Combination Treatment with Estrogen and Calcitriol in the Prevention of Age-Related Bone Loss

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Estrogen deficiency and declining calcium absorption due to reduced calcitriol levels or intestinal resistance to calcitriol, are important factors in the pathogenesis of age-related bone loss. The main objective of this study was to examine the effect of estrogen and 1,25-dihydroxyvitamin D therapy given individually or in combination on bone loss in elderly women. Four hundred eighty-nine elderly women with normal bone density for their age, aged 65–77 yr, were entered into a randomized double blind, placebo-controlled trial. Women were randomized to one of four groups: conjugated estrogens (0.625 mg, daily) to women without a uterus (estrogen replacement therapy) plus medroxyprogesterone acetate (2.5 mg, daily) to women with a uterus (hormone replacement therapy), calcitriol (0.25μg twice daily), a combination of hormone replacement therapy/estrogen replacement therapy plus calcitriol, or placebos for 3 yr. The primary outcome was the change in bone mineral density of the femoral neck and spine. In the intent to treat analysis, hormone therapy (hormone replacement therapy/estrogen replacement therapy) produced a mean (±1 SD) increase in bone mineral density of 2.98% (±5.45%) at the femoral neck (P < 0.0001) and 4.36% (±6.42%) at the spine (P < 0.0001). There were parallel increases in total hip and trochanter bone mineral density. Calcitriol increased bone mineral density 0.10% (±4.27%) at the femoral neck (P = 0.57) and 1.65% (±4.83%) at the spine (P < 0.0124). The combination of hormone replacement therapy/estrogen replacement therapy + calcitriol increased bone mineral density 3.80% (±4.95%) at the femoral neck (P < 0.0001), 4.91% (±6.0%) at the spine (P < 0.0001), and parallel changes at the total hip and trochanter. All three treatment groups differed significantly from placebo at the spine and for the hormone replacement therapy/estrogen replacement therapy groups at the femoral neck, spine, total hip and trochanter. There were no significant differences between combination therapy and hormone replacement therapy/estrogen replacement therapy alone on bone mineral density at any site in the intent to treat analysis. In a secondary analysis of the effect in women who were adherent to treatment, calcitriol had a more significant effect on spine (P = 0.003) and total hip (P = 0.004). The increase in bone mineral density in the adherent groups of women was always higher compared with the intent to treat groups. Combination therapy compared with hormone replacement therapy/estrogen replacement therapy alone produced a significantly greater response in trochanter (P = 0.007) and total hip bone mineral density (P = 0.0017).

In summary, hormone replacement therapy/estrogen replacement therapy alone and in combination with calcitriol therapy was highly effective in reducing bone resorption and increasing bone mineral density at the hip and other clinically relevant sites in a group of elderly women, with normal bone density for their age. Calcitriol was effective in increasing spine bone mineral density. In the adherent women, combination therapy with hormone replacement therapy/estrogen replacement therapy and calcitriol increased bone mineral density significantly more in the total hip and trochanter than did hormone replacement therapy/estrogen replacement therapy alone. (J Clin Endocrinol Metab 86: 3618–3628, 2001)

OSTEOPOROSIS IS A condition characterized by increased skeletal fragility and susceptibility to fractures. It is a significant cause of frailty, morbidity, and even mortality. Osteoporosis and its consequences, particularly vertebral and hip fractures, is a serious public health problem in the aging population.

Estrogen deficiency and changes in vitamin D metabolism are important contributors to the development of osteoporosis in postmenopausal women. Estrogen deficiency at the time of menopause causes rapid bone loss, especially in the first 4 - 7 yr after the menopause, (1–4) and, is one of the main causes of postmenopausal (type 1) osteoporosis. There have been two small prospective studies of the effect of estrogen in postmenopausal osteoporotics with fractures. (5, 6) Both showed significant increases in bone mineral density (BMD) of the spine but not femoral neck. There was no significant reduction in the number of patients having vertebral fracture (5) There is some evidence that low levels of estradiol (<5 pg/ml) continue to play a role in age-related bone loss (7), but there have been few long-term randomized controlled studies of the effects of initiating estrogen therapy in elderly women to prevent or treat senile (type 2) osteoporosis (7).

Another important factor in age-related bone loss is the decrease that occurs in calcium absorption (8) because malabsorption of calcium exacerbates the negative calcium balance in elderly subjects (9). The decrease in calcium absorption can, in part, be attributed to a decrease in serum 1,25-dihydroxyvitamin D (8, 10, 11), but there is also evidence for an age-related impairment in the sensitivity or response of the gut to circulating endogenous serum 1,25-dihydroxyvitamin D (12–15).

Treatment with 1,25 dihydroxyvitamin D of postmenopausal osteoporotics with vertebral fractures (type 1) reverses negative calcium balance (9), increases spine density (16–18), and reduces the incidence of vertebral fractures in some studies (19), but not others (20). However, the use of
1,25-dihydroxyvitamin D has not been studied in the prevention of senile osteoporosis (type 2) in elderly women. The rationale for performing this study was that continuing estrogen deficiency after menopause together with declining calcium absorption are both important factors in the pathogenesis of senile osteoporosis and by combining two therapies one might achieve a synergistic or additive effect on bone. We compared the effects of estrogen in women without a uterus (ERT) plus medroxyprogesterone acetate in women with a uterus (HRT), calcitriol, and the combination of ERT or HRT plus 1,25-dihydroxyvitamin D against placebos, on bone density, biochemical markers of bone remodeling, falls, and fractures. This clinical trial was performed at one of three centers, which examined the effect of different interventions on bone density of the proximal femur as the primary outcome (STOP IT study). The other two centers studied exercise and calcium or vitamin D 700 IU/d plus calcium 500 mg/d. This study is also the first substantive study of the use of combined-continuous hormonal therapy in nonhysterectomized elderly women.

