Ultralow-Dose Micronized 17β-Estradiol and Bone Density and Bone Metabolism in Older Women

A Randomized Controlled Trial

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Context Estrogen therapy is known to prevent osteoporosis, but studies have shown that conventional doses increase adverse events. Whether lower doses, one quarter of standard treatment, prevent bone loss is not known.

Objective To examine the effect of 3 years of treatment with 0.25 mg/d of micronized 17β-estradiol on bone mineral density (BMD) and bone turnover in healthy older postmenopausal women.

Design, Setting, and Participants Randomized, double-blind, placebo-controlled trial conducted from July 24, 1998, through June 14, 2002, at a university general clinical research center in the United States. Healthy, community-dwelling women (N = 167) who were older than 65 years at enrollment.

Intervention Dosage of 0.25 mg/d of micronized 17β-estradiol (n = 83) or placebo (n = 84); all women who had not had a hysterectomy received 100 mg/d of oral micronized progesterone for 2-week periods every 6 months.

Main Outcome Measures The BMD of the hip, spine, wrist, and total body measured annually for 3 years. Serum and urine biochemical markers of bone resorption and formation and sex hormones were measured at baseline, 3 months, and during years 1 and 3 of treatment.

Results Mean BMD increased at all sites for participants taking low-dose estrogen (17β-estradiol) compared with placebo ($P<.001$). Compared with participants receiving placebo, participants taking low-dose estrogen had BMD increases of 2.6% for the femoral neck; 3.6%, total hip; 2.8%, spine; and 1.2%, total body. Markers of bone turnover, N-telopeptides of type 1 collagen, and bone alkaline phosphatase decreased significantly ($P<.001$) in participants taking low-dose estrogen compared with placebo. Estradiol, estrone, and sex hormone–binding globulin levels increased in the estrogen-treated group compared with placebo. The adverse effect profile was similar; specifically, there were no statistically significant differences in breast tenderness, changes in endometrial thickness or pathological effects, or annual mammographic results between the 2 groups. The number of abnormal mammograms over 3 years was 15 for the low-dose estrogen group and 10 for the placebo group (8 occurred at baseline) ($P = .26$). There were no reports of breast cancer during the study.

Conclusions In older women, a dosage of 0.25 mg/d of 17β-estradiol increased bone density of the hip, spine, and total body, and reduced bone turnover, with minimal adverse effects. Future studies evaluating the effect of low-dose estrogen on fractures are indicated.

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OSTEOPOROSIS IS A MAJOR cause of disability and excess mortality in older women. Estrogen therapy has been used for treatment and prevention of osteoporosis; however, many older women are reluctant to use it because of adverse effects. Recent data from the Women's Health Initiative demonstrated that postmenopausal women who took hormone therapy for approximately 7 years had decreased hip fracture risk; however, the dose and preparation of hormone therapy used in the study also increased the risk of breast cancer, heart disease, stroke, and deep venous thrombosis.[1] Previous studies demonstrated that a conventional dose of estrogen therapy (1.0 mg/d of 17β-estradiol or 0.625 mg/d of conjugated equine estrogen [CEE]) reduced bone turnover and bone loss and was associated with reduced fracture incidence in older women.[2-4] Moreover, half the conventional hormone therapy dose (0.3 mg/d of CEE and 2.5 mg/d of medroxyprogesterone acetate [MPA] or 0.5 mg/d of 17β-estradiol) decreased bone turnover and increased bone mass in older women when taken with adequate doses of calcium and vitamin D.[5,6] We previously demonstrated that 0.25 mg/d of 17β-estradiol decreased markers of bone turnover to the same degree as 0.5 mg/d or 1.0 mg/d of 17β-estradiol with an adverse effect profile that was equivalent to placebo.[7] We designed this study to determine the long-term effects on bone and overall safety of treatment with 0.25 mg/d of 17β-estradiol in older women.

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METHODS

Healthy, community-dwelling women who were older than 65 years were recruited to participate in the study. The exclusion criteria were (1) diseases or medications affecting bone metabolism; (2) use of estrogen or calcitonin within the past 6 months; (3) ever use of bisphosphonates or fluoride; (4) history of breast or endometrial cancer within the past 5 years; (5) baseline endometrial thickness greater than 5 mm; (6) any thromboembolic event within 6 months; and (7) bone mineral density (BMD) *t* score of less than –4; or (8) symptomatic vertebral fracture within the past year or past history of low trauma hip fracture. White women were recruited by newspaper advertisement or by letter of invitation from existing databases. Black and Hispanic women were recruited through community presentations on osteoporosis and BMD screening sessions.

