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Prevention of Femoral and Lumbar Bone Loss with Hormone Replacement Therapy and Vitamin D_3 in Early Postmenopausal Women: A Population-Based 5-Year Randomized Trial*

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ABSTRACT

The long term effects of hormone replacement therapy (HRT) and vitamin D_3 (Vit D) on bone mineral density (BMD) were studied. A total of 464 nonosteoporotic early postmenopausal women from the Kuopio Osteoporosis Study (n = 13100) were randomized to four groups: 1) HRT (sequential combination of 2 mg estradiol valerate and 1 mg cyproterone acetate, 2) Vit D_3 (300 and 100 IU/day during the fifth year), 3) HRT and Vit D combined, and 4) placebo. Lumbar (L2–L4) and femoral neck BMD were determined by dual x-ray absorptiometry (DXA) at baseline and after 2.5 and 5 yr of treatment.

Intention to treat analysis (n = 464) showed that after 5 yr, lumbar BMD remained unchanged in the HRT and HRT plus Vit D groups [+0.2% ($P = 0.658$) and +0.9% ($P = 0.117$), respectively], whereas lumbar BMD decreased by 4.6% in the Vit D group and by 4.5% in the placebo group ($P < 0.001$ in both). The loss of femoral neck BMD was less in the HRT (−1.4%; $P = 0.005$) and HRT plus Vit D (−1.3%; $P = 0.003$) groups than in the Vit D and placebo groups (−4.3%; $P < 0.001$ in both). Among those 370 women who complied with the 5-yr treatment, the effect was more pronounced: lumbar BMD had increased by 1.5% in the HRT ($P = 0.009$) and by 1.8% in the HRT plus Vit D group ($P = 0.005$), with a plateau after 2.5 yr, whereas lumbar BMD had decreased in both the Vit D and placebo groups (4.6% and 4.7%; $P < 0.001$, respectively). Femoral neck BMD decreased again less in the HRT (−0.4%) and HRT plus Vit D (−0.6%) groups than in the Vit D and placebo groups (−4.4% in both).

This study confirms the positive long term effect of HRT on BMD also seen in intention to treat analysis. The data suggest that low dose vitamin D_3 supplementation does not prevent bone loss in healthy, nonosteoporotic, early postmenopausal women, and it confers no benefit additional to that of HRT alone. (*J Clin Endocrinol Metab* **84:** 546–552, 1999)

ESTROGEN deficiency imposes postmenopausal women to an increased risk of osteoporosis and osteoporotic fractures. Hormone replacement therapy (HRT) with or without progestin is generally considered to be an effective method for the prevention and treatment of bone loss after menopause (1). Progestins have been added to the therapy to eliminate the risk of endometrial hyperplasia and cancer associated with the unopposed estrogen treatment. 17-Hydroxyprogesterones such as cyproterone acetate (CPA) have been considered to be more favorable progestins in HRT than androgenic 19-nortestosterone as far as lipid changes are concerned (2). However, more prospective data of the effects of prolonged administration of HRT on bone are needed, and the effect of CPA in HRT combination has been studied previously in our 2.5-yr trial for nonosteoporotic (3) and our 4-yr trial for osteoporotic women (4).

Vitamin D (Vit D) supplementation may reduce bone loss (5–7) and decrease the risk of fractures among elderly women (7), although the positive effect on fracture risk has not been shown in all studies (8). Vitamin D supplementation has been recommended for prevention of osteoporosis in the elderly, especially among institutionalized people deficient in Vit D. However, the effect of Vit D supplementation on prevention of bone loss in nonosteoporotic early postmenopausal women has not yet been well established. We have previously reported a minor effect of low dose Vit D_3 supplementation in the prevention of bone loss among nonosteoporotic postmenopausal women in a 2.5-yr trial (3). There are no longer prospective studies in this age group.

The purpose of this randomized trial was to examine the long term effects of a sequential estrogen-progestin combination therapy (estradiol valerate and CPA) and low dose vitamin D_3 supplementation on bone mineral density (BMD) in nonosteoporotic, early postmenopausal women and to determine whether Vit D_3 supplementation can give additional benefit to HRT.

Subjects and Methods

Subjects

The study population was a subsample of the Kuopio Osteoporosis Risk Factor and Prevention Study, which was started in 1989 with a

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postal inquiry sent to all 47- to 56-yr-old women (n = 14,220) of Kuopio Province, Eastern Finland. A total of 13,100 women (92.8%) responded to the questionnaire. In 1990–1991, BMD was measured in 3,220 women, who were a random stratified sample of those willing to undergo bone densitometry. Interest in a 5-yr clinical trial was ascertained, and all 464 willing, recently postmenopausal women without contraindications to HRT were recruited for a 5-yr clinical trial with factorial design. Written informed consent was obtained from the participants, and the trial was approved by the ethics committee of Kuopio University Hospital.