Subjects and Methods

Study subjects were obtained from mailing lists of women in the geographical area of Omaha and the surrounding district. All of the women between age 65–77 yr were sent a letter describing the study, and, after a short time, the letter was followed up with a phone call. Approximately 8005 women were contacted by a mail survey. 1905 agreed to come in for a preliminary screening. Women were excluded if they had severe chronic illness, had primary hyperparathyroidism or active renal stone disease, and were on certain medications, such as bisphosphonates, anticonvulsants, estrogen, fluoride, or thiazide diuretics in the previous 6 months. To complete eligibility, femoral neck density had to be within the normal range (±2 sd) for their age. Four hundred eighty-nine women satisfied eligibility and were enrolled. The distribution of subjects to each treatment group is shown in Fig. 1. There were 480 white, 6 black, and 2 Asian women. The Institutional Review Board approved the protocol at Creighton University and written informed consent was obtained from each subject before enrollment. A

Data, Safety, and Monitoring Board established by the NIH reviewed the conduct of the study.

Study design

This was a 3-yr double blind placebo-controlled trial, with the acronym STOP IT. All investigators and staff conducting the study remained blinded throughout the treatment period. Subjects were randomly assigned to either conjugated estrogens (Premarin) 0.625 mg/daily plus medroxyprogesterone acetate (Provera) 2.5 mg/daily (HRT), calcitriol (Rocaltril) 0.25 µg twice a day, the combination of Premarin plus Provera + calcitriol, or matching placebos; hysterectomized women (n 290) assigned to estrogen were not given the progestin (ERT). An independent statistical group performed the blinding and randomization. Dietary advice was given to increase dietary calcium if the intake was below 500 mg/d and to decrease calcium intake if it was greater than 1000 mg/d.

Measurements

On entry into the study, women underwent a physical examination, and detailed medical history; 7-d dietary intake, alcohol and tobacco use was obtained from a questionnaire. Because the proportion of women who were taking multivitamins containing vitamin D at baseline was only 35% it was decided that women could use other multivitamins that did not contain vitamin D. Previously, vitamin D deficiency was not found to be a significant problem in this geographical area.

BMD measurements of the spine (L1–L4), proximal femur (neck, trochanter, total hip), total body and radial mid shaft were performed on a DPXL scanner using dual-energy x-ray absorptiometry (Lunar Corp. Radiation, Madison, WI), software versions 1.2 and 1.3y were used for acquisition and analysis. The coefficients of variation for BMD measurements were 1.8% for femoral neck, 2.4% for spine, 0.7% for total body and 2.5% for radial mid shaft. During the study, all measurements of the proximal femur were performed in duplicate with repositioning between the scans; these values were averaged for the analysis. An independent phantom was scanned every week as a quality control measure for the scanner. After randomization, subjects came to the clinic at 6 and 12 wk and then every 6 months over 3 yr At each of these visits we measured urine and serum calcium levels. At baseline and at 6 monthly visits, we measured BMD, blood chemistry, and dietary calcium, and we dispensed new supplies of medication and estimated medication compliance by pill counts. At baseline and end of study a fasting blood
Sample was collected between 0700 h and 0900 h for measurement of serum calcium, ionized calcium, PTH, 25 hydroxyvitamin D (25OHD), 1,25-dihydroxyvitamin D and osteocalcin. Twenty-four-hour urine was collected for calcium and creatinine. Each time a 24-h urine was also recorded their calcium intake for the same period. At interim assessments, fasting urine was collected for calcium, creatinine, and N telopeptides. Serum and urine calcium and creatinine were measured using the immunoradiometric assay (INCSTAR Corp., Stillwater, MN), serum PTH was measured by immunometric assay (Nichols Institute Diagnostics, Capistrano, CA), and serum 25OHD and 1,25 dihydroxyvitamin D, as described previously (21). Measurements of serum 25OHD by protein binding assay were cross-checked against direct high-performance liquid chromatography measurements (Shimadzu) (22). Urine N telopeptides were measured by an Elisa assay (INCSTAR Corp.) (21).

The measurements of bone markers were performed at baseline, yr 2 and 3. Calcium absorption was measured fasting at the beginning and end of the study; 100 mg elemental calcium was mixed with 5 μCi calcium 45-200 ml distilled water. Blood samples were collected at 1, 2, and 3 h for estimation of calcium 45 and calcium absorption was expressed as the percent per liter after 3 h. These measurements were corrected for body size (23).

Data were collected prospectively by an interview-administered questionnaire on the incidence of falls and fractures at each visit, 6 wk, 3, 6,12,18, 24, 30, and 36 months. All fractures were confirmed from x-ray reports. Lateral radiographs of the spine were performed at baseline and end of study; morphometric measurements on the vertebral bodies were performed to assess the presence of baseline fractures and the incidence of new fractures. A baseline fracture was defined as a 20% reduction in anterior vertebral height and a new fracture as a 20% reduction in any vertebral height compared with baseline in a previously normal vertebra (24).

For safety assessment, annual radiographs of the abdomen were performed to look for evidence of renal stones or nephrocalcinosis. Mammograms were performed at baseline and at annual intervals. A Pap smear was performed at the beginning and end of study. An endometrial biopsy was not performed at baseline because of the technical difficulties in sampling nonestrogenized elderly women. Therefore, before starting treatment all women who had a uterus underwent a 10-d challenge with medroxyprogesterone acetate 10 mg. (medroxyprogesterone acetate to identify preexisting endometrial hyperplasia (25). Fourteen of the 290 women with a uterus had a withdrawal bleed and underwent an endometrial biopsy using a Pipelle cannula. One of these women was found to have endometrial hyperplasia. Another patient, in whom the biopsy was technically difficult and no cells were obtained, was enrolled but after a bleeding episode several months into the study. She underwent an endometrial biopsy using a Pipelle cannula. One of these subjects had an abnormal biopsy result. The other 12 subjects did not have hyperplasia.