The institutional review board at the University of Connecticut Health Center approved the study, and all women provided written informed consent. Eligible women were randomly assigned to treatment with 0.25 mg/d of 17β-estradiol or placebo using a computer-generated list. The research pharmacist maintained the randomization list; all other staff members, as well as all participants, were blinded to treatment allocations. All women who had not had a hysterectomy received 100 mg/d of micronized progesterone for 2 weeks every 6 months. All participants received 1300 mg/d of elemental calcium and 1000 IU/d of vitamin D. The primary outcome was BMD of the hip, which was measured at baseline and then annually for 3 years. We also evaluated spine, wrist, and total body BMD. Serum and urine markers of bone turnover and sex hormones (estradiol, estrone, and sex hormone–binding globulin [SHBG]) were measured at baseline, 3 months, and during years 1 and 3 of treatment. Dietary calcium intake was estimated at baseline and then yearly using a 4-day food diary. Physical activity was assessed at baseline and then yearly using the Physical Activity Scale for the Elderly.[8] Adverse effects were assessed by questionnaire using a Likert scale and by diary completion. Endometrial thickness was measured by transvaginal ultrasound at baseline and every 6 months prior to progesterone administration. We completed endometrial biopsies in women with endometrial thickness of 8 mm or greater and asked all women to undergo endometrial biopsy at the 3-year visit. Women had annual mammograms while enrolled in the study. We measured adherence to calcium intake and study medication using pill counts every 3 months.

Bone density was measured by dual-energy x-ray absorptiometry (Lunar DPXL, Madison, Wis). The coefficient of variation of BMD measurement, based on reproducibility scans completed in women older than 65 years, are 1.3% for femoral neck; 1.1%, total hip; 1.7%, L1-L2 spine; 2.2%, L2-L4 spine; 3.7%, ultradistal wrist; 1.3%, 33% radius; and 0.5%, total body.

Fasting serum and urine samples were collected between 7 AM and 9:30 AM and stored at –80°C. Bone marker assays were run in duplicate after one thaw; all samples for one individual were assayed using the same kit. We measured bone alkaline phosphatase (Metra Biosystems Inc, Mountain View, Calif) and urinary crosslinked N-telopeptides (Ostex International Inc, Seattle, Wash) using enzyme-linked immunosorbent assay. Intra-assay variability was 8% for N-telopeptides and 5% for bone alkaline phosphatase. We measured estradiol and estrone using radioimmunoassay and SHBG using immunoradiometric assay (Diagnostic Systems Lab Inc, Webster, Tex). The intra-assay variability for these studies in the general clinic research center laboratory is lower than 10%. The detection limit of the estradiol assay is 2 pg/mL. The level of 25-hydroxyvitamin D was measured by competitive protein binding (Esoterix Inc, Calabasas, Calif).

Baseline and clinical characteristics are reported using means and SDs stratified by treatment group. We tested for the difference in baseline characteristics between the treatment groups using 1-way analysis of variance and χ^2 analysis. We tested for normality of distribution of all variables and none required transformation. We analyzed the primary outcome data using mixed models procedural software (SAS PROC MIXED, SAS Institute Inc, Cary, NC). The dependent variables were BMD measurements at baseline, and at 1, 2, and 3 years. The fixed effects were treatment group, time, and treatment group time interaction. The random effects were individual subjects as well as time nested within subject. The main variable of interest was the treatment group time interaction term, which demonstrated whether the changes in BMD were different in the 17β-estradiol treatment group compared with placebo. We completed a separate analysis for each BMD site. We estimated the difference in absolute and percentage BMD change between placebo and treatment group at 3 years and report these for women who completed the study. To account for

Table 1. Baseline Characteristics

Variable	Placebo (n = 84)	17β-Estradiol (n = 83)
	Mean (SD)	
Age, y	74.7 (4.9)	73.8 (5.1)
Age at menopause, y	47.0 (7.6)	47.7 (6.6)
Body mass index*	28.0 (4.9)	28.4 (5.1)
Intake per day		
Calcium, mg	656 (269)	752 (366)
Vitamin D, IU	155 (82)	161 (114)
Endometrial thickness, mm	2.8 (1.4)	3.0 (1.4)
Physical Activity Scale for the Elderly†	101 (51)	122 (60)
	No. (%) of Participants	
Prior estrogen therapy use	0	2 (2)
Hysterectomy	27 (32)	32 (39)
Prior fractures		
Wrist	24 (28)	25 (30)
Vertebral	6 (7)	4 (5)
Hip	0	2 (2)
Smoking status		
Current	4 (5)	2 (2)
Past	26 (31)	33 (40)
Never	54 (64)	48 (58)
Race		
Black	14 (17)	11 (13)
Hispanic	19 (23)	15 (18)
White	50 (59)	56 (68)
Other	1 (1)	1 (1)

*Calculated as weight in kilograms divided by the square height in meters.
†$P = .02$ for comparison. The range of possible values in older persons is 0 to 360. Comparisons of other baseline characteristics were all $P > .07$.

