 mg/day and 3.5 μg/day, respectively) [17]. Different preparations of calcium supplements are available. A recent study has examined the intestinal calcium absorption of a calcium carbonate supplement and found it as good as the uptake from an equivalent amount of milk [18]. In this study we examined the possible effect of calcium carbonate in combination with vitamin D_3 on postmenopausal bone loss.

Subjects and Methods

Subjects

Two hundred and forty women were enrolled in the study. They were randomly selected from the central citizens registry and contacted by letter and telephone. The criteria for entry were Caucasian background, age 58–67 years, good general health and postmenopausal status defined as cessation of menstrual bleeding for at least 6 months.

Patients treated with oestrogen or calcitonin during the previous 12 months or with bisphosphonates in the previous 24 months were not included in the study. Presence of diseases known to affect bone metabolism, renal disease with serum creatinine above 120 μmol/l, and hepatic disease with increased ALAT and/or decreased extrinsic coagulation factors II, VII and X were causes of exclusion. Decreased function of the exocrine pancreas or any other state of malabsorption also led to exclusion.

The study subjects were selected from the general population and included in the study independently of their bone mineral density (BMD) status. They were included as healthy subjects from a clinical viewpoint. No assessments were made regarding the number of years since menopause. From other studies we know that the initial 3–5 years after the menopause seem to be a period with little or no effect of calcium supplementation. At the time of inclusion the participants were aged 58–67 years (mean 62.5 years), and we would therefore not expect to have included a significant number of women in that non-responsive period.

In a subgroup of patients with a minimum participation of 3 months in the main study, we examined the 24-h urinary calcium and creatinine excretion in two consecutive periods. For this substudy 28 patients were randomly selected and invited to participate.

The study was approved by the local ethics committee and was performed in accordance with the Helsinki II Declaration.

Supplements and Study Design

Different preparations of calcium supplements are available. In this study we examined the possible effect of calcium carbonate in combination with vitamin D_3. The patients were randomized equally into two active treatment groups and one group received placebo. One group (80 patients) received a daily supplement consisting of 1000 mg of elementary calcium (calcium carbonate) together with 14 μg (560 IU) of vitamin D_3 (cholecalciferol) contained in two tablets. Eighty patients received in addition to the calcium and vitamin D_3 a multivitamin supplement contained in the same formulation. Eighty patients received placebo in a similar formulation. The compositions of the formulations are described in Table 1. All placebo and active treatment tablets were provided by Lube Ltd. The participants were instructed to take two tablets daily at breakfast and to make no changes in their habitual food intake.

BMD measurements were performed at baseline, after 1 year and after 2 years. Calcium and vitamin D intake was assessed using a 7-day dietary diary at baseline and after 2 years. Calculations were based on the Dankost system (Dansk Catering Centre A/S, Herlev, Denmark).

Patients were asked to take no calcium or vitamin D supplement other than the supplement supplied for the study. Treatment with prescribed medications was recorded. No formal assessment of compliance, such as tablet counting, was made. At each visit the subjects were questioned about their compliance with the study

Table 1. Composition of formulations used and daily dose

	Treatment group		
	Calcium + vitamin D	Calcium + multivitamin	Placebo
Calcium carbonate (mg)	2500	2500	0
Elementary calcium (mg)	1000	1000	0
Cholecalciferol (μg)	14	14	0
Retinol (μg)	0	800	0
Thiamine (mg)	0	1.4	0
Riboflavine (mg)	0	1.6	0
Pyridoxine (mg)	0	2	0
Cyanocobalamine (μg)	0	1	0
Folic acid (μg)	0	100	0
Niacine (mg)	0	18	0
Pantothenic acid (mg)	0	6	0
Biotin (μg)	0	150	0
Ascorbic acid (mg)	0	60	0
D-alpha-tocopherol (mg)	0	10	0
Phylloquinone (μg)	0	70	0

medication and encouraged to comply. No registration of physical activity was made, neither was any programme of exercise undertaken.

The biochemical parameters of efficacy and safety were measured every 6 months.

In the substudy no attempts were made to standardize the calcium intake. The participants were specifically asked to make no changes in their habitual food intake during the days of urine collection.

