ORIGINAL ARTICLE

Effect of calcium and vitamin D supplementation on bone mineral density in women aged 65–71 years: a 3-year randomized population-based trial (OSTPRE-FPS)

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Abstract

Summary The Osteoporosis Risk Factor and Prevention-Fracture Prevention Study (OSTPRE-FPS) was a randomized population-based open trial ($n=593$). The supplementation group ($n=287$) received daily cholecalciferol 800 IU + calcium 1,000 mg for 3 years while the control group ($n=306$) received neither supplementation nor placebo. Daily vitamin D and calcium supplementation have a positive effect on the skeleton in ambulatory postmenopausal women.

Introduction Vitamin D deficiency is common in the elderly, and vitamin D levels are associated with low bone mineral density (BMD). The working hypothesis was that vitamin D and calcium supplementation could prevent bone loss in ambulatory postmenopausal women.

Methods The OSTPRE-FPS was a randomized population-based open trial with a 3-year follow-up in 3,432 women (aged 66 to 71 years). A randomly selected subsample of 593 subjects underwent BMD measurements. The supplementation group ($n=287$) received daily cholecalciferol 800 IU + calcium 1,000 mg for 3 years while the control group ($n=306$) received neither supplementation nor placebo.

Results In the intention-to-treat analysis, total body BMD ($n=362$) increased significantly more in the intervention group than in the control group (0.84% vs. 0.19%, $p=0.011$). The BMD change differences at the lumbar spine ($p=0.372$), femoral neck ($p=0.188$), trochanter ($p=0.085$), and total proximal femur ($p=0.070$) were statistically nonsignificant. Analyses in compliant women (≥80% of use) resulted in stronger and statistically significant effects at the total body and femoral regions.

Conclusion Daily vitamin D and calcium supplementation have a positive effect on the skeleton in ambulatory postmenopausal women with adequate nutritional calcium intake.

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Introduction

Serum levels of 25-hydroxyvitamin D (25[OH]D) are
directly related to bone mineral density (BMD) [1].
Calcium absorption decreases from digestive tract when
25(OH)D levels are inadequate (<75 nmol/l) [2]. Accord-
ingly, low 25(OH)D leads to bone resorption and bone loss
and thus increased risk of fracture [1, 3–5]. Vitamin D
deficiency is common in the elderly since the capacity of
the skin to synthesize provitamin decreases with age [6].
Low vitamin D status is prevalent in elderly women living
in northern latitudes [7], but this deficiency can also be
detected in young adults in northern Europe [8] and in the
housebound elderly [9]. It is believed that in order to
achieve an optimal vitamin D level of >75 nmol/l one
should consume between 800 and 1,000 IU of vitamin D
everyday [10].

In a recent meta-analysis, calcium alone and calcium
with vitamin D were associated with reduced bone loss at
the hip and spine [11]. In another study, the effect of
vitamin D and calcium was nonsignificant at the spine and
in the total body [12]. Dawson-Hughes et al. reported that
the combined supplementation had no effect on proximal
femur in a 3-year follow-up [13]. However, an increase of
femoral BMD during a shorter 18-month follow-up has also
been reported [5]. Chapuy et al. reported a lower rate of
annual bone loss at the proximal femur with combined
supplementation [14], but in a recent trial, vitamin D
treatment alone had no bone effect [15]. These controver-
sial findings could be partly explained by variations in
dosages and different intervals between dosings [16]. In
addition, different forms of vitamin D and the heterogeneity
of study populations might confound the results.

There is still no definitive answer about how vitamin D
and calcium supplementation can influence bone status,
and in particular, there are no population-based trials to
settle the issue. In this 3-year population-based Osteopo-
rosis Risk Factor and Prevention Study-Fracture Preven-
tion Study (OSTPRE-FPS) randomized trial, we
hypothesized that vitamin D and calcium supplementation
might be able to reduce bone loss in ambulatory
postmenopausal women over the age of 65. The open
population-based approach demonstrates the effect of the
intervention in the general population, in comparison with
the situation in women who simply continue their normal
diet, perhaps using nonprescription sources of vitamin D
and calcium supplementation.