All women recruited were postmenopausal according to the criterion that 6–24 months had elapsed since their last menstruation. Contraindications for the clinical trial were history of breast or endometrial cancer, thromboembolic diseases, and medication-resistant hypertension. However, women taking other medication were accepted (Table 1). In the four treatment groups, there were no significant differences in the frequency of diseases or drugs possibly affecting BMD.

The women were randomized to four treatment groups: A) HRT group: sequential combination of 2 mg estradiol valerate (E_2Val; days 1–21) and 1 mg CPA (days 12–21) and a treatment-free interval (days 22–28; Climen, Schering AG, Germany); B) Vit D group: Vit D_3 (300 IU cholecalciferol) plus 93 mg Ca^{2+}/day, no intake during June-August (D-Calsor, Orion Ltd., Finland), the Vit D_3 dosage was lowered to 100 IU/day after 4 yr of treatment because of adverse lipid changes noticed during the first years of the trial (9); C) HRT + Vit D group: treatments A and B combined; and D) placebo group: calcium lactate (500 mg/day, equivalent to 93 mg Ca^{2+}/day; Calcium Lactate, Rohto Ltd., Finland).

Those women who wanted to change treatment groups were excluded from the study. The personnel involved were unaware of the group allocations until the initiation of the treatments. However, the group allocation was masked in data analysis.

The power of the clinical trial to demonstrate the effect of treatments was calculated with the assumption that HRT prevents bone loss totally (100%), vitamin D prevents bone loss by 25%, and risk factors modify the hormone effect by 33%. The analyses were computed using the means and variances obtained from a cross-sectional study of a healthy female population (10) with the one-sided test of two independent samples based on normal distribution.

Of the 464 women enrolled in the study, 435 (94%) eligible women completed it. Among the 29 drop-outs were 20 women who could not be contacted in the end of the study and 3 who died from unrelated causes during the study period. In addition, 6 osteoporotic women were withdrawn from the study after enrollment when subject eligibility data were available (baseline lumbar or femoral BMD above −2 SD of the mean of the whole study population).

TABLE 1. Diseases and medications potentially affecting BMD of the 458 postmenopausal women at baseline by treatment group

	HRT (n = 115)	Vit D (n = 112)	HRT + Vit D (n = 116)	Placebo (n = 115)
Diseases				
Lactose intolerance	12	4	6	5
Asthma bronchialis	6	4	3	6
Hypothyroidism	4	5	7	4
Hyperthyroidism	1	4	5	2
Rheumatoid arthritis	7	5	2	1
Diabetes (type I and II)	0	2	2	3
Hyperparathyroidism	0	2	0	0
Total	30	26	25	21
Medication				
T_4	5	7	7	4
Thyrostatic drugs	0	1	1	0
Glucocorticoids	5	5	5	6
Anticonvulsants	0	1	0	2
Tiazide	1	2	3	1
Loop diuretics	3	2	1	2
Total	14	18	17	15

None of the diseases or medications, individually or combined, differed significantly between groups. HRT, E_2Val/CPA.

Methods

Each subject underwent a gynecological examination at baseline and each completed year during the 5-yr follow-up. Fasting venous blood samples were taken concurrently. Body height and weight were measured at the time of each BMD measurement. After discontinuation of the study medication the noncompliant subjects received only the 5-yr close-out examination. The 2.5 yr BMD measurement was not performed if the medication was discontinued before that point.

Lumbar (L1–L4) and left proximal femur BMD were measured using DXA (Lunar Corp., Madison, WI) at baseline and after 2.5 and 5 yr of treatment, at Kuopio University Hospital by trained personnel. The short term reproducibilities [coefficient of variation (CV), percentage] of the spine and femoral neck measurements were 0.9% and 1.5%, respectively (10). The long term reproducibility (CV) of our DXA instrument for the entire study period based on regular phantom measurements was 0.4% (n = 60).