During the first year of the study, any women who had excessive or troublesome bleeding was first given an increased additional daily dose of medroxyprogesterone acetate 20 mg for 1 wk. This was stepped down to 10 mg daily for another week, then 5 mg daily for a week before returning to the maintenance dose. If bleeding still persisted, then the estrogen dose could be reduced to alternate days until bleeding stopped. An independent gynecologist reviewed the bleeding data and drug assignment list and could recommend an endometrial biopsy for safety. At the end of the study, women from all groups were recommended to undergo an endometrial biopsy for safety evaluation because this was the first study of continuous combined hormone therapy in elderly women; 85% of women on hormones agreed to the procedure.

The protocol for management of hypercalcemia (ionized calcium >5.28 mg/dl or 1.32 nmol/liter) and hypercalcuria (24 h urine calcium > 400 mg or 10 mmol) was to repeat the sample 1 wk later without a change in dose or diet. If either parameter remained high then the dietary calcium intake was reviewed and adjusted to 800 mg/day. If either parameter remained elevated after a third week, then the blinded dose of calcitriol was reduced to 0.25 μg/day.

Subjects were dispensed medication every 6 months, and all returned pills were counted at each 6 monthly visit to estimate compliance for that time period.

### Statistical Methods

The study assigned subjects to treatment groups using simple randomization stratified on hysterectomy status. Because the study hypotheses compared each of the three treatment groups to placebo, analyses corresponded to a four-group design rather than a factorial design. Sample size was based on an expected placebo group decrease in femoral neck BMD of 1.5% over three years and assumed losses from morbidity and dropout of 8% per year. Based on these assumptions, the study was designed to have 90% power to detect 3-yr changes in BMD with a two-sided α = 0.05 significance level as follows; a 1% increase on the combination of HRT/ERT and calcitriol, zero percent change on HRT/ERT only or calcitriol only, and a 1.5% decrease on placebo. Similar calculations were performed for spine, using an estimated loss on placebo of 3% over 3 yr, an increase of 2% on calcitriol, an increase of 4% on estrogen and an increase of 5% on the combination.

For the primary outcome analysis of change in BMD over time, all randomized subjects (n = 489) were included in the group to which they were assigned regardless of compliance with treatment. For analyses of BMD, missing values were imputed according to a “last observation carried forward” algorithm. Mean BMD measurements presented in the tables are raw means of subjects seen at the 36-month visit. Statistics are based on the intent-to-treat analyses that included all randomized subjects. (26) Primary outcome hypothesis testing for the 3-yr change in BMD of the femoral neck and spine included three contrasts of the active treatment groups against the placebo group for each of the two skeletal sites. Because these six contrasts were prespecified as primary outcomes, a critical value of P = 0.017, corresponding to a Bonferroni correction for the three pair-wise comparisons, was used for the three contrasts at each skeletal site. For all statistical tests, the tables give actual p-values. Interpretations in the text declare comparisons vs. placebo to be significant using the three-comparison Bonferroni. Tests of all pair-wise comparisons use a five-comparison Bonferroni adjustment.

We compared treatment groups on baseline mean values for age, weight, height, body mass index, intact and D intake, serum osteocalcin, serum PTH, N-telopeptide/creatinine ratio, serum 25OHD, serum 1,25-hydroxyvitamin D, calcium intake, calcium absorption, total body BMD, BMD of the femoral neck, trochanter, total hip, spine and radius using general linear models ANOVA from the SAS General Linear Models procedure (27). Because the hormone replacement regimen differed for hysterectomized women vs. women with intact uterus, we compared the two strata on these baseline characteristics.

Analyses included an overall test of difference between treatment groups, and contrasts between each active treatment group and placebo only if the overall test was significant. To address the study’s primary objective of comparing the four randomized groups on the change in femoral neck BMD over time, we used repeated measures mixed models with an interaction among treatment group, time, and baseline BMD. An interaction was also tested for to determine if the treatment groups differed over time on BMD. In the presence of overall differences between groups we also tested three contrasts, one for each active intervention vs. placebo. We considered as covariates, age, hysterectomy status, body mass index, baseline calcium absorption, and baseline 1,25-dihydroxyvitamin D. Initial models included, in addition to treatment by time effect, interaction terms of the covariates with both treatment and time. If a three-way interaction was not found to be a significant effect it was dropped from the model, and a term for the two-way interaction of the covariate with time was then tested. If the two-way interaction was dropped according to the step-down criterion, we included a main-effect term for the covariate. To maintain consistency across models for all skeletal sites, main effect terms for each covariate were kept in the final model for adjustment purposes regardless of whether the covariate was prognostic.

Three-year changes in BMD for those who completed 3 yr on blinded study medication were assessed by analysis of covariance with the 3-yr change as the dependent variable, and effects for treatment group and the same covariates as used in the primary analysis as independent variables.
Changes in mean values for laboratory measurements from baseline to 36 months were tested using general linear models ANOVA computed using the SAS General Linear Models procedure, and included only subjects having both assessments. Serum 1,25-dihydroxyvitamin D was not remeasured at the end of study because of the variable times that subjects took their medication on the day before they came to clinic and because of the short half-life of serum 1,25-dihydroxy vitamin D in the blood.

The occurrence of falls was analyzed using recurrent event analysis. Since falls were ascertained at regular visits, the exact times of falls were not known, so homogeneity of the counting process over time could not be assessed directly. To compare the treatment groups on the rate of occurrence of falls over time we used a homogeneous Poisson regression model (28), computed with the SAS GENMOD procedure. The cumulative number of falls was modeled as a function of treatment group with age and BMI as covariates. The improvement in fit from adding terms to the model was assessed by the likelihood ratio test between successive models. The $\chi^2$ test of the parameter estimate associated with each treatment group represented the contrast of the treatment group vs. placebo (29).