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dropouts using an intention-to-treat approach, we used Gaussian linear mixed models to use all available data points without imputation. In a secondary analysis, we controlled for age, height, and weight as fixed effects. We used the same methods to test for differences in bone turnover and sex hormones. We compared adverse effects in the 2 groups using either repeated measures analysis of variance or χ^2 tests (SPSS Version 10.0, SPSS Inc, Chicago, Ill). For all analyses, the level of significance was $P<.05$.

RESULTS

The baseline characteristics of the 167 women included in the study from July 24, 1998, through June 14, 2002, and randomized to treatment are listed in TABLE 1 and TABLE 2. Baseline characteristics did not differ significantly between groups except for activity level on the Physical Activity Scale for the Elderly, which was higher in the 17β-estradiol treatment group ($P=.02$). Over the 3-year study period, 31 women (36%) discontinued participation in the placebo group and 24 (29%) in the 17β-estradiol group (FIGURE 1). Adherence was the same for both groups and ranged from 87% to 94% for study medication and 78% to 92% for calcium supplementation (compliance over 3 years and checked every 3 months).

Low-dose estrogen significantly increased bone density at all BMD sites compared with placebo (TABLE 3 and FIGURE 2). Controlling for age, weight, and height did not change our findings, although the placebo group had a 2% weight loss (67.2 kg at baseline to 65.9 kg at 3 years) while the 17β-estradiol group remained stable (68.5 kg at baseline to 68.3 kg at 3 years). Women who received progesterone had similar BMD changes as women who did not. Compared with the placebo group, participants who received 17β-estradiol treatment had significantly decreased markers of bone turnover (TABLE 4). In the 17β-estradiol treatment group, crosslinked N-telopeptides of type 1 collagen, a marker of bone resorption, decreased 28% at 3 months, 43% at 1 year, and 24% at 3 years compared with placebo. The difference in the change in crosslinked N-telopeptides of type 1 collagen between placebo and 17β-estradiol treatment was statistically significant at all time points. The bone alkaline phosphatase level decreased significantly in the treatment group compared with the placebo group at 1 year and 3 years, but not at 3 months ($P=.79$). Estrone and estradiol levels increased significantly ($P<.001$) in the treatment group compared with the placebo group (Table 4). Both mean estradiol and estrone levels increased in the treatment group by 3 months and then remained constant for the duration of the study. Levels of SHBG increased by 13% in participants after 3 years of treatment compared with the placebo group. Because of the large increase in estrone and estradiol levels and the relatively smaller change in SHBG, the free estrogen index (17β-estradiol + estrone/SHBG) increased over time.

Endometrial thickness increased slightly in both groups over the 3-year study but there was no significant difference between groups except at year 2 (TABLE 5). Over 3 years, 5 women reported bleeding in the 17β-estradiol group. Three women reported scheduled bleeding after taking progesterone and 2 reported unscheduled bleeding. Of the 2 women with unscheduled bleeding, one had 3 episodes of spotting within 4 months of her 18-month visit and the other had 1 week of bleeding prior to her 6-month visit and then again following an endometrial biopsy at 12 months. There was no statistically significant dif-

Table 2. Baseline Bone Mineral Density, Bone Turnover Markers, and Hormone Measurements*