Subjects and methods

Study design and participants

This study was based on the OSTPRE-FPS which
began in 2003 in Kuopio, Finland. The primary aim
of the study was to determine whether vitamin D and
calcium supplementation would be effective in prevent-
ing falls and fractures in postmenopausal women. The
Ethics Committee of Kuopio University Hospital ap-
proved the OSTPRE-FPS study in October 2001.
Written informed consent was collected from the study
participants at the baseline enquiry.

The targeted OSTPRE-FPS population (n=5,407) was
selected from the population-based OSTPRE cohort (n=
13,100) of women born in 1932–1941 [17]. The inclusion
criteria for OSTPRE-FPS were age at a minimum of
65 years at the end of November 2002, living in the
Kuopio Province at the onset of the trial, and not belonging
to the former OSTPRE bone densitometry sample. The
willingness to participate in a calcium and vitamin D trial
was enquired via a postal enquiry between August and
December 2002. The 3,432 volunteers out of 5,407 (63.5%)
were randomized into two groups of equal size without
stratification or random allocation sequence by an indepen-
dent statistician. The subjects were assigned to their groups
by postal notification according to the randomization.
OSTPRE-FPS was conducted as an open-label trial. The
power analysis was done based on incidence of fractures.
There was no a priori power analysis to calculate the size of
the subsample.

In the entire trial population, the intervention and control
groups had 1,718 and 1,714 subjects, respectively. A subsam-
ple of 750 women was randomly selected from the 3,432
women at the baseline. In the subsample, the supplementation
and control groups had both 375 subjects. The subsample
underwent a detailed measurement program including the
BMD and serum 25(OH)D measurements. The baseline
measurements took place between February 2003 and May
2004 and the follow-up measurement between January 2006
and May 2007 (mean (SD) follow-up time 2.8 (0.4) years).
Between the randomization and the actual start of the
intervention, 237 subjects withdrew, and ten were lost during
the follow-up. At the end of the trial, 306 and 287 subjects in
intervention and control groups of the subsample, respectively,
completed the follow-up (Fig. 1).

Baseline characteristics

The baseline postal questionnaire included questions about
health disorders, medication, duration of hormone therapy