Measurements

The primary end-point was changes from baseline in BMD in the lumbar spine (L2–4). Other effect parameters were: hip BMD, forearm BMD, serum calcium, serum phosphate and serum intact PTH.

BMD (g/cm^2) of the lumbar spine, left hip and non-dominant distal forearm were determined using dual-energy X-ray absorptiometry (DXA; Norland XR-26, Norland MZ Weesp, The Netherlands). All measurements were performed by the same two, experienced technicians. At the lumbar spine BMD was measured in the anteroposterior (AP) projection for the second, third and fourth vertebrae. When scanning the AP spine the vertebral column was centred at the scan table and the subject's legs raised on a block to straighten the curvature of the spine. At the hip, the BMD measurement was performed at the femoral neck. A fixation device for rotation and correct positioning of the legs was used. At the forearm, calculations were made on the distal part (scan length 2.4 cm; located proximal to the distal junction of radius and ulna). The short-term precision for the AP spine (L2–4) was 1.0% (data based on 42 scans on 7 subjects). For femoral neck the short-term precision was found to be 1.2% (36 scans on 6 subjects). No data on precision are available for the distal forearm measurements. During the study daily phantom calibrations were performed. No longitudinal drift occurred.

Serum intact PTH (1–84) was measured using a chemiluminescence assay (Immulite Diagnostic Products Corporation (DPC), Los Angeles, CA). The total coefficient of variation for the method was 10.0%. Serum calcium was calculated as the albumin-corrected value.

The biochemical safety parameters included: blood haemoglobin, serum albumin, serum creatinine, serum urea, serum sodium, serum potassium and serum ALAT.

In the substudy the concentrations of creatinine and calcium were measured. Urinary calcium excretion was expressed as mmol/24 h.

Statistical Analysis

The results are presented as percentage changes from baseline values and were evaluated using a paired *t*-test. Level of significance was 5%.

Results

For all parameters measured, we observed no differences between the two active treatment groups. In presenting the results we therefore consider the two groups as one group receiving active treatment.

During the study, 41 of the 240 women dropped out (Table 2). No difference in drop-out rate was found between the groups. One hundred and ninety-nine women completed all visits. In the analysis an additional two women were excluded due to development of radiologically verified vertebral fractures in the lumbar spine. This left 197 women who were included in the statistical analysis.

In the treatment group a significant increase (0.0195 g/cm^2; $p < 0.0001$) in the BMD of the lumbar spine was seen in the first year. A small decrease was observed in the second year compared with the first year. The overall increase after 2 years was 1.6% ($p < 0.002$). In the placebo group no significant changes were observed during the 2 years. A statistically significant difference between the treatment and placebo groups at both 1 year ($p < 0.01$) and 2 years ($p < 0.05$) was found (Fig. 1). At the femoral neck, we found an insignificant increase in BMD in the treatment group compared with the placebo group at both 1 and 2 years. At the distal forearm, no significant changes from baseline values were observed in either group.

In the treatment group an increase in serum calcium was seen during the first year, followed by a smaller decrease in the second year. A statistically significant difference was detected between the placebo and treatment groups at both 1 and 2 years ($p < 0.0001$). In the placebo group a significant increase in serum calcium was observed during the first year ($p < 0.0001$), but combined with a similar decrease in the second year, this resulted in no overall change from baseline to 2 years in the placebo group (Fig. 2).

Regarding serum PTH, a significant decrease of 26.5% was observed in the treatment group during the first year ($p < 0.0001$), followed by a slight increase during the second year. Statistically significant differences between the two groups were found at both 1 year

Table 2. Status of excluded subjects

Reason for exclusion	Treatment			
	Calcium + vitamin D	Calcium + multivitamin	Placebo	Total
Personal	9	4	12	25
Other illness	3	2	1	6
Constipation	2	2	0	4
Death	0	1	1	2
Osteoporosis	1	1	0	2
Nausea	0	0	2	2
Total	15	10	16	41