To ensure that bone edges and intervertebral markers were set consistently in the spine, all analyses were reviewed by one investigator. Primarily, the BMD of vertebrae L2-L4 were used for the analyses. However, if there were marked changes (arthrosis, suspicion of a fracture, scoliosis, operations, *etc.*), L1–L3 (n = 15), L1–L2 (n = 7), L2–L3 (n = 26), or L3–L4 (n = 1) were used instead. In the follow-up, the BMDs of the same vertebrae were reported. Altogether 11 lumbar measurements were excluded [arthrosis (n = 6), scoliosis (n = 2), operation (n = 1), and unrepeatable measurements (n = 2)]. In addition, 7 femoral measurements were disqualified because of previous hip operation (n = 3), poor quality scan (n = 3), and severe hip arthrosis (n = 1). From the 2.5 and 5 yr data, 1 additional femoral measurement was excluded from each time point because of poor quality scans. Of 464 subjects, 447 lumbar BMD and 451 femoral neck BMD measurements were accepted in the final analysis at baseline.

Dietary habits and other life-style factors were determined by the baseline postal inquiry. The estimate for daily dietary calcium intake was based on the consumption of milk products and was calculated as the sum of calcium intake from milk, sour milk, yogurt (120 mg/dL), and cheese (87 mg/slice). The monthly number of meals containing fish as the main course was registered. Weekly hours of physical activity were noted, and if they exceeded 3 h, the subject was considered physically active. The duration of regular smoking and the number of cigarettes consumed currently per day were registered. Lifetime smoking was presented in pack-years (lifetime number of cigarettes/20 × 365). Alcohol consumption was registered as absolute ethanol intake. No attempt was made to alter the women's diet or activity level.

Serum concentrations of estradiol (E_2) were measured using RIAs (Sorin Biomedica, Milan, Italy; interassay CV, <8.5%), and serum levels of FSH were determined by luminescence immunoassay (Byk-Sangtec, Germany; interassay CV, <5.6%). All assays were carried out at Department of Clinical Chemistry, Kuopio University Hospital. The serum concentrations of intact PTH and vitamin D metabolites (25-hydroxyvitamin D and 1,25-dihydroxyvitamin D) were measured from 18 women from each group; the results of the substudy were previously reported (11).

Statistical analysis

The data were analyzed with valid BMD measurements in two ways, first by the intention to treat analysis including all randomized eligible participants and second by the valid case analysis, which was restricted to the women who complied with the trial medication.

The frequencies of certain diseases and medications in the groups were compared using χ^2 test (exact test). Student's *t* test for independent samples was used for comparing baseline BMDs between drop-outs and the subjects who completed the study. One-way ANOVA or the nonparametric Kruskal-Wallis test was used to analyze differences in the baseline characteristics. Longitudinal BMD changes were analyzed by repeated measures ANOVA (MANOVA). The changes in BMD were analyzed using paired differences, *e.g.* individual changes in BMD. Student's *t* test for paired observations was used to test for the significance of differences within a group if there was a time-related change within the group by MANOVA.

Secondly, the data of the women who complied with the therapy, were analyzed analogously, as mentioned above (valid case analysis). In

addition, one-way ANOVA was used to test the significance of differences between the BMD changes achieved during the 2.5 and 5 yr of treatment.

Statistical analysis was performed using SPSS for Windows and StatXact statistical packages. The results are reported as means with 95% confidence intervals and are regarded as statistically significant if $P < 0.05$. The 2.5 yr BMD changes in the compliant women (valid case analysis) were reported previously (3).

Results

Of the 458 eligible women, 23 could not be met at the close-out visit. In all, 370 (80%) complied with the 5-yr treatment. Compliance was considered adequate if the participants reported that they had used the medication 80% or more of the planned trial period (at least 4 yr of treatment). Most of noncompliers were from the HRT (n = 42) and HRT + Vit D groups (n = 28). The most common reasons for noncompliance were menstrual disorders such as hypermenorrhea, dysmenorrhea, or metrorrhagia (n = 19) and headache (n = 14). After discontinuation of the study medication, 17 women initiated another HRT preparation (HRT group, n = 7; Vit D group, n = 4; HRT + Vit D group, n = 2; placebo group, n = 4). One woman began to use intranasal calcitonin (placebo group). The serious adverse events during the treatments were evenly distributed in the 4 treatment groups: HRT group, 4 malignancies (endometrium, ovary, rectum,* and breast), 1 myocardial infarction, and 1 cerebral infarction; Vit D group, 2 malignancies (endometrium and cervix), 1 endometrial hyperplasia, 1 myocardial infarction, and 1 coronary by-pass operation; HRT + Vit D groups, 1 breast cancer, 1 venous thrombosis in a leg, 1 myocardial* and 2 cerebral infarctions, and 1 coronary by-pass operation; and placebo group, 3 malignancies (breast, ventricle, and melanoma*) and 1 endometrial hyperplasia. Three women died during the trial (marked with an *asterisk*).