	Placebo (n = 84)	17β-Estradiol (n = 83)
Bone Mineral Density, g/cm²		
Femoral neck	0.813 (0.114)	0.825 (0.140)
t Score	−1.39 (0.95)	−1.29 (1.17)
No. (%) with osteoporosis	10 (12)	11 (13)
No. (%) with osteopenia	42 (50)	45 (54)
Total hip	0.874 (0.134)	0.901 (0.156)
t Score	−1.05 (0.12)	−0.82 (0.14)
L2-L4 spine	1.082 (0.203)	1.080 (0.232)
t Score	−0.98 (1.69)	−0.99 (1.93)
No. (%) with osteoporosis	16 (19)	16 (19)
No. (%) with osteopenia	24 (29)	31 (37)
L1-L2 spine	0.979 (0.167)	0.989 (0.224)
t Score	−1.42 (1.39)	−1.34 (1.87)
Ultradistal wrist	0.308 (0.055)	0.311 (0.063)
t Score	−1.93 (1.50)	−1.87 (1.74)
33% Radius	0.567 (0.077)	0.573 (0.081)
t Score	−2.11 (1.08)	−2.02 (1.14)
Total body	1.068 (0.092)	1.077 (0.099)
t Score	−0.71 (1.15)	−0.59 (1.24)
Markers of Bone Turnover		
Bone alkaline phosphatase, U/L	27.9 (11.6)	27.7 (10.1)
N-telopeptides of type 1 collagen, nmol BCE per mmol/L	40.7 (28.5)	42.9 (29.4)
Hormone Measurements		
Estrone, pg/mL	20.8 (11.6)	18.8 (11.1)
Estradiol, pg/mL	9.8 (3.8)	9.7 (4.3)
Sex hormone–binding globulin, nmol/L	41.2 (16.3)	39.8 (15.4)
Parathyroid hormone, pg/mL	49.0 (37.2)	46.5 (34.5)
25-Hydroxyvitamin D, ng/mL	22.7 (8.1)	21.9 (7.7)

Abbreviation: BCE, bone collagen equivalents.
*Values are presented as mean (SD). $P\geq.24$ for all comparisons.

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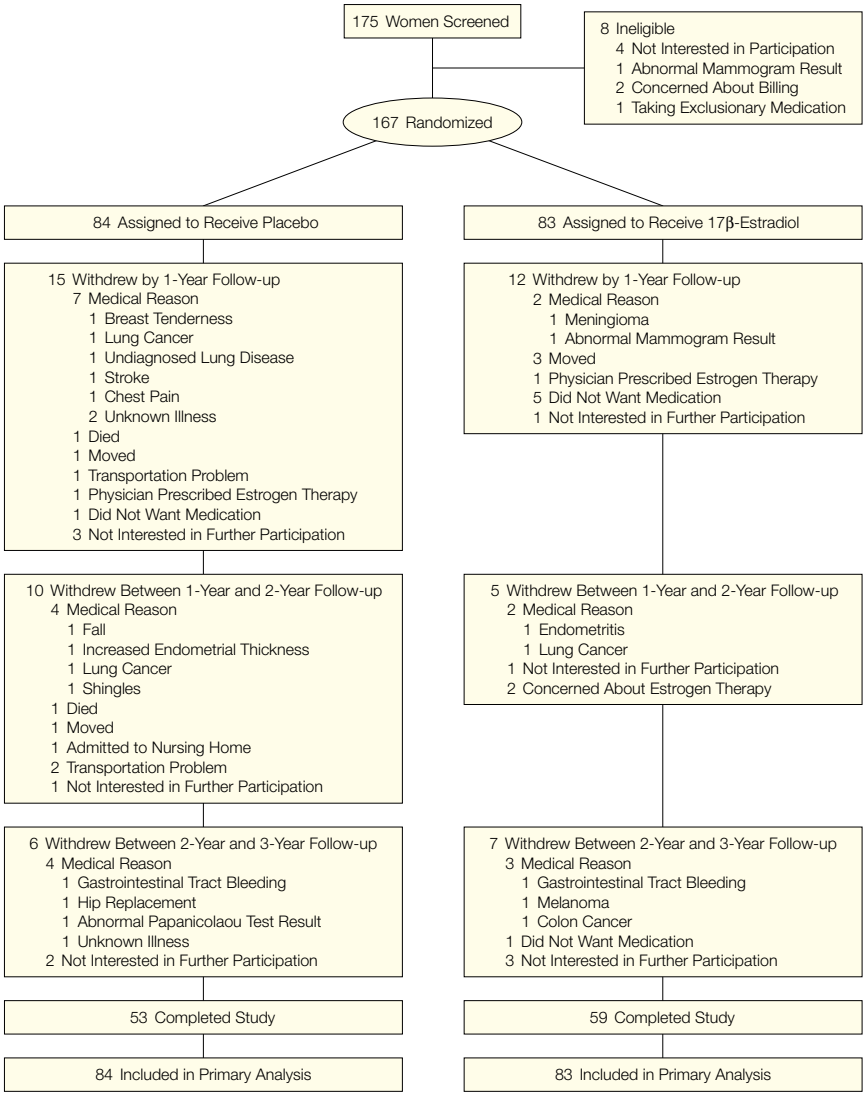
ference in endometrial biopsy categories between the 2 groups. Four women (3 in the 17β-estradiol group and 1 in the placebo group) required interim endometrial biopsies. Of these women, 1 woman (in the 17β-estradiol group) had 4 biopsies—2 biopsies showed atrophic endometrium and 1 showed hyperplastic endometrium; the final biopsy (after progesterone treatment for hyperplasia) demonstrated atrophic endometrium. Another woman (in the placebo group) had hyperplasia and was treated; the repeat biopsy demonstrated atrophic endometrium, but she dropped out of the study. The other 2 women in the 17β-estradiol group had proliferative endometrial tissue on one biopsy and atrophic endometrium on the final biopsy. One additional woman had weakly proliferative endometrium on the final biopsy with an endometrial thickness of 9 mm. By χ^2 analysis, breast tenderness, fluid retention, bloating, and headache were not significantly different between the groups (Table 5) except more headaches were reported in the placebo group at 3 years. There was no significant difference in the total number of abnormal mammogram results (n=15 in the 17β-estradiol group vs n=10 in the placebo group; $P=.26$) or in the number of abnormal follow-up mammogram results (n=12 in 17β-estradiol group vs n=5 in placebo group; $P=.07$). One woman in the 17β-estradiol group dropped out of the study secondary to an abnormal mammogram result at 6 months. There were no reports of breast cancer during the study.