Fig. 1 Flowchart of the subsample of the OSTPRE-FPS trial
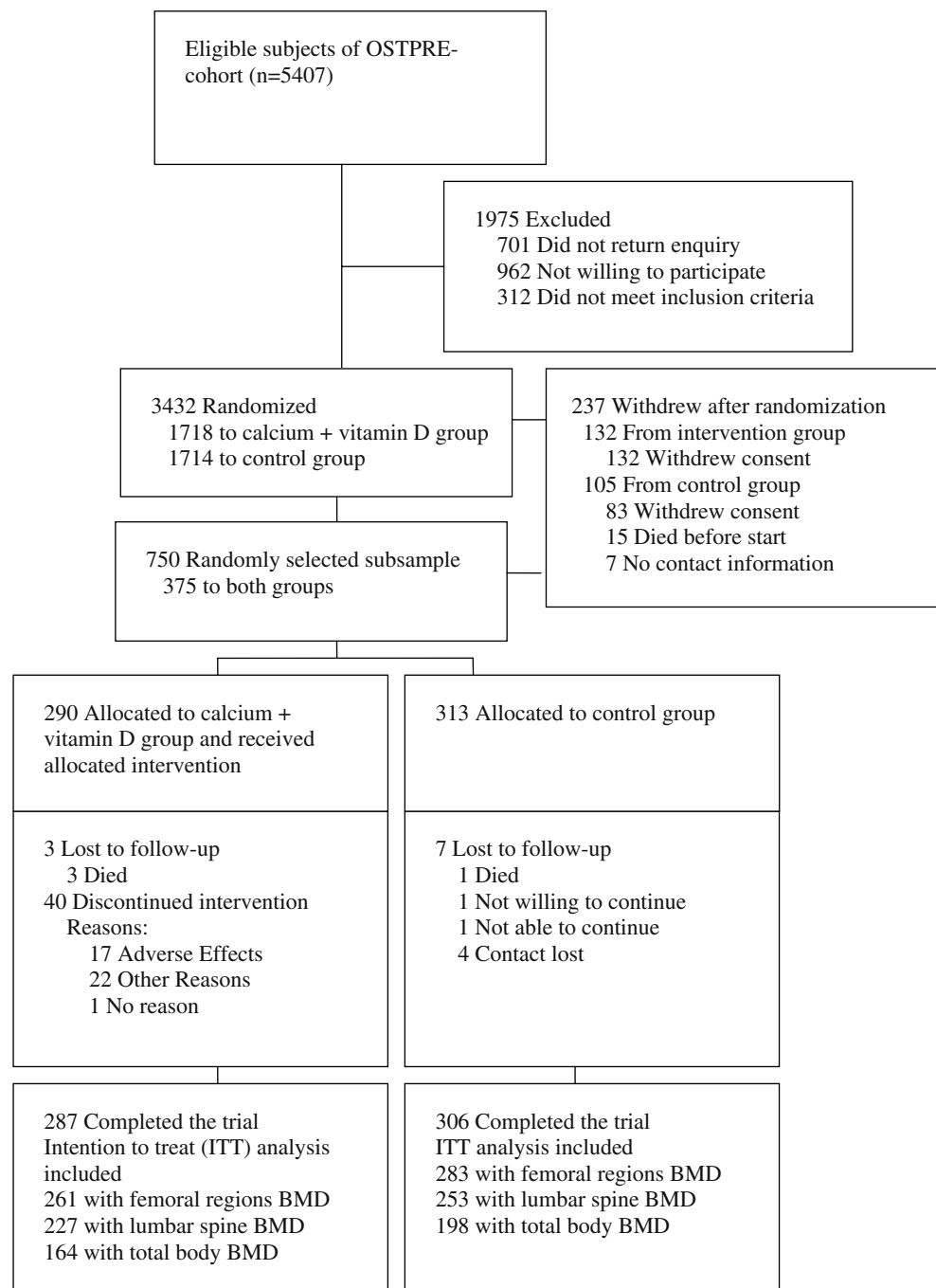
(HT; years), gynecological history, calcium questionnaire (milligram per day, the total dietary calcium was calculated as summing up calcium derived from dairy products (cheese, milk, sour milk, yogurt, sour whole milk), juices including calcium and supplements (both prescription and natural products)), physical activity (type of activity and duration in hours per week), alcohol consumption (one alcohol unit was based on calculation that one bottle of beer has been estimated to contain 12 g and one glass of wine

(12 cl) and hard liquor (4 cl) 14 g of absolute alcohol), and smoking (present status). The time since menopause (years) was calculated using the self-reported beginning of amenorrhea at baseline. In Eastern Finland, 75% of dietary calcium has been estimated to come from dairy products [18]. The self-reported calcium intake has been validated, and it showed moderate correlation against the 4-day food record [18]. The medications possibly affecting bone metabolism included corticosteroids, diuretics, cytotoxic

drugs, anticonvulsive drugs, anabolic steroids, calcitonin, bisphosphonates, and vitamin D. Weight was measured with a digital calibrated scale (Phillips, type HF 351/00) and height with a calibrated wall meter.

Intervention

The intervention group obtained daily cholecalciferol 800 IU and calcium carbonate 1,000-mg supplementation (Cal-cichew-D3 Forte, Leiras-Nycomed Ltd.) divided into two daily doses for 3 years, and the control group received no supplementation. The vitamin D and calcium was a combined formula that was chewable, and subjects took it twice a day. Both groups were asked to continue with their previous diet. The supplements were distributed via pre-scriptions which were written and supervised by one of the authors (MT), and local pharmacies arranged the distribution of the supplements. The prescriptions were written for 1-year period at the beginning of the trial. The pharmacies contacted one of the authors (MT) once a year to renew the prescriptions. The pharmacies provided the supplements for 3 months at a time for the subjects of the intervention group. Compliance was calculated as the percentage of the delivered tablets of predicted consumption during the follow-up period.