We compared the treatment groups on the time to first nonvertebral fracture using the log rank test. Subjects who did not experience a fracture were censored as of their last follow-up visit.

All data were submitted at regular intervals during the study to a Data, Safety, and Monitoring Board.

**Results**

Subjects ranged in age from 65-77 yr, average age was 71(±4) yr. The four groups were comparable on their baseline characteristics as shown in Table 1, except for the percentage of women with vertebral fractures, which was subsequently found to be higher in the group randomized to calcitriol. The T scores for the femoral neck were below −2.5 in 19% and between −1.5 and −2.4 in 42% of the women. For the spine the T scores were below −2.5 in 28% and between −1.5 and −2.4 in 27% of the women. The Z score (mean ± sp) for the spine was 0.4 ± 1.4 and for the femoral neck was −0.2 ± 0.7.

Serum 25OHD was below 37.5 nmol/liter in 5.7% of subjects at baseline and 13.3% at 36 months. Of the 489 women randomized to treatment, 416 women completed the study and 337 remained on active medication (Fig. 1). At 36 months, treatment group differences in adherence to assigned therapy were evident, with 78% of those assigned to placebo, 70% of those assigned to calcitriol, 65% of those assigned to HRT/ERT and 62% of those assigned to HRT/ERT + calcitriol still adherent to their assigned medication. Among those still on medication the compliance for the groups calculated at 6 months and compared with 36 months, respectively, was: conjugated estrogens, 86% and 92%; medroxyprogesterone acetate, 91% and 94%; calcitriol, 87% and 93%; placebos, 94% and 92%.

The major reasons given for discontinuing medication were bleeding problems (21), breast tenderness (13), other significant health problems (21), lost interest in the study (19), cerebrovascular incident, cerebral thrombosis, cerebral hemorrhage, transient ischemic attack (15), and gastrointestinal problems (14). Five of the subjects died during study from causes unrelated to study medication. There were four deaths from congestive heart failure, one from each treatment group, and one case of sudden death due to myocardial infarct on the combination treatment.

Unadjusted percentage changes in mean BMD at 3 yr in response to the various treatment regimens and based on the 416 participants completing the study are shown in Table 2. a and b, and Fig. 2. The significance levels given for each skeletal site reflects adjustment for baseline covariates age, calcium absorption, serum 1,25-dihydroxy vitamin D, and BMI. In the placebo group there were significant declines of 1.8%, 1.7%, and 1.99% in total hip, trochanter, and total body BMD, respectively. BMD in the spine and radius also decreased significantly by 0.91% and 2.69% over 3 yr. The changes in BMD in the placebo group were analyzed by tertiles of serum 25OHD (< 50 nmol/liter, 50–75 nmol/liter, and >75 nmol/liter), and then by tertiles of serum PTH (< 28 ng/liter, 28–39 ng/liter, and >39 ng/liter). There were no significant differences in the rates of bone loss among the tertiles of serum 25OHD or PTH. Treatment with estrogen therapy with or without calcitriol led to rapid increases in spine BMD during the first 6–12 months. BMD continued to increase during the remainder of the intervention period, but at a lower rate. In the 416 subjects measured at 36 months, the change of mean BMD over time differed significantly in the groups receiving HRT/ERT ($P < 0.001$), calcitriol ($P = 0.0126$), and the combination of HRT/ERT + calcitriol ($P < 0.0001$) compared with placebo. Mean BMD of the spine increased by 4.36% in the HRT/ERT group, 1.65% in the

<table>
<thead>
<tr>
<th>TABLE 1. Baseline characteristics of the randomized study subjects</th>
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<tr>
<td>Characteristic</td>
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<tr>
<td>Age (yr)</td>
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<td>Weight (kg)</td>
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<td>Height (cm)</td>
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<tr>
<td>Total calcium intake (mg/d)</td>
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<tr>
<td>Dietary vitamin D (IU/d)</td>
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<tr>
<td>BMD (g/cm²)</td>
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<tr>
<td>Femoral neck</td>
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<tr>
<td>Spine (L2–L4)</td>
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<tr>
<td>T score (femoral neck)</td>
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<tr>
<td>T score (spine)</td>
</tr>
<tr>
<td>Vertebral fractures (%)</td>
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<tr>
<td>Serum PTH (ng/ml)</td>
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<tr>
<td>Serum 25OHD (nmol/liter)</td>
</tr>
<tr>
<td>Serum 1,25-dihydroxyvitamin D (pmol/liter)</td>
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<tr>
<td>Serum creatinine (μmol/liter)</td>
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Plus-minus values are means ± SD.
calcitriol only group and 4.91% in the HRT/ERT plus calcium group, compared with a mean decrease of 0.91% for placebo. Review of Fig. 2 shows that the increase in BMD on HRT/ERT therapy was more rapid and larger than in the other calcium group.

In the femoral neck, trochanter, and total hip, the change in mean BMD in the 416 subjects measured at 36 months differed significantly in those groups receiving HRT/ERT and the combination of HRT/ERT plus calcitriol (P < 0.0001 compared with placebo (P < 0.0001, not statistically larger). Mean BMD of the femoral neck increased by 2.98% in the HRT/ERT only group and 3.80% in the HRT/ERT plus calcitriol group. Although the difference in mean BMD between the calcitriol and placebo groups at 36 months was not statistically significant, the calcitriol-treated group showed a slightly positive change in mean femur neck BMD of 0.1% whereas the placebo group showed a significant decrease in mean femoral neck of −0.47%. The decrease in BMD at the trochanter and total hip, although less on calcitriol, was not significantly different compared with placebo. However, whereas the decrease in BMD was not significantly different, the decrease was significantly less from baseline in the calcitriol group, which was for the placebo group.