The number of low trauma fractures was higher in the placebo group than in the 17β-estradiol group, but there were only 8 incident fractures. The placebo group had 2 wrist, 1 patella, 1 rib, and 2 hand/finger fractures while the treatment group had 1 humerus and 1 hand/finger fracture. There were no symptomatic vertebral or hip fractures.

COMMENT

Our study demonstrates that 0.25 mg/d of 17β-estradiol increased BMD at the hip, spine, and wrist and decreased markers of bone turnover in older women. The long-term adverse effect profile of this dose of estrogen was similar to placebo. These data suggest that this low dose of estrogen had a beneficial effect on bone without the adverse effects typically associated with estrogen therapy. These data confirm and expand our previous study, which demonstrated an equivalent short-term effect with dosages of 0.25 mg/d, 0.5 mg/d, and 1.0 mg/d of 17β-estradiol on markers of bone turnover.[7] The dose of estrogen used in this study was equivalent to 0.15 mg/d of CEE or one quarter of the usual estrogen dose and half the dose used in previous studies in older women.[6] Our BMD changes are similar to those published by Recker et al[6] who demonstrated that 0.3 mg/d of CEE, half the usual estrogen dose, plus 2.5 mg/d of MPA increased BMD of the hip and spine in older women (mean age, 73 years) who received adequate vitamin D. The only other well-controlled study to specifically examine the effect of estrogen in older women (mean age, 82 years) used 0.625 mg/d of CEE plus 2.5 mg/d of MPA for 9 months and demonstrated a beneficial effect.[4] Previous studies, conducted in younger postmenopausal

Figure 1. Flow Diagram



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women, support the use of low-dose estrogen for prevention of bone loss.[9-11] Most recently, Lindsay et al[12] compared 0.3 mg/d, 0.45 mg/d, and 0.625 mg/d of CEE with placebo (mean age, 52 years) and showed that all 3 doses prevented bone loss and decreased markers of bone turnover over 2 years; similar results were seen for CEE plus MPA. Spine BMD, but not hip BMD, differed significantly between the group that received 0.625 mg/d and 0.3 mg/d of CEE. Mizunuma et al[13] also demonstrated that 0.3 mg/d of CEE increased BMD of the spine by about 3% over 2 years, again similar in magnitude to our results. Our results also were similar to those reported in a recent meta-analysis of the efficacy of hormone therapy on bone.[14] This study is the first, to our knowledge, to examine an ultra-low estrogen dose in a long-term randomized placebo-controlled study.