Main outcome measure

BMD measurements were performed at the lumbar spine (L2–L4), left proximal femur, and total body with dual X-ray absorptiometry (DXA; Lunar Prodigy, GE, Madison, WI, USA) twice, at the baseline and at the end of the trial. The regions of interest at the proximal femur included femoral neck, trochanter, Ward's triangle, and total proxi-mal femur. The four vertebrae between L1 and L4 ($n=395$) were used in the analyses, and whenever it was not possible (e.g., degenerative changes), we substituted it with any two or three consecutive vertebrae between L1 and L4 ($n=85$). The phantom control of the DXA instruments was run daily. The in vitro long-term reproducibility (CV) for total femur region was 0.3%. The technical quality of each DXA measurement and analysis was carefully checked, and those with measurement errors were not included in the statistical analyses.

Serum 25(OH)D measurements

Blood samples ($n=593$) were obtained in a nonfasting state at the baseline and the 3-year follow-up visits. Serum was frozen, and all 25(OH)D samples were analyzed promptly after the study closure. Serum 25(OH)D was determined by radioimmunoassay method using 25(OH)D RIA kit from DiaSorin (Stillwater, MN, USA). Total variation (CV%) for

the Diasorin method was from 8.2% to 11.0% according to the manufacturer.

Statistical analysis

Data were analyzed on an intention-to-treat basis by retaining groups according to randomization (Fig. 1; SPSS, version 14.0.1; SPSS, Chicago, IL, USA). Differences between the intervention and control groups were analyzed with independent-samples t test for normally varied and with the Mann–Whitney test for nonnormally varied variables. Pearson chi-square test was used for the univariate analyses of category variables. The change in BMD within the group was defined with paired-samples t test. The difference of change of BMD between the groups was defined with general linear repeated-measures model. The results are presented time×treatment interaction from the repeated-measures analysis of variance. The differences of vitamin D levels and change between the groups were analyzed with independent-samples t test. All analyses were unadjusted. The secondary analysis was performed in adherent women (i.e., those who took at least 80% of their supplementation). Associations between serum 25(OH)D level, total calcium intake at the baseline, and the annual BMD change were analyzed with linear regression model.

Results

The characteristics of the study population at the baseline are shown in Table 1. All differences between the intervention and control groups were nonsignificant. There were no statistically significant differences between the entire study population and the subsample (data not shown).

Main outcome results

Table 2 shows the change in BMD during the trial. Total body BMD increased significantly more in the interven-tion group than in the control group (0.84% vs. 0.19%, $p=0.011$). The BMD decrease at Ward's triangle was lower in the intervention group (-2.69% vs. -2.83%, $p=0.003$). The BMD changes at the spine ($p=0.372$), femoral neck ($p=0.188$), trochanter ($p=0.085$), and total proximal femur ($p=0.070$) were statistically nonsignificant between the groups.

Secondary outcome results

The mean compliance was 79%. The proportions for 70%, 80%, and 90% compliance were 71.8%, 63.8%, and 47.4% of the intervention group, respectively.

Table 1 Baseline characteristics of the study population (n=593)

Characteristic	Intervention group (n=287)	Control group (n=306)
Age (years)	67.4 (2.0)	67.4 (1.9)
Weight (kg)	70.3 (12.3)	69.9 (10.3)
Height (cm)	160 (5.3)	160 (5.1)
BMI	27.5 (4.5)	27.4 (3.9)
Age at menopause (years)	49.3 (5.3)	49.4 (4.6)
Years since menopause	18.1 (5.7)	18.1 (5.2)
History of HT use (%)	52.9%	56.5%
Duration of HT use (years)	10.6 (6.6)	11.4 (7.6)
Use of calcium supplements (%)	15.0%	19.0%
Calcium/milk products (mg/day)	928 (487)	888 (490)
Calcium/supplements (mg/day)	310 (179) (n=57)	319 (180) (n=72)
Calcium total (mg/day)	988 (490)	965 (489)
Number of prescribed medications	2.8 (2.6)	2.5 (2.5)
Use of bone affecting medication (%)	11.1	6.9
Alcohol units/week	0.7 (1.5)	0.8 (1.4)
Smoking status current smoker (%)	6.2%	3.6%
Amount of physical activity (hours/week)[a]	11.0 (10.8)	12.4 (12.8)

There were no statistically significant differences between the groups at the baseline

