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Anabolic Effect of Estrogen Replacement on Bone in Postmenopausal Women with Osteoporosis: Histomorphometric Evidence in a Longitudinal Study*

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ABSTRACT

It is well recognized that estrogen (E_2) prevents postmenopausal bone loss by suppressing bone resorption. Despite evidence that E_2 may also stimulate bone formation in animals, an anabolic effect in humans is still controversial. To investigate this, we studied 22 older postmenopausal females, with a mean age of 65.4 yr and mean interval of 16.9 yr since menopause and low bone mineral density. Transcortical iliac bone biopsies were performed before and 6 yr after E_2 replacement therapy (ERT) [75 mg percutaneous E_2 replaced 6-monthly plus oral medroxy progesterone acetate (5 mg daily) for 10 days each calendar month]. The mean serum E_2 level after 6 yr of treatment was 1077 (range, 180-2568) pmol/L. Bone mineral density improved in every patient, with a median increase of 31.4% at the lumbar spine and 15.1% at the proximal femur. Bone histomorphometry showed an increase in cancellous bone volume from 10.75% to 17.31% ($P < 0.001$). The wall thickness after 6 yr of E_2 treatment was 38.30 μm compared with 31.20 μm before commencement of ERT ($P < 0.0005$), indicating net bone gain. This is the first report showing histological evidence for an increase in cancellous bone volume, together with an increase in wall thickness, in a longitudinal follow-up study of ERT in older postmenopausal women. Our results show that E_2 is capable of exerting an anabolic effect in women with osteoporosis, even when started well into the menopause. (*J Clin Endocrinol Metab* **86:** 289–295, 2001)

OSTEOPOROSIS IS CHARACTERIZED by bone loss and disruption of cancellous architecture, resulting in bone fragility and increased susceptibility to fracture (1). Currently available therapies, such as estrogen, bisphosphonates, and calcitonin inhibit bone resorption and lead to moderate increases in bone density and reduction in fractures. In patients with severe osteoporosis, the ideal treatment would be one that stimulates bone formation, increases bone mass, and restores trabecular connectivity.

Despite the established role of estrogen (E_2) in the treatment of postmenopausal osteoporosis, its mechanism of action on bone is still uncertain. E_2 is generally considered to act by suppression of bone resorption, and whether or not E_2 exerts an anabolic effect on the human skeleton remains controversial.

There is some evidence to suggest that E_2 may exert an anabolic effect in bone. E_2 has been shown to stimulate the differentiation and activity of osteoblasts (2, 3). E_2 replacement therapy (ERT) has also been shown to increase bone formation and bone mass in animal models (4, 5). In humans, an anabolic effect of E_2 before skeletal maturation has been suggested by the low peak bone mass in E_2-deficient adolescent girls and in males with rare genetic syndromes of E_2 deficiency (6, 7). In postmenopausal women, raised serum osteocalcin 2 weeks after E_2 treatment suggests that E_2 may stimulate bone formation (8).

Previous studies with ERT, mainly given orally, have failed to demonstrate an anabolic effect of E_2 in postmenopausal women (9–12). We have shown that the anabolic effect of E_2 demonstrates dose-responsiveness in rats (5). It is, therefore, possible that a similar anabolic effect in humans may be evident with sc estradiol implants, which produce a higher circulating estradiol level and increase bone mineral density (BMD) substantially more than other routes of administration of ERT in postmenopausal women (13). The effect of estradiol implants on the BMD continues as long as the therapy is given (14) and also exhibits a positive dose response (15). It is, however, unclear whether the rise in BMD is due to new bone formation resulting in greater bone mass or due to increased mineralization of the preexisting osteoporotic bone. A recent cross-sectional study of women on long-term high-dose sc E_2 implants found substantial increases in BMD (16), and histomorphometric analysis of their bone biopsies showed a nonsignificant increase in cancellous bone volume but an increase in wall thickness, raising the possibility that E_2 may exert an anabolic effect in bone (17).