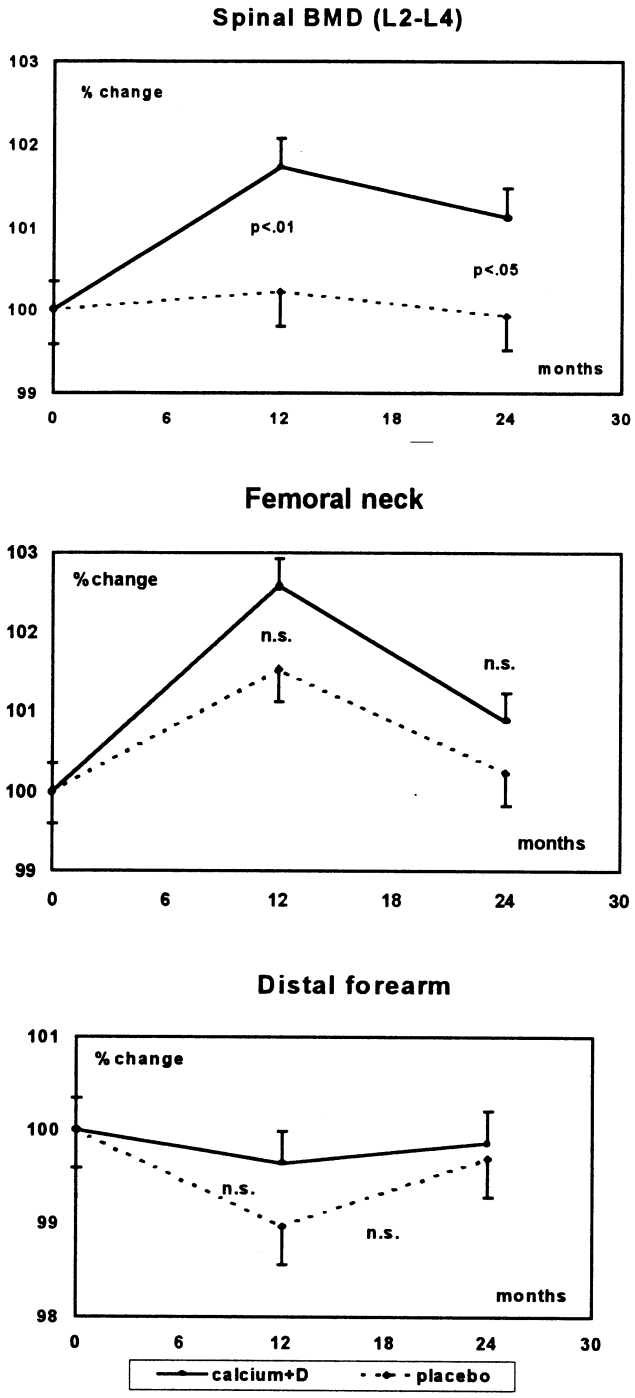

Fig. 1. BMD at the lumbar spine, hip and distal forearm in active treatment (*continuous line*) versus placebo (*dashed line*) groups.

($p < 0.0001$) and 2 years ($p < 0.001$). No changes were observed in the placebo group during the two years (Fig. 2).

In the active treatment group we found a mean urinary calcium excretion of 6.73 mmol/24 h compared with 4.69 mmol/24 h in the placebo group. This constitutes a significant ($p < 0.05$) difference between the two groups of 44%.