The baseline characteristics and the laboratory data for the 458 women are shown in Table 2. Potential confounding variables were similarly distributed among the four treatment groups at baseline. Body weight and body mass index did not change significantly during the study in any group. There were more treatment-free months in the HRT than in the non-HRT groups, but there were no differences in the medication-free periods between the HRT and the HRT + Vit D groups.

Table 3 shows the lumbar and femoral neck BMD values at baseline and the means of individual BMD changes after 5 yr of treatment for all eligible women in the study (intention to treat principle). There were no significant differences in lumbar or femoral neck BMDs at baseline. After 5 yr of treatment, lumbar BMD slightly increased in the HRT group (+0.2%; $P = 0.658$) and the HRT + Vit D group (+0.9%; $P = 0.117$). In contrast, lumbar BMD decreased by 4.6% in the Vit D group ($P < 0.001$) and by 4.5% in the placebo group ($P < 0.001$). The relative increases were significant in both the HRT and HRT + Vit D groups compared with those in the placebo (4.7% and 5.4%, respectively) and Vit D (4.8% and 5.5%, respectively) groups. These time-related changes were statistically significant between the groups ($P < 0.001$, by MANOVA).

Femoral BMD decreased significantly in all treatment groups (intention to treat analysis). However, the decrease was significantly less in the HRT group (−1.4%; $P = 0.005$) and the HRT + Vit D group (−1.3%; $P = 0.003$) than in the Vit D and placebo groups (−4.3% in both groups; $P < 0.001$). Again, the BMD changes showed statistically significant difference between the groups ($P < 0.001$, by MANOVA). The baseline lumbar or femoral neck BMDs did not differ in any treatment group between those who completed the trial and the 23 women who could not come to the close-out visit.

Figure 1 illustrates the BMD changes in the valid case analysis, which consisted of data from all of the women who complied with the study treatment during at least 80% of the trial (over 4 yr of therapy). The baseline BMDs in these analyses were similar to those shown in the intention to treat analysis (Table 3), and there were no significant BMD differences between the groups at baseline ($P = 0.784$ and $P = 0.299$, respectively). The time-related changes in both lumbar and femoral neck BMDs were statistically significant between the groups ($P < 0.001$ in both, by MANOVA).

At the spine, the time-related change was significant in all treatment groups (HRT, $P = 0.004$; Vit D, $P < 0.001$; HRT + Vit D, $P = 0.003$; placebo, $P < 0.001$; by MANOVA). Lumbar

TABLE 2. Baseline characteristics and laboratory measures of the 458 nonosteoporotic postmenopausal women by treatment group

	HRT (n = 115)	Vit D (n = 112)	HRT + Vit D (n = 116)	Placebo (n = 115)	*P* value[a]
Age (yr)	52.9 (52.5–53.3)	52.9 (52.4–53.3)	52.5 (52.1–53.0)	52.6 (52.2–53.0)	0.608
Time since menopause (yr)	1.1 (1.0–1.2)	1.1 (1.0–1.2)	1.1 (1.0–1.2)	1.1 (1.0–1.2)	0.989
Wt (kg)	70.5 (68.2–72.9)	70.7 (68.7–72.8)	70.0 (67.8–72.2)	67.9 (65.9–69.9)	0.206
Ht (cm)	162 (160.8–162.7)	161 (160.5–162.4)	162 (160.5–162.5)	160 (159.4–161.2)	0.077
BMI (kg/m^2)	26.9 (26.1–27.7)	27.1 (26.4–27.9)	26.8 (26.0–27.6)	26.5 (25.7–27.2)	0.593
Smoking (pack-yr)[b]	9.6 (5.8–13.3)	6.0 (3.6–8.3)	8.5 (5.2–11.8)	8.3 (5.3–11.3)	0.571
Physically active persons (%)[c]	36 (31)	39 (35)	42 (36)	46 (40)	0.680[d]
Dietary Ca intake (mg/day)	785 (715–854)	827 (750–903)	871 (785–957)	843 (765–921)	0.707
Alcohol (absolute ethanol g/week)	25.0 (9.3–40.8)	19.5 (12.3–26.7)	16.2 (8.4–24.0)	22.5 (15.3–29.7)	0.566
Dietary fish (meals/month)	5.4 (4.5–6.3)	6.0 (5.1–6.8)	5.1 (4.4–5.9)	5.0 (4.2–5.8)	0.181
Previous HRT use (yr)	0.8 (0.5–1.1)	0.6 (0.3–0.8)	0.6 (0.3–0.9)	0.4 (0.2–0.5)	0.627
FSH (IU/L)	60.2 (55.3–65.1)	59.1 (54.6–63.6)	60.4 (55.2–65.6)	62.4 (57.4–67.4)	0.783
E_2 (nmol/L)	0.15 (0.11–0.18)	0.14 (0.10–0.17)	0.14 (0.12–0.17)	0.18 (0.10–0.25)	0.698

Values are given as the mean, with the 95% confidence interval in *parentheses*. HRT, E_2Val/CPA.