Because bone resorption and formation are coupled (18) and E_2 suppresses bone resorption, any anabolic effect of E_2

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on bone formation is likely to be masked and limited. E_2 also reduces the bone turnover rate and, therefore, clear evidence of a positive bone balance may take some time to develop. With the high bone turnover rate in rodents the anabolic effect of E_2 on bone has been shown after short-term therapy (5). In humans, the cancellous bone surface is normally renewed completely every 2–3 yr, whereas the bone turnover period is doubled with ERT (9, 19). Hence, a minimum period of 4–6 yr is likely to be required to demonstrate a clear anabolic effect of E_2 on the human skeleton.

The anabolic effect of E_2 is ideal for elderly women, who are often deemed too old or their bones too osteoporotic to benefit from ERT. We, therefore, conducted a longitudinal study to evaluate the changes in cancellous bone mass, architecture, and turnover in older postmenopausal women with osteoporosis following sc estradiol implants for 6 yr. The purpose of this study was to elucidate the putative anabolic role of E_2 on bone by histomorphometric analysis. The findings have significant implications for the understanding of the action of E_2 and for the treatment of osteoporosis.

Materials and Methods

Patient selection and follow-up

Previously untreated postmenopausal women with suspected osteoporosis were invited to participate in the study. Those who had any high risk factor for osteoporosis other than ovarian failure, previous hip fracture or replacement, suffered from medical disorders, or used any drugs known to affect calcium or bone metabolism were excluded. After an initial screening with dual-energy x-ray absorptiometry (DEXA) scan, 36 women of white European origin, who had osteoporosis according to the WHO criteria (1), were selected. Their BMD either in the lumbar spine or proximal femur was more than 2.5 SD below the mean for young female adults (T score, <-2.5). The demographic features including age, parity, height, weight, body mass index (BMI) of these women were recorded. The interval since natural menopause was noted, but those who had a hysterectomy were considered menopausal from the onset of climacteric symptoms or from the time of surgery if ovaries were removed.

The study was approved by the hospital ethics committee, and informed consent was obtained from the patients before each bone biopsy. We could not obtain ethical approval and consent for a placebo/nontreatment control group in a long-term study because the preventative role of ERT in postmenopausal women with osteopororis is well established. After the initial bone biopsy all women received a 75-mg estradiol implant (Organon Laboratories Ltd., Cambridge, UK), inserted sc in the anterior abdominal wall and replaced at 6-month intervals. Those with an intact uterus were initially given oral medroxy progesterone acetate (MPA) (Upjohn Ltd., Crawley, UK), 5 mg daily for 10 days in each month to protect against endometrial hyperplasia. All women were advised to continue ERT for the long term and avoid any other treatment that alters bone metabolism, including calcium supplementation.

Three women withdrew from the study at 6 months, and five women between the first and second year, either due to side effects of ERT or with an illness unrelated to ERT. The side effects included heavy withdrawal bleeding, mastalgia, and headaches. Among those who continued on ERT, three women later developed heavy withdrawal bleeding. This was managed by increasing the dose and duration of MPA as well as reducing the dose of ERT. At the 6-yr follow-up visit, 24 women remained on long-term ERT and agreed to have another bone biopsy, which was successful in 22 women. The dose and duration of estradiol implantation in these women were: 75 mg for 6 yr (n = 19) and 75 mg for 5 yr, followed by 25 mg for the last year (n = 3). The three women in whom the dose of ERT was reduced after 5 yr to avoid heavy withdrawal bleeding were retained in the study because their serum estradiol levels were still within the premenopausal range. In 19 of these women, a second bone biopsy had been taken from the contralateral side after 1 yr of ERT (10) and the third bone biopsy was taken from the ipsilateral iliac crest as the first biopsy. The final bone biopsy was performed 2 months after the insertion of the last implant when the estradiol level was expected to peak, and serum samples were taken on the same day to measure hormone levels. The DEXA scan was repeated to measure the changes in BMD both at the lumbar spine and the proximal femur.

Bone biopsy and histomorphometry

Before each bone biopsy the patients were given two courses of tetracycline spaced 12 days apart, and the transcortical iliac crest biopsy was performed at a standard site 4 days after the second course of tetracycline. The specimens were fixed in 70% alcohol, then dehydrated through graded alcohols and embedded undecalcified in resin (London Resin Co. Ltd., Basingstoke, UK). To reduce intrasample variation in the results, sections were cut from three levels separated by 200 μm, and four nonconsecutive sections were selected for study. Seven-micrometer sections were stained with Goldner's trichrome and toluidine blue, and 12-μm sections were prepared unstained for fluorescence microscopy. For each sample, two sections were examined with bright field illumination and two other sections under ultraviolet light using a semiautomated computer-assisted image analyzer (Osteomeasure; Osteometrics, Inc., Atlanta, GA).