[a] Includes walking, gardening, cycling, cross-country skiing, and other more strenuous activity

The women who were adherent (i.e., those who took at least 80% of their supplementation) showed significantly lower bone loss in femoral neck (−1.26% vs. −1.73%, p=0.002), Ward's triangle (−1.63% vs. −2.83%, p<0.0001), trochanter (0.25% vs. −0.88%, p=0.001), and total proximal femur (−0.84% vs. −1.47%, p<0.0001) than in the control group. Further, total body BMD increased more in the intervention group (+1.31% vs. +0.19%, p=0.002). In contrast, lumbar spine bone loss was greater in the intervention group than in the control group (+0.67% vs. +0.76%, p=0.033; Table 3).

Baseline serum 25(OH)D associated with Ward's triangle annual BMD change (p=0.033), but it did not associate with trochanter (p=0.726), total hip (p=0.845), proximal femur (p=0.099), lumbar spine (p=0.060), or total body (p=0.343) annual BMD change.

The total intake of calcium at the baseline did not predict annual BMD change at Ward's triangle (p=0.497), trochanter (p=0.665), total hip (p=0.720), proximal femur (p=0.339), lumbar spine (p=0.729), or total body (p=0.668).

Table 2 Change in BMD in the intervention and control groups during the 3-year trial (general linear repeated-measures model)

Measured site	Baseline BMD (g/cm² (SD))	3-year BMD (g/cm² (SD))	Percentage change	Difference between the groups, p value
Total body				
Intervention	1.069 (0.09) (n=195)	1.078 (0.10) (n=200)	0.84%	0.011
Control	1.079 (0.09) (n=238)	1.081 (0.10) (n=230)	0.19%	
Femoral neck				
Intervention	0.866 (0.13) (n=280)	0.848 (0.13) (n=264)	−2.08%	0.188
Control	0.865 (0.12) (n=311)	0.850 (0.12) (n=284)	−1.73%	
Trochanter				
Intervention	0.783 (0.14) (n=280)	0.779 (0.13) (n=264)	−0.51%	0.085
Control	0.797 (0.13) (n=310)	0.790 (0.13) (n=284)	−0.88%	
Ward's triangle				
Intervention	0.670 (0.15) (n=280)	0.652 (0.14) (n=264)	−2.69%	0.003
Control	0.672 (0.13) (n=310)	0.653 (0.13) (n=284)	−2.83%	
Total proximal femur				
Intervention	0.948 (0.14) (n=280)	0.934 (0.14) (n=264)	−1.48%	0.070
Control	0.953 (0.13) (n=310)	0.939 (0.13) (n=283)	−1.47%	
Lumbar spine				
Intervention	1.039 (0.17) (n=259)	1.047 (0.17) (n=230)	0.77%	0.372
Control	1.052 (0.17) (n=285)	1.060 (0.17) (n=253)	0.76%	

Table 3 Change in BMD in the intervention with the adherent subjects (those who took at least 80% of their supplementation) and in the control group during the 3-year trial (general linear repeated-measures model)

Measured site	Baseline BMD (g/cm² (SD))	3-year BMD (g/cm² (SD))	Percentage change	Difference between the groups, p value
Total body				
Intervention	1.071 (0.09) (n=119)	1.085 (0.10) (n=129)	1.31%	0.002
Control	1.079 (0.09) (n=238)	1.081 (0.10) (n=230)	0.19%	
Femoral neck				
Intervention	0.873 (0.13) (n=176)	0.862 (0.13) (n=171)	−1.26%	0.002
Control	0.865 (0.12) (n=311)	0.850 (0.12) (n=284)	−1.73%	
Trochanter				
Intervention	0.792 (0.13) (n=176)	0.794 (0.13) (n=171)	0.25%	0.001
Control	0.797 (0.13) (n=310)	0.790 (0.13) (n=284)	−0.88%	
Ward's triangle				
Intervention	0.675 (0.15) (n=176)	0.664 (0.14) (n=171)	−1.63%	<0.0001
Control	0.672 (0.13) (n=310)	0.653 (0.13) (n=284)	−2.83%	
Total proximal femur				
Intervention	0.955 (0.14) (n=176)	0.947 (0.14) (n=171)	−0.84%	<0.0001
Control	0.953 (0.13) (n=310)	0.939 (0.13) (n=283)	−1.47%	
Lumbar spine				
Intervention	1.050 (0.16) (n=162)	1.057 (0.16) (n=147)	0.67%	0.033
Control	1.052 (0.17) (n=285)	1.060 (0.17) (n=253)	0.76%	