[a] Kruskal-Wallis test.

[b] Smoking = life-time number of cigarettes/20 × 365.

[c] Physically active person = 3 h or more of physical activity/week.

[d] By χ^2 test.

TABLE 3a. Lumbar spine BMD according to study group at baseline and the BMD changes (percentage) after 5-yr treatment among the 458 women (intention to treat analysis)

Study group	n	At baseline (g/cm^2)	BMD change in 5 yr (%)	*P* Value[a]
HRT	112	1.135 (1.107–1.162)	+0.2 (−0.8 to 1.3)	0.658
Vitamin D	110	1.147 (1.120–1.174)	−4.6 (−5.5 to −3.6)	<0.001
HRT + vitamin D	111	1.153 (1.123–1.183)	+0.9 (−0.2 to 2.1)	0.117
Placebo	114	1.154 (1.126–1.182)	−4.5 (−5.4 to −3.6)	<0.001

Data available from 447 women. HRT, E_2 Val/CPA.
[a] By paired *t* test.

TABLE 3b. Femoral neck BMD according to study group at baseline and the BMD changes (percentage) after 5-yr treatment among the 458 women (intention to treat analysis)

Study group	n	At baseline (g/cm^2)	BMD change in 5 yr (%)	*P* value[a]
HRT	114	0.940 (0.917–0.963)	−1.4 (−2.4 to −0.5)	0.005
Vitamin D	108	0.937 (0.916–0.958)	−4.3 (−5.3 to −3.4)	<0.001
HRT + vitamin D	115	0.942 (0.921 (0.963)	−1.3 (−2.3 to −0.4)	0.003
Placebo	114	0.955 (0.935–0.975)	−4.3 (−5.2 to −3.4)	<0.001

Data available from 451 women. HRT, E_2 Val/CPA.
[a] By paired *t* test.

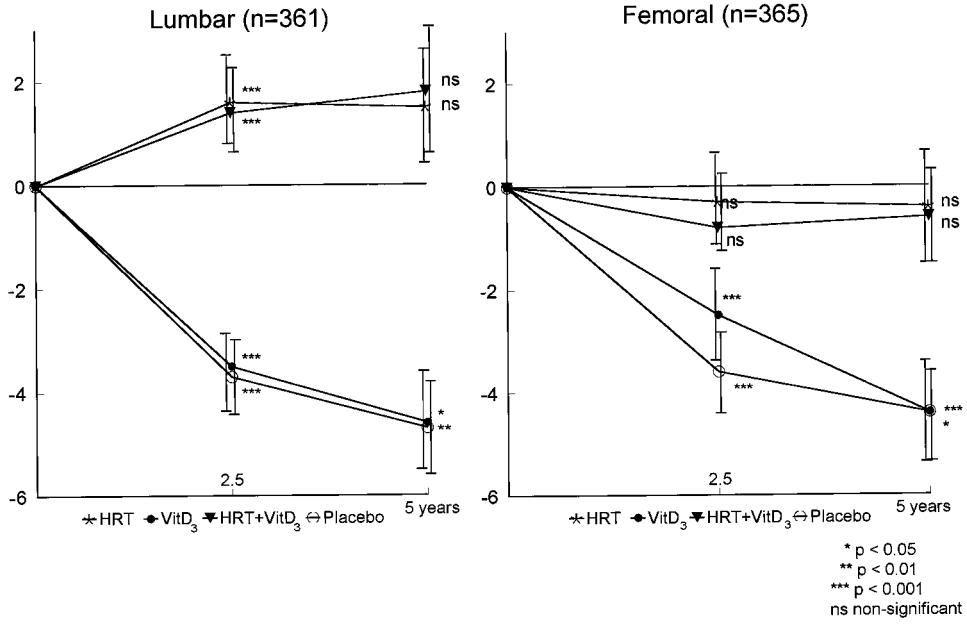

FIG. 1. Mean changes (percentages) in lumbar spine and femoral neck BMDs after 2.5 and 5 yr of treatment in the four study groups. The *P* values indicate the significance between 0–2.5 yr and 2.5–5 yr data (valid-case analysis, Student's *t* test for paired observations with 95% confidence intervals; HRT, n = 74; Vit D, n = 101; HRT + Vit D, n = 88; placebo, n = 104).