A similar pattern was seen for radius BMD and total body calcium. Significant differences vs. placebo were seen in the groups receiving HRT and the combination of HRT/ERT + calcitriol (both P < 0.0001). The decrease in mid radial BMD on calcitriol was significantly less than that for placebo (−0.89 vs. −2.69%, respectively, P = 0.003). The group on calcitriol showed a smaller decrease in total body, however, the change in BMD was less than that for placebo (−0.59 vs. −1.99%, P = 0.06). In the intent to treat analysis there was no significant difference between combination therapy and HRT/ERT alone.

Table 2b shows the unadjusted mean percentage changes in BMD for the 337 participants who were still adherent to their assigned treatment regimen at the conclusion of the study. In the subjects on HRT/ERT or HRT/ERT vs. the placebo group in the intent-to-treat analysis of the change in BMD over time (n = 489).

Numbers vary per group: a = 108, b = 99, c = 95.

a P values correspond to the contrast of each intervention group vs. the placebo group in the intent-to-treat analysis of the change in BMD over time (n = 489).

b Placebo group 36 month BMD change from baseline is statistically significant at P < 0.01.
including age, weight, height, BMI, BMD at any site, number of vertebral fractures, daily calcium intake, intestinal calcium absorption, serum 25OHD, and 1,25-dihydroxyvitamin D. It is not possible using this design to evaluate the effects due to the addition of a progestin, because the potential effects of the progestin are confounded by hysterectomy status. How-

FIG. 2. Percentage change in mean BMD of the 416 women who came in for final visit (from Table 2a).
However, hysterectomy status was examined as a covariate in the models of BMD change over time for each skeletal site. In our models of BMD, we found no significant interaction effects between hysterectomy status and treatment group, indicating that treatment effects were similar for women on combination HRT and ERT. Thus, a significant effect on BMD by a progestin is unlikely because the effects of treatment over time on BMD did not differ by hysterectomy status.

**Biochemistry**

The data are summarized in Table 3. Mean serum ionized calcium showed no significant change on placebo, HRT/ERT, or the combination and increased 0.01 mmol/liter on calcitriol (P < 0.001). Mean 24-h urine calcium increased by 0.46 mmol (19 mg) in the placebo group, 0.17 mmol (7 mg) on HRT/ERT, 1.83 mmol (72 mg) on calcitriol (P < 0.001), and 1.98 mmol (79 mg) on the combination (P < 0.001). Mean serum osteocalcin showed 35% and 38% decreases, respectively, in the two groups given HRT and HRT plus calcitriol, which were significantly different from change in the placebo group. The small decrease (8%) in mean serum osteocalcin in the calcitriol group was not significantly different from the placebo group change.

Mean 24-h urine N-telopeptide/creatinine showed 28% and 30% decreases, respectively, in the HRT/ERT and HRT/ERT plus calcitriol-treated groups, which were significantly different from the placebo group, P < 0.0001). The calcitriol group showed a mean decrease of 9%, which was not statistically significant from the placebo group change after adjusting the P value for multiple comparisons.

The three intervention groups showed no significant differences from placebo in the change in total calcium intake over the 36-month period of study. Mean calcium absorption in both of the calcitriol groups increased significantly compared with the small negative change seen in the placebo group. There was no effect of HRT/ERT on calcium absorption and calcium absorption decreased similarly in both the placebo and HRT only groups.

There were no significant differences in the changes in serum 25OHD levels between the treatment and placebo groups. Mean serum PTH values declined significantly in the hormone therapy groups, which were significantly different from change after adjusting the P value for multiple comparisons.