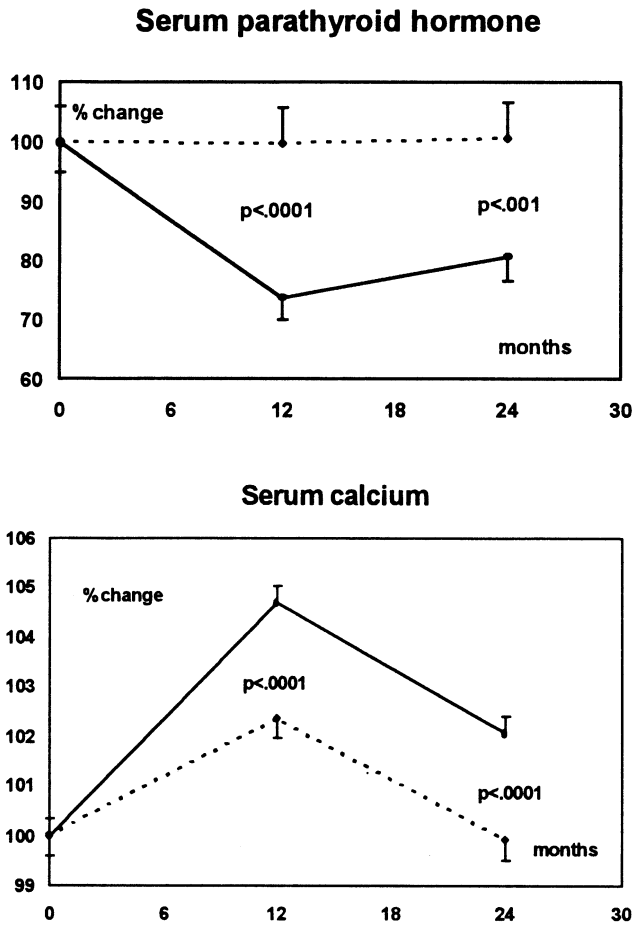

Fig. 2. Serum parathyroid hormone and serum calcium concentration in active treatment (*continuous line*) versus placebo (*dashed line*) groups.

Based on information obtained from the 7-day dietary diary, mean calcium intake, mean vitamin D intake and total energy intake were calculated for the treatment and the placebo groups. There were no statistically significant difference between the two groups. Dietary intakes of calcium and vitamin D were recorded twice during the study (before the start of treatment and after 2 years) and showed no difference between the first and second measurements. The mean calcium intake was 919 versus 844 mg/day (p = 0.06), and the mean vitamin D intake was 3.8 versus 3.4 μg/day (p = 0.18). The cumulated intakes of calcium and vitamin D are shown in Fig. 3.

No changes were observed in the safety parameters.

Discussion

The main finding of the present study was a significant increase of 1.6% in spinal BMD in the women treated with calcium and vitamin D compared with the placebo group, while no significant changes were observed at the hip or the distal forearm. Our findings are in accordance

Table 3. Baseline characteristics

	Treatment			
	Calcium + vitamin D	Calcium + multivitamin	Placebo	*p* value
Number	65	69	63	
Age (years)	62.9	62.9	61.8	NS
Spine BMD (g/cm^2)	0.902 (0.615–1.409)	0.921 (0.64–1.606)	0.926 (0.6–1.262)	NS
Hip BMD (g/cm^2)	0.726 (0.514–1.033)	0.739 (0.547–1.121)	0.727 (0.501–0.973)	NS
Forearm BMD (g/cm^2)	0.369 (0.272–0.535)	0.377 (0.254–0.626)	0.373 (0.197–0.499)	NS
Serum calcium (mmol/l)	2.34 (2.12–2.48)	2.35 (2.11–2.48)	2.36 (2.16–2.52)	NS
Serum PTH (pmol/l)	2.21 (0.5–7.3)	2.19 (0.5–4.5)	2.42 (0.3–8.7)	NS
Calcium intake (g/day)	889 (303–1917)	1003 (225–2396)	863 (285–1842)	NS
Vitamin D intake (μg/day)	4.0 (0.6–13.7)	3.9 (0.6–16.1)	3.5 (0.3–14.8)	NS