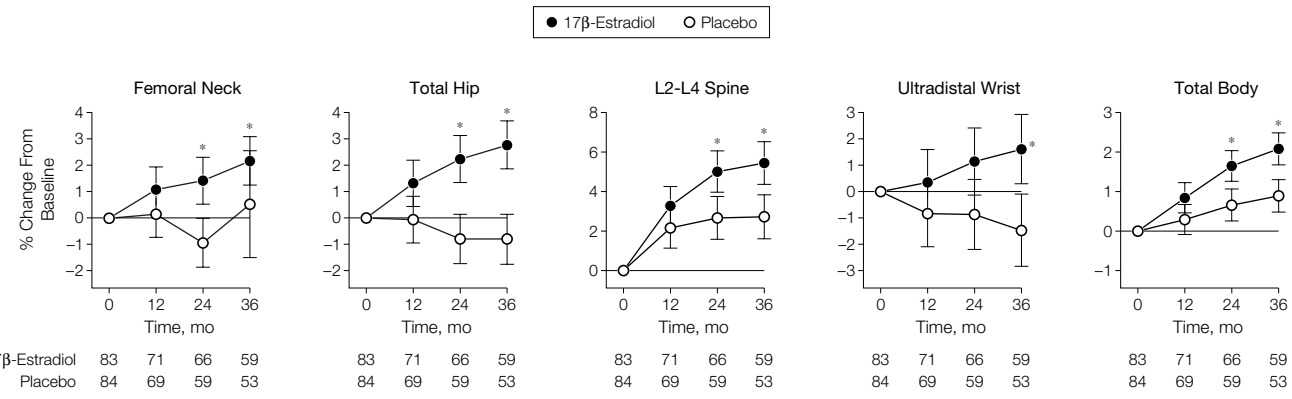
The increase in BMD, compared with placebo, in the current study (femoral neck 2%, total femur 4%, lumbar spine 3%) are somewhat smaller than BMD changes seen with antiresorptive agents that have been shown to reduce fractures in postmenopausal women.[15-17] However, most fracture studies were completed in postmenopausal women with established osteoporosis (*t* score < −2.5 or presence of vertebral fracture at baseline) who were younger than women in the current study. In the only prospective study of BMD and fracture using estrogen, Lufkin et al[15] reported decreased vertebral fracture risk after 1-year treatment with transdermal 17β-estradiol plus medroxyprogesterone acetate with a relative risk of 0.39; the mean increase in lumbar spine BMD, compared with placebo, was 5%. In a 3-year study of raloxifene on vertebral fracture risk, the increase in lumbar spine

Table 3. Estimates of the Absolute Difference in Bone Mineral Density Change Over 3 Years (Intention to Treat Analysis)*

Bone Mineral Density Site, g/cm²	Final Bone Mineral Density, Mean (95% CI), g/cm²		Absolute Difference Over 3 Years Between Groups		Absolute Difference From Baseline Within Groups, Mean (95% CI), g/cm²	
	Placebo	17β-Estradiol†	Mean (95% CI), g/cm²	*P* Value	Placebo	17β-Estradiol†
Hip						
Femoral neck	0.809 (0.780 to 0.837)	0.843 (0.814 to 0.871)	0.0218 (0.0107 to 0.0330)	<.001	−0.0016 (−0.0102 to 0.007)	0.0183 (0.0079 to 0.0287)
Adjusted‡	0.822 (0.796 to 0.849)	0.830 (0.816 to 0.868)	0.0174 (0.0061 to 0.0286)	.003		
Total hip	0.867 (0.835 to 0.900)	0.926 (0.893 to 0.958)	0.0318 (0.0211 to 0.0425)	<.001	−0.0042 (−0.0137 to 0.0052)	0.0250 (0.0162 to 0.0338)
Adjusted‡	0.883 (0.854 to 0.912)	0.924 (0.895 to 0.953)	0.0248 (0.0149 to 0.0349)	<.001		
Trochanter	0.746 (0.718 to 0.773)	0.792 (0.765 to 0.819)	0.0357 (0.0221 to 0.0494)	<.001	−0.0063 (−0.0174 to 0.0047)	0.0258 (0.0145 to 0.0371)
Adjusted‡	0.746 (0.718 to 0.773)	0.792 (0.765 to 0.819)	0.0269 (0.0141 to 0.0396)	<.001		
L2-L4 Spine	1.11 (1.06 to 1.16)	1.14 (1.09 to 1.19)	0.0283 (0.0121 to 0.0445)	<.001	0.0310 (0.0177 to 0.0444)	0.0609 (0.0462 to 0.0756)
Adjusted‡	1.10 (1.06 to 1.16)	1.13 (1.09 to 1.18)	0.0279 (0.0116 to 0.0442)	<.001		
Ultradistal wrist	0.306 (0.293 to 0.319)	0.317 (0.304 to 0.330)	0.0092 (0.0035 to 0.0144)	.001	−0.0051 (−0.0102 to 0)	0.0044 (−0.0002 to 0.0090)
Adjusted‡	0.311 (0.298 to 0.323)	0.317 (0.305 to 0.329)	0.0073 (0.0020 to 0.0126)	.007		
Total body	1.08 (1.06 to 1.09)	1.10 (1.07 to 1.12)	0.0126 (0.0067 to 0.0186)	.001	0.0102 (0.0047 to 0.0158)	0.0234 (0.0182 to 0.0286)
Adjusted‡	1.08 (1.04 to 1.11)	1.09 (1.05 to 1.13)	0.0104 (0.0041 to 0.0167)	.001		

Abbreviation: CI, confidence interval.
*Intention-to-treat analysis used Gaussian linear mixed models to account for missing data; absolute difference between groups is adjusted.
†Dosage of 0.25 mg/d.
‡Adjusted for height, weight, and age.