### TABLE 3. Initial laboratory values and changes at 3 yr in subjects who completed the study

<table>
<thead>
<tr>
<th>Initial value</th>
<th>Change</th>
<th>P*</th>
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<tr>
<td><strong>Serum 25OHD (nmol/liter)</strong></td>
<td></td>
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<tr>
<td>Placebo (n = 112)</td>
<td>80.5 ± 27.4</td>
<td>−17.3 ± 20.5</td>
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<tr>
<td>Calcitriol (n = 101)</td>
<td>78.0 ± 21.6</td>
<td>−17.3 ± 19.7</td>
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<tr>
<td>HRT/ERT (n = 100)</td>
<td>77.3 ± 24.4</td>
<td>−15.2 ± 24.0</td>
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<td>HRT/ERT + calcitriol (n = 101)</td>
<td>80.0 ± 29.4</td>
<td>−16.1 ± 23.4</td>
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<td><strong>Serum PTH (ng/liter)</strong></td>
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<td>HRT/ERT + calcitriol (n = 101)</td>
<td>37 ± 13</td>
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<td><strong>Serum osteocalcin (µg/dl)</strong></td>
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<td>4.0 ± 1.5</td>
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<td>Calcitriol (n = 101)</td>
<td>3.7 ± 1.1</td>
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<td>HRT/ERT (n = 100)</td>
<td>3.7 ± 1.3</td>
<td>−1.4 ± 1.3</td>
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<td>HRT/ERT + calcitriol (n = 101)</td>
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<td><strong>24-h urine N-telopeptide/creatinine (nmol bce/mmol Cr)</strong></td>
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<td>HRT/ERT + calcitriol (n = 101)</td>
<td>48.2 ± 22.2</td>
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</tr>
<tr>
<td><strong>Calcium absorption (% actual dose/liter)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 112)</td>
<td>2.55 ± 0.55</td>
<td>−0.10 ± 0.48</td>
</tr>
<tr>
<td>Calcitriol (n = 101)</td>
<td>2.59 ± 0.64</td>
<td>0.14 ± 0.63</td>
</tr>
<tr>
<td>HRT/ERT (n = 100)</td>
<td>2.53 ± 0.68</td>
<td>−0.11 ± 0.55</td>
</tr>
<tr>
<td>HRT/ERT + calcitriol (n = 101)</td>
<td>2.57 ± 0.63</td>
<td>0.12 ± 0.63</td>
</tr>
<tr>
<td><strong>Total calcium intake mg/d</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 107)</td>
<td>771 ± 293</td>
<td>11 ± 280</td>
</tr>
<tr>
<td>Calcitriol (n = 97)</td>
<td>776 ± 303</td>
<td>0 ± 274</td>
</tr>
<tr>
<td>HRT/ERT (n = 98)</td>
<td>769 ± 361</td>
<td>15 ± 326</td>
</tr>
<tr>
<td>HRT/ERT + calcitriol (n = 101)</td>
<td>703 ± 278</td>
<td>1 ± 265</td>
</tr>
<tr>
<td><strong>Serum ionized calcium (nmol/liter)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 112)</td>
<td>1.24 ± 0.03</td>
<td>−0.004 ± 0.04</td>
</tr>
<tr>
<td>Calcitriol (n = 101)</td>
<td>1.23 ± 0.04</td>
<td>0.01 ± 0.03</td>
</tr>
<tr>
<td>HRT/ERT (n = 100)</td>
<td>1.23 ± 0.04</td>
<td>−0.01 ± 0.04</td>
</tr>
<tr>
<td>HRT/ERT + calcitriol (n = 101)</td>
<td>1.23 ± 0.04</td>
<td>0.0007 ± 0.04</td>
</tr>
<tr>
<td><strong>24-h urine calcium (mmol)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 112)</td>
<td>3.89 ± 1.64</td>
<td>0.46 ± 1.51</td>
</tr>
<tr>
<td>Calcitriol (n = 101)</td>
<td>3.82 ± 1.60</td>
<td>1.83 ± 2.46</td>
</tr>
<tr>
<td>HRT/ERT (n = 100)</td>
<td>3.53 ± 1.68</td>
<td>0.17 ± 1.71</td>
</tr>
<tr>
<td>HRT/ERT + calcitriol (n = 101)</td>
<td>3.23 ± 1.66</td>
<td>1.98 ± 2.44</td>
</tr>
</tbody>
</table>

*P represents contrast of the mean 36-month change for the intervention vs. placebo if the overall test of difference between groups was statistically significant. One outlier with a baseline urine NTx/Cr of 755 in the HRT group was excluded. ns, Not significant.*
whereas PTH increased from baseline in both the placebo and HRT/ERT groups.

**Fractures**

The study was not designed to have power to detect a difference in the incidence of fractures between treatment groups. During the 3-yr follow-up on treatment, the cumulative incidence of nonvertebral fractures (percentage of subjects having experienced at least one fracture) was: 10.7% on placebo, 4.9% on calcitriol, 11.9% on HRT alone, and 7.8% on HRT + calcitriol. The relative risks for the occurrence of any nonvertebral fracture during the study period were not different from 1.0 for any of the intervention groups vs. placebo: 1.06 (0.69–1.63, \( P = 0.83 \)) for HRT/ERT, 0.83 (0.47–1.44, \( P = 0.63 \)) for HRT and calcitriol, 0.60 (0.28–1.27, \( P = 0.13 \)) for calcitriol alone, and 0.7 (0.44–1.1, \( P = 0.08 \)) for both calcitriol groups combined.

Vertebral fractures occurred after 3 yr in nine patients. There were four patients on calcitriol, two on HRT/ERT, two on HRT/ERT + calcitriol, and one on placebo.

**Falls**

During the study, 254 subjects experienced a total of 440 falls. The percentage of subjects in each group having a fall at any time during the study was 63% on placebo, 48% on calcitriol, 56% on HRT/ERT, and 56% on HRT/ERT + calcitriol. The rate of falls per year was 0.43 for placebo, 0.27 for calcitriol alone, 0.39 for HRT alone, and 0.35 for the combination HRT/ERT + calcitriol group. The overall test for a difference among groups in the rate of falls over time was significant (\( P = 0.025 \)). The only group differing significantly from placebo on the rate of falls was the calcitriol only group (\( P = 0.0015 \)).

**Adverse events and side effects**

The incidence of major diseases occurring in the 3 yr is given in Table 4. Eleven patients had gallstones or cholecystitis, eight in the two estrogen groups compared with three patients in the other two groups. There were five cases of deep venous thrombosis: four in the two estrogen groups vs. one in the other two groups. There were 12 cases of cancer: 1 case in the two estrogen groups (colon cancer) vs. 11 cases (6 colon, 4 breast, 1 endometrial) in the other two groups (the endometrial case was probably present at baseline). There were two lymphomas, one developed after 6 wk on HRT and one on placebo and one case of anal skin cancer. There were 15 cases of myocardial infarct: 8 in the two estrogen groups, 4 on calcitriol, and 3 on placebo. There were 17 cerebrovascular accidents: 10 in the estrogen groups, 4 on calcitriol, and 3 on placebo. There were two cases of kidney stones: one on placebo and one on calcitriol, and in both cases the stone was visible on the baseline radiograph.

Significant bleeding and spotting (defined as >7 d) were common events. Bleeding occurred in 39 of 144 of the patients (27%) in the first year. Among women continuing in the study on HRT into the second and third years, 4 of 97 (4%) and 3 of 88 (3%), respectively, experienced bleeding. For women who had spotting only, the number was 29 of 144 (20%) in the first year, 16 of 97 (16%) in the second year, and 7 of 88 (8%) in the third year. During the study, any woman who had heavy bleeding or prolonged spotting underwent endometrial biopsy. This was performed in 67 women. Forty-five biopsies showed an atrophic endometrium, 13 had proliferative endometrium, 1 had adenomatous hyperplasia, and 1 had an endometrial carcinoma (on placebo), 3 had endometrial polyps, 1 had a pyometra, and 3 had insufficient tissue. At the end of the study 170 women also underwent an endometrial biopsy for general evaluation (85% of women still on HRT had a final biopsy). One hundred fifty-five showed atrophic changes, four proliferative, and eleven had insufficient tissue. There were no cases of hyperplasia or cancer.