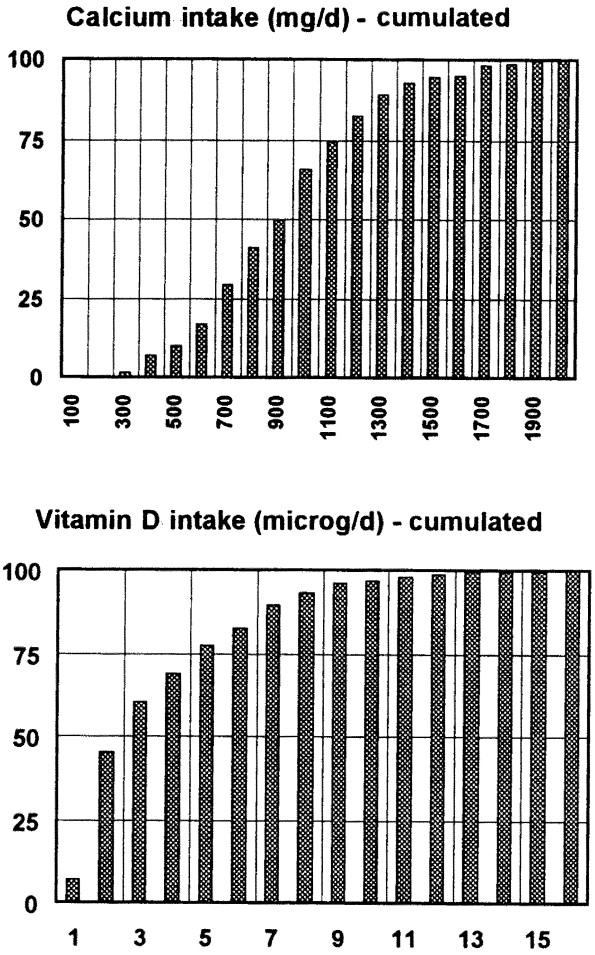

Fig. 3. Cumulated intakes of calcium and vitamin D.

with previous studies on calcium supplementation [5,7], showing a positive effect on bone mass. Previous studies [9,14] have demonstrated an effect of vitamin D on bone mass for both peri- and postmenopausal women. The effect on spinal BMD was mainly restricted to the first year. This was in accordance with the findings of Elders et al. [8]. Relative insufficiency of calcium and vitamin D will lead to an increase in bone turnover that will be reversed by supplementation. The subsequent reduction in bone turnover with a decreased activation frequency leads to a filling of the remodelling space and an increase in bone mass as seen in the first year of supplementation. Later, the simultaneous decrease in bone resorption and bone formation will be reflected in the decreased response in BMD observed during the second year of supplementation.

In our study we found no significant changes in BMD in the placebo group after 2 years. Mean dietary intake of calcium was 844 mg/day and of vitamin D 3.4 μg/day in the placebo group. In a study by Dawson-Hughes et al. [11], the group having a total intake of 950 mg calcium/day and 5 μg/day of vitamin D_3, very similar to our placebo group, experienced no net change in spinal BMD over 2 years. The dietary intakes of calcium and vitamin D_3 in the placebo group might have been too high to observe a decrease during the 2 years. In postmenopausal Danish women the mean dietary intakes of both calcium and vitamin D were found to be quite high (887 mg/day and 3.5 μg/day, respectively). The intakes of calcium and vitamin D in the placebo group were similar to the intake in the general population.

In the lumbar spine, where the composition of bone is predominantly trabecular, we saw a positive effect on BMD. In our study we found no significant effect on BMD at the hip or distal forearm – sites mainly composed of cortical bone. Due to the higher metabolic activity of trabecular bone, the effect on BMD at sites with mainly trabecular bone might therefore be more pronounced. At the median forearm, which is 85% cortical bone, Prince et al. [6] found a reduced bone loss (but still a loss) after 2 years of treatment with exercise + calcium compared with a pure exercise group (-1.3%/year vs -2.4%/year, respectively).

In the study by Dawson-Hughes et al. [11] the mean age of the study population was similar to that in our study. It was found that in healthy calcium-supplemented postmenopausal women, mean age 63.5 years, a daily intake of 5 μg vitamin D_3 was sufficient to limit bone loss from the spine but not adequate to minimize bone loss from the femoral neck. At a dosage of 20 μg/day vitamin D_3 the bone loss from the hip was reduced though still present. The findings of a larger response in the spine compared with the hip were in accordance with

our results, where an effect on BMD was significant only in the spine. In the study by Ooms et al. [10] significant increases in hip BMD were observed, but in an older study population (aged 70 years and over).

In this study calcium carbonate was used as the calcium source. Other studies [18] have indicated that this calcium salt is absorbed as well as is calcium from dairy products. As a measure of absorption, urinary calcium excretion was measured in a subgroup of subjects and found to be significantly higher in the treatment group than the placebo group.

During the 2 years of the study we saw a similar variation in serum calcium in the treatment and placebo groups. An increase was observed in the first year, followed by a decrease in the second year. The variation observed in the placebo group might reveal drift of the analysis, since samples were analyzed immediately. However, a significant difference in serum calcium between the treatment and placebo groups after both 1 year ($p < 0.0001$) and 2 years ($p < 0.0001$) was observed, reflecting the physiological response in serum calcium to calcium and vitamin D supplementation.