BMD increased significantly in the HRT group (+1.6%; $P = 0.001$) during the first 2.5 yr of treatment and remained constant thereafter (−0.1% BMD change during the last 2.5 yr; $P = 0.982$). The BMD changes in the HRT + Vit D group were similar [+1.4% increase after 2.5 yr of treatment ($P = 0.001$) and a plateau during the next 2.5 yr—an additional increase of 0.4% ($P = 0.395$)]. There were no differences in the lumbar BMDs between the HRT and HRT + Vit D groups. In contrast, BMD decreased significantly in both the Vit D and placebo groups (−3.5% and −3.7%, respectively) during the first 2.5 yr of treatment ($P < 0.001$ in both). The bone loss decreased in both the Vit D and placebo groups during the next 2.5 yr (−1.1%/2.5 yr for both), but was still significant ($P = 0.016$ and $P = 0.008$, respectively). The relative increases were significant in both the HRT and HRT + Vit D groups compared with those in the Vit D (6.1% and 6.4%, respectively) and placebo groups (6.2% and 6.5%, respectively) during the 5-yr treatment.

Accordingly, in the valid case analysis, femoral neck BMDs decreased significantly in the Vit D and placebo groups during the 5-yr trial ($P < 0.001$, by MANOVA). The decrease was constant in the Vit D group [−2.5%/first 2.5 yr ($P < 0.001$) and −1.9% during the next 2.5 yr ($P < 0.001$)]. The decrease in the placebo group was greater during the first half (−3.6%; $P < 0.001$) than during the second half (−0.8%; $P = 0.018$) of the study. The tendencies for decreases in the HRT group (−0.4%/5 yr) and HRT + Vit D group (−0.6%/5 yr) were not significant (by MANOVA). However, the BMD changes in the HRT and HRT + Vit D groups differed significantly compared with those in the Vit D and placebo groups at both 2.5 and 5 yr of treatment.

There were no significant differences in the baseline characteristics among the four treatment groups in the valid case analysis (data not shown). In addition, the women who discontinued the study treatment did not differ from those who complied with it according to the baseline variables listed in

Table 2 (data not shown) except for smoking, which was more common among the noncompliers; however, the difference could not be seen in any particular study group. Baseline lumbar and femoral BMDs were similar in the HRT group and the HRT + Vit D group between the compliers and noncompliers. However, the lumbar and femoral BMDs were lower ($P = 0.009$ and $P = 0.006$, respectively) in the women who discontinued the Vit D_3 treatment (n = 12) than in the compliers in the same group. In addition, baseline femoral neck BMD was lower in noncompliant than in the compliant women in the placebo group ($P < 0.001$; n = 11).

Discussion

This randomized study evaluated the effects of four different treatment schedules on BMD in 458 early postmenopausal women, a subsample from the population-based Kuopio Osteoporosis Risk Factor and Prevention Study. With regard to the baseline data, the randomization was successful.

The results of the present trial demonstrate that HRT, as a sequential regimen of E_2 (2 mg) and CPA (1 mg), is effective in the long term prevention of osteoporosis in postmenopausal women. The benefits of HRT were observed not only among the women who complied with the treatment but also at the population level according to the intention to treat analysis. HRT had a more dynamic effect on the axial than on the peripheral skeleton, a finding in harmony with previous studies. HRT preserved the lumbar BMD in the HRT group and in the HRT + Vit D group and decreased bone loss significantly in femoral neck (intention to treat analysis). However, the increase in lumbar BMD and the stabilization of bone mass in femoral neck were more clearly shown among the women who complied with the treatment (valid case analysis). Previously, the data on the long term effects of HRT in postmenopausal women have been based mostly on observational studies (12) or prospective studies up to 2–3 yr in duration, but there are few randomized trials beyond that point (13–15). In addition, earlier studies evaluating the bone effects of E_2Val combined with CPA took 2.5 yr or less (2, 3, 16, 17) or were performed on osteoporotic women (4).

In this study, the HRT effect was mainly obtained during the first 2.5 yr and plateaued for the last 2.5 yr. This finding is consistent with the observations of Lindsay and colleagues (15) using unopposed estrogen treatment and of Nielsen and colleagues (14) using continuous and sequential HRT. In the PEPI trial (Postmenopausal Estrogen/Progestin Interventions study), the increase in BMD was greatest (70–90%) during the first 12 months of the 3-yr HRT trial, with a slight BMD increase during the next 2 yr (18). These findings suggest that the peak BMD increase will be obtained between 1–3 yr of HRT. The BMD changes after the onset of the study may reflect the bone remodeling transient. HRT inhibits bone resorption and thereby decreases the rate of bone loss. In the beginning of the treatment, in-filling of previously existing erosion cavities on the bone surface will continue so that the skeletal mass may increase transiently until a new steady state is achieved (19). This study was long enough to attain a steady state in bone remodeling. Whether the BMD remains stable after 5 yr of HRT or increases, as in the Danish 10-yr trial with E_2 and norethisterone (13), remains to be shown.