At least one episode of hypercalciuria (>400 mg or 10 mmol) occurred in 8% of the patients on placebo, 26% on calcitriol, 3.3% on estrogen, and 14.8% on the combination. There were 58 cases of hypercalciuria occurring on calcitriol alone; 44 returned to normal in the second week, and 12 returned to normal in the third week. In two cases hypercalciuria was unresolved. In the combination group there were 25 cases of hypercalciuria of whom 20 returned to normal in the second week, 4 returned to normal in the third week, and 1 persisted. If several episodes of hypercalciuria occurred in the same patient, then the blinded dose was reduced. This occurred in two patients. As a percentage of the tests done over 3 yr, hypercalciuria >400 mg/d or 10 mmol occurred in 1.2% on placebo (\( n = 830 \)), 4% on calcitriol (\( n = 772 \)), 0.6% on HRT/ERT (\( n = 698 \)), and 2.8% on HRT/ERT and calcitriol (\( n = 648 \)). If one used a cut off of 300 mg/d, then the incidence of hypercalciuria was 7% on placebo, 6% on HRT/ERT, 18% on the combination of HRT/ERT + calcitriol and 29% on calcitriol alone. An episode of hypercalcemia occurred in 6% of patients on placebo, 12% on calcitriol, 5% in the estrogen group, and 5% on the combination. In all cases, it was mild and rarely exceeded 10.4 mg/dl or 2.6 nmol/liter (ionized 5.48 mg/dl or 1.32 nmol/liter). The protocol allowed a continuation of treatment with a repeat blood 1 wk later; in 66% of the cases serum calcium had returned to normal after 1 wk. In those in whom it remained above the normal range, an additional blood sample was done after a second week, and in 98% it had returned to normal. If hypercalcemic episodes occurred on more than two or three times throughout the 3 yr of study, then the blinded dose of calcitriol was reduced to 0.25 \( \mu \)g (1 capsule) a day. This occurred in 3 of the 250 patients treated with calcitriol during the 3 yr of study.

---

**TABLE 4. Major adverse events**

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 123)</th>
<th>Calcitriol (n = 123)</th>
<th>HRT/ERT (n = 121)</th>
<th>HRT/ERT + calcitriol (n = 122)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallbladder</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Cancer</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CVA</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>22</td>
<td>20</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Psychiatric</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Kidney stone</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Deep venous thrombosis</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Death</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
Discussion

In this study of elderly women with normal bone mass for their age, all three therapies increased BMD in the spine whereas only the groups receiving HRT/ERT significantly increased BMD in the proximal femur. The combination of HRT/ERT and calcitriol consistently produced the greatest gains in BMD at all skeletal sites compared with single therapy, and the effects were seen as early as the 6-month visit. However, the difference between combination HRT/ERT therapy with calcitriol and HRT/ERT alone was significant only in the adherent group and only in the total hip and trochanter. These early increases in hip BMD have implications for treating elderly women at risk of bone loss because it shows that combination therapy can reverse bone loss rapidly within the first 6 months and produce gains in BMD that are sustained for at least 3 yr.

A dose of 0.625 mg/d conjugated estrogens is the most commonly used dose in the early postmenopausal period (30). There are no other comparable studies of this dose in a population similar to ours. However, in younger osteoporotic women aged 61–62 yr, increases in femoral neck and spine BMD were similar to those seen in this study (6). The increase in spine and femur BMD on HRT/ERT is similar to that seen with risedronate 5 mg/daily (31), larger than with raloxifene (32) and slightly less than with alendronate (33) However, in all of these studies subjects were supplemented with calcium (500–1000 mg/d). Calcitriol therapy increased spine density by 1.8%, which is similar to the effect of 60 mg/daily raloxifene (32) and nasal 200–400 mcg/daily calcitonin (34), both of which have been reported to reduce the incidence of vertebral fractures.

An issue to consider is the vitamin D nutritional status of our study group at baseline and throughout the study and whether the positive effects of 1,25-dihydroxyvitamin D are only the results of correction of age-related vitamin D insufficiency with calcitriol. This is unlikely because at baseline the mean serum 25OHD in our full cohort was 77.5 nmol/liter (31 ng/ml), which is identical to the mean level seen in normal young and elderly women in this area and similar to that in other areas of North America (35). These vitamin D levels are much higher than those seen in Northern Europe where serum 25OHD may average only 37.5 nmol/liter in elderly (36,37), half of those seen in this country. During the 3 yr of study, serum 25OHD levels fell slightly but few women had levels below 37.5 nmol/liter, levels that can be associated with subclinical vitamin D deficiency. Furthermore, our results did not show significantly higher bone loss in spine or femoral neck for those women in the lowest tertile of serum 25OHD. Nor was there any relationship found between serum 25OHD and rates of bone loss in the Framingham study (38). A recent multinational study in which serum 25OHD was stratified into tertiles, less than 25, 25–50, and more than 50 nmol/liter in 7000 women, was unable to demonstrate any relationship between serum 25OHD levels and BMD for spine and femoral neck though trochanteric BMD was 3.6% lower in the lowest tertile of serum 25OHD (39).