The reported dietary intake of calcium and vitamin D revealed an intake of calcium that was only slightly lower than the recommended daily intake: 50% of subjects received more than 900 mg calcium per day. Regarding vitamin D, 25% reached the recommended intake of 5 μg per day. However, the influence of vitamin D synthesis in the skin was not known, since serum measurements of vitamin D were not performed.

No formal assessment of compliance was performed. However, significant differences in serum calcium and urinary calcium excretion between the treatment and the placebo group do not indicate that lack of compliance has influenced the results of the study.

In conclusion, we found an increase in lumbar spine BMD of 1.6% in a group of healthy, postmenopausal women after 2 years of calcium and vitamin D supplementation. Together with relevant changes in serum calcium and serum PTH, this indicates that a long-term calcium and vitamin D supplementation of 1 g elementary calcium and 14 μg vitamin D_3 has a positive influence on bone mass, even in a population of that age who had a fairly good initial calcium and vitamin D status.

Acknowledgements. We are indebted to laboratory technicians Mrs Britta Skotte and Mrs Lena Vind, who have been largely responsible for the care of the subjects during the study and performed all clinical tests with great skill and enthusiasm.

References

1. Kanis JA, Passmore R. Calcium supplementation of the diet: not justified by present evidence. BMJ 1989;298:137–40.
2. Nordin BEC, Heaney RP. Calcium supplementation of the diet: justified by present evidence. BMJ 1990;300:1056–60.
3. Heaney RP. Effect of calcium on skeletal development, bone loss, and risk of fractures. Am J 1991;91:23–8.
4. Reid IR, Ames RW, Evans MC, et al. Long-term effects of calcium supplementation on bone loss and fractures in postmenopausal women: a randomized controlled trial. Am J Med 1995;98:331–5.
5. Dawson-Hughes B, Dallal GE, Krall EA, et al. A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. N Engl J Med 1990;323:878–83.
6. Prince RL, Smith M, Dick IM, et al. Prevention of postmenopausal osteoporosis: a comparative study of exercise, calcium supplementation and hormone-replacement therapy. N Engl J Med 1991;325:1189–95.
7. Riis B, Thomsen K, Christiansen C. Does calcium supplementation prevent postmenopausal bone loss? A double-blind, controlled clinical study. N Engl J Med 1987;316:173–7.
8. Elders P, Lips P, Netelenbos JC, et al. Long-term effect of calcium supplementation on bone loss in postmenopausal women. J Bone Miner Res 1994;9:963–70.
9. Heikinheimo RJ, Inkovaara JA, Harju EJ, et al. Annual injection of vitamin D and fractures of aged bones. Calcif Tissue Int 1992;51:105–10.
10. Ooms ME, Roos JC, Bezemer PD, et al. Prevention of bone loss by vitmain D supplementation in elderly women: a randomized double-blind trial. J Clin Exp Med 1995;80:1052–8.
11. Dawson-Hughes B, Harris SS, Krall EA, et al. Rates of bone loss in postmenopausal women randomly assigned to one of two dosages of vitamin D. Am J Clin Nutr 1995;61:1140–5.
12. Khaw K-T, Scragg R, Murphy S. Single-dose cholecalciferol suppresses the winter increase in parathyroid hormone concentrations in healthy older men and women: a randomized trial. Am J Clin Nutr 1994;59:1040–4.
13. Chapuy MC, Arlot ME, Duboeuf F, et al. Vitamin D_3 and calcium to prevent hip fractures in elderly women. N Engl J Med 1992;327:1637–42.
14. Lukert B, Higgins J, Stroskopf M. Menopausal bone loss is partially regulated by dietary intake of vitamin D. Calcif Tissue Int 1992;51:173–9.
15. NIH Consensus Conference: optimal calcium intake. JAMA 1994;272:1942–8.
16. Nordiska Näringsrekommandationer, Nordiska Ministerrådet, 1996.
17. Danskernes kostvaner 1995, Hovedresultater. Levnedsmiddelstyrelsen, publikation no. 235.
18. Mortensen L, Charles P. Bioavailability of calcium supplements and the effect of vitamin D: comparisons between milk, calcim carbonate, and calcium carbonate plus vitamin D. Am J Clin Nutr 1996;63:354–7.

Received for publication 2 July 1997
Accepted in revised form 28 October 1997