In the present study, at the end of 5 yr, lumbar BMD had increased significantly in the HRT group (+1.5%) and the HRT + Vit D group (+1.8%). Previously, only the Danish investigators (14) had conducted a 5-yr placebo-controlled trial assessing the effects of HRT (E_2 and norethisterone) on axial bone. They observed a net bone gain in lumbar BMD of 15–19% in early postmenopausal women treated with either sequential or continuous HRT compared with those treated with placebo. In our study the net gain in lumbar BMD was 6.2% in the HRT group and 6.5% in the HRT + Vit D group. Our study has the advantage of having a larger study population and a higher compliance (62–72%) with HRT. In addition, our results seem accurate, firstly because the BMD changes were large compared with the precision of our DXA method, and secondly because the BMD changes in the placebo group were similar to the changes reported earlier for the same postmenopausal period at both spine and femoral neck (20). The placebo group of the Danish study lost somewhat more bone, which might also be partly due to the higher noncompliance. Our study was designed for nonosteoporotic women to investigate the preventative effect of HRT; it is unclear whether the Danish study had the same criteria.

In the present study, we found no increase in the BMD of the femoral neck in the HRT or HRT + Vit D groups. However, the effects of HRT and HRT + Vit D on femoral neck BMD were notable, as they partially prevented bone loss. This is the first 5-yr trial reporting the effects of E_2Val and CPA on femoral BMD. The stronger effect on lumbar BMD is most likely due to the relatively high bone turnover in the vertebrae, which consists mainly of cancellous bone, whereas femoral neck area contains both cortical and cancellous bone. Whether the minor bone loss during HRT at the femoral neck could be prevented with higher doses of estrogen or the addition of a second agent is not known.

Vit D deficiency can lead to osteomalacia and secondary osteoporosis (21). Therefore, Vit D supplementation has been suggested as a nonhormonal alternative in the prevention of osteoporotic fractures in the elderly. Our study showed that low dose Vit D_3 supplementation (300 IU) had no effect on lumbar or femoral BMD in nonosteoporotic early postmenopausal women. In our earlier report, 2.5-yr Vit D_3 supplementation (3) showed a minor effect on femoral BMD in the same study group, but it disappeared during the following years. There are no previous reports of 5-yr Vit D supplementation in postmenopausal women. Previous randomized trials of 1.5- to 2-yr treatment with Vit D (400–800 IU/day) have shown some positive effect on femoral BMD in elderly people (mean age, 80 yr) (6, 7, 22). In addition, Dawson-Hughes *et al.* (5) conducted a 2-yr study comparing the effects of 2.5 μg (100 IU) and 17.5 μg (700 IU) Vit D supplementation. They observed only a minimal change in lumbar BMD (−0.11–0.31%) in both groups, and the effect on femoral BMD with 17.5 μg Vit D supplementation was greater than what we obtained during the 2.5-yr follow-up (−2.5%). The differences between the results could partly be due to the younger age of our study population. It is most likely that our low dose Vit D_3 supplementation was ineffective in the prevention of bone loss because our study subjects may not have

been deficient in Vit D, even though the dietary Vit D intake of this age group in the Kuopio Province is rather low (3.6 μg/day) (23). This suggestion is supported by the results of the 1-yr substudy of our trial (11): Vit D_3 supplementation increased serum 25OHD concentrations significantly but did not affect serum 1,25-dihydroxyvitamin D or PTH levels.

However, recently it has been suggested that for optimal bone effect, Vit D status should be much higher than what previously has been believed to be sufficient (24). The serum PTH levels tend to remain unchanged after Vit D treatment only in patients with much higher baseline 25OHD levels (50 nmol/L or higher) than previously used 25OHD thresholds (25, 26). It may be predicted that increasing Vit D intake would be beneficial in individuals with suboptimal Vit D levels (24–26). In our substudy, the serum mean 25OHD level was 27.4 nmol/L, which has previously been considered to be within the normal range. Unfortunately, we have not measured the Vit D metabolites from the whole study group, and it is not possible to evaluate the benefit of Vit D treatment for those with suboptimal levels of Vit D, which may be possible in Finland during the winter when the sunshine exposure is low.