In another clinical trial of women of comparable age and femoral neck BMD, supplemental Vitamin D 700 IU/daily plus calcium 500 mg/daily increased spine BMD by a non-significant 1.4% compared with an increase on placebo of 0.8%. In this study, there was a small but significant change in total body BMD but no significant change in femoral neck BMD (40). However, despite these unremarkable changes in BMD, there was a significant reduction in nonvertebral fractures, although the study sample is small. Another study of vitamin D and calcium in 80-yr-old women living in nursing homes in France showed a reduction in fractures in the first 2 yr but not in yr 3 and 4 (37). In this group mean baseline serum 25OHD was only 32.5 nmol/liter, which suggests that the majority of the elderly French women in this study had marked vitamin D deficiency. Certainly providing vitamin D 400–600 U/d to a high-risk group to maintain normal vitamin D nutrition and avoid osteomalacia is sensible but there is no data to show that this dose will reduce osteoporotic fractures in women with normal vitamin D nutrition. A 4-yr study that compared 25 hydroxyvitamin D₃, 15 micrograms daily to calcium supplements 750 mg/d or placebo (41) found that the 25OHD treatment increased serum 25OHD to the same level as that in the vitamin D 700 IU/daily study (40). It was ineffective in reversing bone loss at the hip and spine and had no significant effect on fractures.

Because a major effect of calcitriol is to increase calcium absorption, another important question is whether calcium treatment alone would be just as effective as calcitriol therapy. Unfortunately there is little clinical trial data comparing the effects of calcium supplements against placebo on BMD and fractures in elderly women (41, 42). In these studies, calcium supplements, 500 or 750 mg/d, reduced the rate of bone loss in the femoral neck and spine compared with placebo. However, these studies were not powered as fracture studies and there was no effect on fracture rates.

Calcium absorption was significantly increased in both groups receiving calcitriol whereas estrogen had no effect on absorption. Some studies have previously shown an increase in calcium absorption on conjugated equine estrogens (43, 44), probably because estrogen increases serum PTH and calcitriol (43, 45). In another study, calcium absorption increased on estrogen in younger women but not in older women (45), and a decrease in absorption on estrogen was also noted in another study (46). It may be that the reason for the lack of response in these other groups can be attributed to the intestinal resistance to endogenous calcitriol that occurs with aging. Serum PTH was significantly reduced in both groups treated with calcitriol, but increased in the estrogen and placebo groups. It is likely that the antiresorptive action of calcitriol is due to a decrease in secondary hyperparathyroidism, mediated either by a direct effect of calcitriol on the gland or via increased calcium availability subsequent to enhanced intestinal absorption of calcium.

The incidence of nonvertebral fractures was a secondary outcome and the study was not powered to detect significant differences between treatments. It is interesting that the incidence of fractures was about 50% lower in the two groups on calcitriol compared with HRT/ERT and placebo, and this deserves further study. In two other recent large-scale studies of antiresorptive agents, of raloxifene in 7700 osteoporotic women (32) and alendronate (33) in 4400 women with low bone mass, neither study demonstrated a significant reduc-
tion in the incidence of nonvertebral fractures. However, in a post hoc analysis of the alendronate study subgroup with a T score less than −2.5 at the hip, there was a 25% reduction in fractures (33).

All treatments used in this study significantly reduced bone resorption but HRT/ERT was more potent than calcitriol. Although calcitriol is the most potent bone-resorbing agent in vitro in bone cells (47), the opposite is true in humans as shown in this study. Antiresorptive agents have minimal, if any, positive effects on the peripheral cortical bone compared with their effect in trabecular-rich sites such as the spine and trochanter, so it is not surpassing that elderly women continue to sustain nonvertebral fractures. Importantly, HRT/ERT and the combination HRT/ERT with calcitriol not only increased BMD in the radius, but also led to a 3.5–4.0% greater radial BMD than placebo after 3 yr. If this effect were to continue over several years there is the potential to significantly strengthen important cortical sites such as the calcar femorale in the femoral neck. In this respect, these therapies could provide better protection to the cortical sites than do alendronate and raloxifene.

Falls were a secondary outcome in the study and the observation that the women in the calcitriol group but not the combination group fell significantly less frequently than those on placebo is intriguing and could be one explanation for the trend toward lower fracture incidence on calcitriol. While it is not clear how calcitriol might reduce falls or why the combination with HRT attenuates the effect of calcitriol alone, this deserves further study. Although the information on falls was collected prospectively at each visit, it would be more accurate in future studies to collect information on falls using a more frequent diary.

The common side effects associated with estrogen therapy were not insignificant in these older women, particularly in the first few months. Bleeding and breast tenderness were the main symptoms. Bleeding led to discontinuation from the study of 21 women (11%), and of 13 women (4.9%) for breast tenderness. These two problems accounted for much of the difference between the drop out of 18% in the placebo groups and 36% in the estrogen treated arms. Because initiating hormones for the first time in elderly women can produce significant side effects and cause discontinuation of therapy, strategies to reduce the frequency and severity of side effects, such as starting on a lower dose for the first few weeks could increase acceptability and compliance.

The adverse events related to calcitriol were not serious and had little impact on retention and compliance with medication. Hypercalcemia that was mild occurred infrequently over 3 yr of treatment and resolved quickly. Hypercalcemia although more frequent, was not a persistent problem. Most episodes of hypercalcemia were probably related to temporary perturbations of dietary calcium intake. However, to avoid significant clinical problems with calcitriol, it may be advisable to limit the total calcium intake to 800–1000 mg daily. Both conditions occurred less frequently in the groups on combination HRT/ERT + calcitriol.

Both HRT/ERT and HRT combined with calcitriol prevent bone loss at all skeletal sites in elderly women. Combination treatment for osteoporosis is emerging as a promising modality because two agents can produce a greater effect on bone than a single agent (48, 49) can. Combination treatment increases the likelihood that more women will experience gains in bone density, and should be considered for the treatment of patients who continue to lose bone or fracture on single therapy.

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