Serum 25(OH)D level

The mean (SD) baseline serum 25(OH)D levels were 50.1 (18.8) nmol/l and 49.2 (17.7) nmol/l in the intervention and control groups (p=0.544), respectively. The absolute changes in 25(OH)D levels were 24.7 (24.1) nmol/l and 6.8 (19.3) nmol/l in the intervention and control groups (p< 0.001), respectively. At the end of the trial, the mean levels were 74.6 (21.9) nmol/l and 55.9 (21.8) nmol/l (p<0.001), respectively.

Adverse effects of the supplementation

In total, 40 out of 290 women discontinued the intervention: 17 due to adverse effects, 22 due to other reasons, and one due to no specific reason. The adverse effects responsible for discontinuation were gastrointestinal symptoms (nine), exacerbation of diseases (two), and mouth irritation (one), skin symptoms (one), nausea (one), cough (one), backache (one), and weight increase (one).

Discussion

The daily cholecalciferol 800 IU and calcium carbonate 1,000-mg supplementation increased the total body BMD in women aged 66 to 71 years in this randomized 3-year population-based trial. However, no site-specific changes at the femoral region or at the lumbar spine were detected in the main analyses. In further analyses, the

intervention effect appeared to be greater in total body and femoral regions among women who adhered to the supplementation.

It has been reported earlier that there is a progress of about 1% annual bone loss in the femoral neck in the elderly [19]. Furthermore, inadequate intakes of vitamin D and calcium lead to reduced calcium absorption, higher bone remodeling rates, and increased bone loss [3, 14]. The combination of these factors increases the risk of osteoporosis and consequently the risk for osteoporotic fractures. There is evidence to suggest that adequate sunlight exposure and the use of supplements are needed to meet human vitamin D needs [20]. Vitamin D supplementation has been shown to have long-term effects on the skeleton as a whole [13]. Similarly, the positive effect on total body BMD that was seen in this study may reflect the general skeletal effect. However, we cannot exclude the possibility that total body and spinal BMD increase could be confounded because of age-related degenerative changes, such as osteoarthritis and aortic calcification [21, 22]. On the other hand, vitamin D and calcium supplementation may have stronger effect on cortical bone [23]. Consequently, the positive effect seen in the total body BMD might be explained by higher proportion of cortical bone in the skeleton.

In a large RCT trial, 1,000 mg daily calcium alone showed 1.6% smaller bone loss in hip over 5-year follow-up in elderly women [24]. The relatively small changes detected in BMD between the supplemented and control groups in the present and earlier studies raise the question

of the clinical relevance of the positive results. At the population level, the supplementation would not be sufficient to prevent osteoporosis, but it could at least decelerate the age-related bone loss to some extent. Additional nonpharmacological and pharmacological interventions should be considered, especially in subjects at a high risk of suffering osteoporotic fractures [25]. Dawson-Hughes et al. have reported that 1.1% net increase in total body BMD during the 3-year vitamin D (700 IU) and calcium (500 mg) trial was associated with relative risk of 0.4 of first osteoporotic fracture [13]. Accordingly, in our study, BMD increase of 0.9% but with slightly greater dose of vitamin D (800 IU) and calcium (1,000 mg) might point out for similar relative risk reduction.

Daily oral dosing with vitamin D seems to be a more effective way of administration than weekly or monthly ones [16]. However, recent study demonstrated the effect of large vitamin D loading and intermittent dosages to normalize serum levels [26]. Furthermore, vitamin D supplementation is effective in preventing the seasonal-related vitamin D level decrease and subsequent bone loss [27, 28]. In fact, vitamin D sufficiency might be more important than high calcium intake to maintain desirable serum parathyroid hormone levels [29]. BMD improvements in the vitamin D and calcium-supplemented subjects are largely lost if the supplementation is discontinued [30]. This is in agreement with the present study where women who were adherent had the most benefit from the supplementation. These findings argue in favor of the importance of regularity in the supplementation. Further, vitamin D supplementation supports other therapies, since optimal vitamin D repletion is necessary for achieving the maximal benefits from antiresorptive pharmacological therapies [31].