Food is not fortified with Vit D in Finland with the exception of margarine. The Vit D intake in the study age group in Finland is slightly higher (3.6 μg) than that among the subjects reported in previous studies (2.5–3.1 μg) (5, 7, 27). However, the dietary Vit D intake decreases with increasing age in Finland (23) to the same level as in the previous studies (5, 7, 27), which were conducted in countries from slightly lower latitudes and more sunshine exposure. Once again, the effect of Vit D may be better among the elderly with inadequate Vit D intake. Secondly, the dose of Vit D_3 supplementation was low in our study, and the effect of a higher dose might have been more pronounced.

Some changes in BMD have been reported to occur with seasons in both untreated women and women with Vit D supplements (27). However, the seasonal BMD changes reported have been small and probably of minor importance in this study when considering the large number of subjects, the long duration of the study, and the large overall BMD changes in our study. Additionally, the 5-yr data could not have affected by seasonal BMD changes because they were obtained in the same season as the baseline measurements.

Our results show that the addition of Vit D_3 to HRT did not confer any additional benefit. This finding is in contrast with the data achieved in our previous study with osteoporotic postmenopausal women who seem to benefit from combining Vit D with HRT (4). Hence, if a woman with normal BMD decides to use HRT for prevention of osteoporosis during her early postmenopausal years, it seems unnecessary to add Vit D to the treatment unless a clear Vit D deficiency is present. In addition, combining Vit D to HRT might adversely affect the positive lipid effects of HRT (9).

In the placebo group lumbar and femoral BMDs decreased at rates of -1.5% and -1.4%/yr during the first 2.5 yr of the study. The decline approached a lower rate during the second half of the study (lumbar, -0.4%/yr; femoral, -0.3%/yr). To date, the age-related loss of bone is well established, but the rate and pattern of loss have been only partially characterized. It is difficult to interpret data across previous prospective studies (28–33) due to differences in study protocols. Our results are consistent with those reported by Pouilles *et al.* (20) in their retrospectively gathered longitudinal study. Our study population is a subsample of a larger population-based study and may indicate a realistic pattern of bone loss typical of the general population in our community.

The placebo group received calcium (93 mg) as lactate daily. Even though the latest studies have indicated that calcium supplementation can reduce the rate of bone loss (34, 35), the effect of 93 mg Ca^{2+} in the placebo and Vit D groups is of minor importance. Firstly, the average dietary calcium intake in our participants (830 mg/day) exceeded the Finnish recommendation for daily calcium intake (800 mg), and secondly, the supplements used for prevention of bone loss are usually much greater (up to 2 g daily) (34). Accordingly, it has been reported that women in the early postmenopausal years do not benefit even from a 500-mg calcium supplement (36). The Food and Nutrition Board has recently recommended that the daily adequate intake for calcium should be 1200 mg (37). The mean intake of calcium in our study population was less and could have been slightly low to reach the threshold for optimal bone maintenance (38). Therefore, it may be argued that the women in this study received too little calcium for maximal benefit of the treatments.

We assume that our results were not biased by the withdrawals or excluded subjects. The number of subjects lost from the follow-up was small, and these women did not generally differ from those who finished the 5-yr trial. As expected, the number of noncompliers was higher in the HRT groups. However, in the valid case analysis, the baseline characteristics of the noncompliers did not differ from those of subjects who complied with the trial treatment, except for the small differences in the baseline BMDs observed in the Vit D and placebo groups. However, the number of dropouts in these groups was small. The Vit D_3 dosage was reduced to 100 IU during the last year of the study. However, this dosage was considered adequate at the time of the study, but the recommendation for daily Vit D intake has been doubled (10 μg = 400 IU daily) (37) since the trial. It is possible that the reduction of the dosage might have had some effect on the results, but it most likely is of minor importance. The exclusion criteria of the study were few so as to keep the study population the best representative of the larger population; therefore, the women taking different medications were not excluded. However, we analyzed the data also by excluding the women taking corticosteroids; this did not affect the BMD results.

In conclusion, this large, population-based randomized trial shows the beneficial effects of prolonged administration of HRT (E_2Val/CPA) on bone density and for the first time demonstrates the effects with the intention to treat principle. This implies that HRT intervention may be profitable also at a population level; hence, the data support the hypothesis that long term HRT prevents postmenopausal bone loss and thereby can protect from fractures, as was shown in this trial (39). The results also suggest that low dose Vit D_3 supplementation does not prevent bone loss in nonosteoporotic early postmenopausal women, and it does not confer any

additional benefit to HRT alone in nonosteoporotic women in this age group.

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