Figure 2. Effect of Low-Dose 17β-Estradiol and Placebo on Bone Mineral Density



Values are mean percentage change with 95% confidence intervals. Asterisk represents difference compared with the placebo group: femoral neck, *P*=.03 for 24 months and *P*<.001 for 36 months; total hip, *P*<.001 for 24 and 36 months; L2-L4 spine, *P*=.02 for 24 months and *P*<.001 for 36 months; ultradistal wrist, *P*<.001 for 36 months; and total body, *P*=.02 for 24 months and *P*<.001 for 36 months.

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BMD was 2.6% compared with placebo and this was associated with approximately 30% reduction in new vertebral fractures; the incidence of nonvertebral fractures did not decrease with raloxifene.[16] Risedronate treatment resulted in BMD increases of 4.3% and 2.8% for the lumbar spine and femoral neck, respectively; these changes were associated with a 41% decrease in new vertebral fractures and a 39% decrease in nonvertebral fractures.[17] The initial alendronate study[18] reported somewhat larger BMD changes, however a later study in women with low BMD without prevalent vertebral fractures over 3 years were more comparable with the current study (femoral neck 3%, total femur 4%, lumbar spine 6%).[19] In the latter study, fracture reduction only occurred in the subset of women with osteoporosis by BMD. Given the different populations involved in the studies, it is difficult to predict whether BMD changes in the current study will result in decreased fracture rates. Nonetheless, the BMD changes seen in this study are comparable with changes seen in fracture studies of approved agents for prevention and treatment of osteoporosis, particularly raloxifene and risedronate.

The change in markers of bone turnover is consistent with BMD changes. N-telopeptides, a marker of bone resorption, decreased within 3 months in participants receiving 17β-estradiol treatment while bone alkaline phosphatase levels did not decrease until 1 year. This is similar to previous studies of 17β-estradiol that demonstrated an immediate effect of 17β-estradiol on bone resorption with a later effect on bone formation, presumably due to coupling of bone formation and resorption.[3,4,7,12] Furthermore, while estrogen increased estradiol and estrone levels, it had little effect on SHBG levels, resulting in an increased free estrogen index over time.

Cummings et al[20-22] reported that women with undetectable levels of endogenous estradiol (<5 pg/mL) had an increased risk for bone loss and fracture. Women with estradiol levels lower than 5 pg/mL experienced 0.8% per year

Table 4. Estimates of the Difference in Markers of Bone Turnover and Hormones Over 3 Years

Marker or Hormone	Final Value (95% CI)		Absolute Difference at 3 Years Between Groups (95% CI)†	Difference at 3 Years Between Groups, %	*P* Value for Absolute Difference
	Placebo	17β-Estradiol*			
Estrone, pg/mL	20.5 (10.1 to 30.9)	80.4 (70.7 to 90.1)	61.8 (45.0 to 78.7)	326	<.001
Estradiol, pg/mL	9.1 (6.2 to 12.0)	26.1 (23.4 to 28.8)	17.1 (12.3 to 21.9)	177	<.001
Sex hormone–binding globulin, nmol/L	43.8 (39.9 to 47.7)	45.5 (41.8 to 49.3)	3.2 (0.46 to 5.9)	13	.02
N-telopeptides of type 1 collagen, nmol BCE per mmol/L	37.1 (33.0 to 44.0)	32.4 (26.4 to 36.8)	–9.1 (–15.9 to –2.41)	24	.008
Bone alkaline phosphatase, U/L	25.5 (23.3 to 27.7)	22.5 (20.3 to 24.6)	–2.8 (–4.5 to –1.03)	10	.002

Abbreviations: BCE, bone collagen equivalents; CI, confidence interval.
*Dosage of 0.25 mg/d.
†Intention-to-treat analysis used Gaussian linear mixed models to account for missing data; absolute difference between groups is adjusted.