There is evidence of an age-related intestinal resistance to the actions of 1,25-dihydroxyvitamin D [32]. Accordingly, this has led to the question about the sufficiency of daily 1,000-mg calcium intake even in the old age. It is known that calcium absorption increases with higher vitamin D levels [2], and accordingly optimal calcium intake in people with adequate vitamin D status might be lower [33]. Nonetheless, it has been suggested that the vitamin D requirement is not greatly dependent on calcium intake [34], and a daily intake of 1,200 mg calcium in the general population should cover calcium needs [11, 34]. In the present study, the mean daily intake of calcium was 980 mg at baseline, which is nearly at the current Finnish recommendation (1,000 mg/day). Thus, the high nutritional calcium intake might have suppressed the positive effect of the supplementation. Further, recent findings suggest that calcium supplementation is associated with adverse cardiovascular events [35, 36]. Thus, the recommendation for supplying additional calcium for those subjects with a nutritional supply of over 1,000 mg needs to be evaluated

in further studies. More studies are needed to study whether daily vitamin D confer additional health benefits.

Mean serum 25(OH)D levels were 50 nmol/l, which suggests that subjects were slightly deficient in terms of vitamin D [20]. In fact, it has recently been reported that only women with 25(OH)D levels below 50 nmol/l benefit from a higher calcium intake [37]. Therefore, we suggest that the positive effect of the supplementation might be attributable to greater extent to the adequate vitamin D intake than calcium intake. This is further supported by the earlier finding that BMD increases with higher 25(OH)D concentrations [37]. Since February 2003, following the recommendation by the Finnish Ministry of Social Affairs and Health, fluid milks (0.5 mg/100 ml), margarines, and spreads (10 mg/100 g) have been fortified by vitamin D in Finland. Indeed, this action in general has improved the mean vitamin D intake and status in Finland, but low 25(OH)D levels still occur [38].

The strengths of this study were its population-based design and the long follow-up. However, as only two thirds of individuals volunteered to participate (3,432 out of 5,407), the population-based benefit was diminished. The subsample of 593 women was randomly selected from those 3,432 subjects, and therefore it is a representative cohort. One limitation was that the compliance with the intended intervention was not complete (79% of the prescribed calcium–vitamin D tablets that were dispensed from the pharmacies). However, the present compliance was quite similar as in the previous study [12]. Further, the compliance was estimated based on prescriptions and not on exact count of tablets consumed. Since vitamin D and calcium supplementations are available over the counter and are routinely included in standard multivitamins, the subjects in the control group might well have increased their use of calcium and vitamin D supplementation. In fact, 3.8% of control subjects reported using prescribed vitamin D with or without calcium supplements at baseline, but this has risen to 16.1% by the end of the trial (data not shown). However, serum 25(OH)D levels increased significantly more in the intervention group (24.7 nmol/l) than in the control group (6.8 nmol/l). This confirms that women in the intervention group had used the study supplementation. The open population-based approach demonstrates the effect of the intervention in the general population, in comparison with the situation in women who simply continue their normal diet perhaps using nonprescription sources of vitamin D and calcium supplementation.

In conclusion, combined daily cholecalciferol 800 IU and calcium carbonate 1,000 mg has a positive effect on the skeleton in ambulatory postmenopausal women with adequate mean nutritional calcium intake. Whether there is effect without calcium supplementation needs to be confirmed in future studies.

Authors' contributions Author M.K. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Authors M.T., R.H., E.A., and H.K. designed the study and directed its implication. Authors M.K., K.S., L.S., R.H., and H.K. analyzed and interpreted the data. Author K.S. helped to prepare the materials and methods section. Author M.K. drafted the manuscript. Authors M.T., K.S., T.R., J.S., R.H., J.A., E.A., and H.K. made critical revision of the manuscript for the important intellectual content.

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