We measured both static and dynamic histomorphometric parameters as defined by the American Society of Bone and Mineral Research (20) and performed strut analysis to assess cancellous bone architecture (21). Trabecular thickness, separation, and number were derived from measurements of cancellous bone area and surface assuming a parallel plate model (22): 1) cancellous bone volume (%), volume of mineralized bone and nonmineralized bone (osteoid) to total bone tissue volume; 2) trabecular thickness (μm), mean trabecular plate thickness; 3) trabecular separation (μm), mean distance between trabeculae; 4) trabecular number (no./mm^2), number of trabeculae in a defined area; 5) termini (no./mm^2), free ends of trabecular network in a defined area; 6) nodes (no./mm^2), junction or branch points of trabecular network in a defined area; 7) terminus to node ratio; 8) wall thickness (μm), distance from the cement line to the quiescent cancellous bone surface of the completed bone packet; 9) osteoid volume (%), volume of osteoid to cancellous bone volume; 10) osteoid thickness (μm), mean osteoid thickness; 11) osteoid surface (%), osteoid-covered surface to total cancellous bone surface; 12) eroded surface (%), extent of resorption lacunae to cancellous bone surface; 13) double-labeled surface: dLS (%), extent of double-labeled surface to cancellous bone surface; 14) single-labeled surface: sLS (%), the extent of single-labeled surface to cancellous bone surface; 15) mineralizing surface: MS/BS (%), the extent of labeled (dL + 1/2sL) surface to cancellous bone surface; 16) mineral apposition rate (μm/day), mean distance between double-labeled lines divided by the labeling interval of 14 days; 17) adjusted appositional rate [AjAR = MAR * MS/OS (μm/day)], amount of new bone mineralized per day per unit of osteoid-covered surface; 18) bone formation rate: BFR/BS = (MS/BS * MAR)/100 (μm^3/μm^2/day $\times 10^{-2}$), amount of new bone mineralized per day per unit of cancellous bone surface: 19) activation frequency: AcFrq = BFR/W.Th (yr^{-1}), frequency by which new remodeling cycles are initiated at a random location on the cancellous bone surface; 20) formation period: FP = WTh/AjAR (day), time required for an individual remodeling site to complete bone formation; and 21) Active formation period: AcFP = WTh/MAR (days), osteoblast lifespan.

Assessments were confined to the center of the cancellous bone, avoiding the transitional zone. Length measurements were made at $\times$100, and width measurements at $\times$400. Osteoid was measured only when it exceeded 3 μm in thickness. Four equidistant width measurements were taken for osteoid thickness and wall thickness. All resorption cavities (eroded surface) were measured. These were excavations in the bone surface that often had a scalloped contour and were measured regardless of whether they contained cells provided they were 30 μm in length. The measurements were corrected for obliquity of sections and presented in three-dimensional terms. To avoid the interobserver variation in the result, all samples were analyzed independently by one histomorphometrist (S.F.) who was blinded to the patient's identification, their BMD results, and the time of biopsy with the treatment.

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Hormone assay

Serum estradiol and FSH were measured in an automated enzyme-linked immunosorbent assay using the ES700 kits (Roche Diagnostics Ltd., Lewes, East Sussex, UK). The interassay precision for estradiol was 14.9%, 6.5%, and 8.0% at serum levels of 148 pmol/L, 856 pmol/L, and 2135 pmol/L, respectively. The interassay precision for FSH was 2.9%, 2.7%, and 3.0% at serum levels of 7.6 U/L, 16.7 U/L, and 46.3 U/L, respectively.