Table 5. Number of Women Reporting Specific Adverse Effects

	Baseline			Year 1			Year 2			Year 3		
	Placebo (n = 84)	Estradiol (n = 83)*	*P* Value	Placebo (n = 69)	Estradiol (n = 71)*	*P* Value	Placebo (n = 59)	Estradiol (n = 66)*	*P* Value	Placebo (n = 53)	Estradiol (n = 59)*	*P* Value
Headache												
Mild	10	9		8	10		8	4		7	3	
Moderate	3	7	.56	4	5	.97	3	3	.46	7	1	.03
Severe	3	4		2	2		3	2		1	1	
Bloating												
Mild	8	12		9	3		4	6		3	8	
Moderate	4	1	.27	1	1	.22	0	3	.22	1	0	.41
Severe	0	0		0	1		0	0		1	1	
Fluid retention												
Mild	8	8		3	9		2	6		0	4	
Moderate	1	2	.48	0	2	.08	4	4	.64	0	1	.10
Severe	0	2		0	0		1	1		0	0	
Breast tenderness												
Mild	3	5		7	10		6	5		3	5	
Moderate	1	1	.75	2	4	.56	0	5	.12	1	0	.52
Severe	0	0		0	0		1	0		0	1	
Endometrial thickness, mm												
Mean (SD)	2.8 (1.4)	3.0 (1.4)	.71	3.5 (1.9)	4.4 (2.7)	.12	3.6 (1.4)	4.6 (2.8)	.04	3.4 (1.7)	4.8 (3.6)	.40
No. of women with thickness >5 mm prior to progesterone therapy†	3	2		4	12		5	12		5	7	

*Dosage of 17β-estradiol of 0.25 mg/d.
†Endometrial thickness of 5 mm or less has not been associated with endometrial pathology in clinical trials; if endometrial thickness is greater than 8 mm, a biopsy is universally recommended.

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bone loss at the hip, while those with levels higher than 10 pg/mL experienced only 0.1% per year loss. In the same study, high SHBG levels were also associated with increased bone loss at the hip. Our study demonstrates that low-dose estrogen, which increased estradiol and estrone, with a smaller effect on SHBG, had a beneficial effect on bone at all important fracture sites. Our 3-year intervention study supports the epidemiological data from the Study of Osteoporotic Fractures[20-22] that estradiol levels need not be in the premenopausal range to protect bone and suggests that the usual dose of estrogen may increase adverse effects with minimal additional benefit to bone.

The adverse effect profile in the 17β-estradiol treatment group in our study was similar to placebo, confirming the tolerability of low-dose estrogen over 3 years. We used lower dose progesterone every 6 months and that regimen protected the endometrium of women who had not had a hysterectomy, although further studies are required to fully determine the safety of this progesterone dose. Only 2 women (one in each group) had endometrial hyperplasia on biopsy and both of these women had increased endometrial thickness. The reasons for discontinuation were similar in both groups suggesting that low-dose estrogen did not result in adverse health outcomes for older women.

This study has limitations. It is a fairly small randomized controlled study with the primary outcome of BMD. The length of the study is similar to previous studies in this population but was neither long enough nor large enough to discern the effect of low-dose estrogen on fracture incidence or major adverse effects such as cardiovascular disease, stroke, or breast cancer. However, despite these limitations, the positive effect on BMD is promising. The preparation of estrogen used was 17β-estradiol while most previous studies used CEE. Conjugated equine estrogen and 17β-estradiol may not have the same effects on bone or produce the same adverse events, although studies have not directly compared these 2 different estrogen preparations. In a previous 3-year study using 0.3 of mg/d of CEE plus 2.5 mg/d of MPA in older women, the adverse effect profile was also favorable.[6]

This study demonstrates that 0.25 mg/d of 17β-estradiol increased BMD at important fracture sites and decreased bone turnover in older women with minimal adverse effects. The data from the Women's Health Initiative regarding the potential adverse effects of hormone therapy are a concern. However, our study supports a beneficial effect of lower dose estrogen on BMD and we hypothesize that lowering the dose of estrogen may also reduce the number of adverse events over a longer study period. Because BMD is an intermediate outcome, studies of fracture incidence are essential to fully evaluate the use of ultralow-dose estrogen in older women.

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Author Contributions: As principal investigator, Dr Prestwood had full access to all study data and takes responsibility for the integrity of the data and accuracy of the data analysis.
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Acquisition of data: Prestwood.
Analysis and interpretation of data: Prestwood, Kleppinger, Kulldorff.
Drafting of the manuscript: Prestwood, Kleppinger.
Critical revision of the manuscript for important intellectual content: Prestwood, Kenny, Kulldorff.
Statistical expertise: Kulldorff.
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Study supervision: Prestwood.

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