BMD

The BMD was measured at the lumbar spine and the proximal femur using a Hologic 1000 QDR DEXA scanner (Hologic, Inc., Waltham, MA). The mean coefficient of variation for the densitometer calculated with the daily use of a spinal phantom was 0.67% during the course of the study. The precision *in vivo* was assessed by serial scans in 10 healthy premenopausal volunteers both before pretherapy and 6 yr posttherapy BMD measurements. The coefficients of variation were 0.98% and 0.96% at the lumbar spine and 1.21% and 1.17% at the proximal femur, respectively. There were no major repairs or alterations to the DEXA scanner during the 6 yr of the study. BMD results were presented as absolute values (g/cm^2) but also as the number of SD and percentages above or below the mean result of young female adults (T score) and age-matched female population (Z score). The T and Z scores enabled assessment of the severity of osteoporosis and degree of improvement with therapy.

Statistical analysis

The majority of bone histomorphometry and DEXA scan variable results were not normally distributed and, thus, presented as median with interquartile range. Similarly, the changes in these variables with therapy were measured as median difference with 95% confidence intervals (CIs), and the significance was assessed by Wilcoxon matched-pairs signed-ranks test. The Spearman correlation coefficient was used to analyze the relation between variables. Multiple regression analysis was performed for those histomorphometric variables that significantly changed with therapy. Pretherapy histomorphometry results and posttherapy serum estradiol levels were used as covariates to assess their individual influence on the posttherapy histomorphometry results. The models were appropriate as the residuals were normally distributed, in all cases except for osteoid volume, where the relationship between variables remained the same even after transformation.

Results

The results of those 22 women who had satisfactory pre- and posttreatment transcortical iliac crest biopsies after 6 yr of ERT were analyzed. At the beginning of the study their mean age was 65.4 yr (range, 55–76), and the mean interval since menopause was 16.9 yr (range, 10–27). Eighteen (82%) of them were parous with a median parity of 2 (range, 0–6), and none had been on oral contraceptive in the past. The mean height, weight, and BMI before therapy were 1.62 m (range, 1.50–1.76), 65.67 kg (range, 44–89.5), and 25.49 (range, 18.08–33.28), which changed minimally after 6 yr to 1.61 m (range, 1.50–1.76), 66.31 kg (range, 47–84), and 25.76 (range, 19.56–32.09), respectively. Twelve women have had hysterectomies, including four women who also had bilateral oophorectomy, eight women suffered from one or more osteoporotic fractures either at the spine or at the distal radius, and four women had a family history of osteoporosis. The clinical characteristics of those women who stopped ERT or did not have a repeat biopsy were similar to those who completed the study.

Table 1 summarizes the bone histomorphometric results. The cancellous bone volume showed a significant increase with a median percentage change (95% CI) of 46.9 (12.7–94.3) after 6 yr of ERT. This was accompanied by architectural changes in cancellous bone, which included a significant increase in trabecular thickness and trabecular number, and a decrease in trabecular separation. Assessment of cancellous connectivity showed a significant decrease in the number of termini, but the increase in number of nodes and decrease in terminus to node ratio did not reach statistical significance. The increase in cancellous bone volume was accompanied by

TABLE 1. Changes in bone histomorphometry with sc oestradiol replacement therapy for 6 yr

Histomorphometry	Pretherapy[a]	Posttherapy[a]	Median difference (95% CI)	*P*
Structural parameters				
Cancellous bone volume (%)	10.75 (7.72–14.89)	17.31 (12.66–21.30)	5.70 (2.05–7.35)	0.0001
Trabecular thickness (μm)	95.58 (90.67–122.35)	131.52 (120.89–151.28)	41.08 (5.28–45.67)	0.0005
Trabecular separation (μm)	874.30 (640.34–1115.75)	665.00 (562.50–859.50)	−263.80 (−513.02 to −69.24)	0.0129
Trabecular number (no./mm^2)	1.05 (0.81–1.29)	1.30 (1.06–1.45)	0.18 (0.03–0.42)	0.0165
Termini (no./mm^2)	1.32 (1.04–1.88)	1.06 (0.73–1.27)	−0.18 (−0.63 to 0.09)	0.0106
Nodes (no./mm^2)	0.23 (0.14–0.41)	0.33 (0.17–0.50)	0.03 (−0.14 to 0.24)	0.1913
Terminus/node ratio	6.10 (3.83–10.79)	2.40 (1.61–7.24)	0.21 (−6.01 to 0.86)	0.2043
Static parameters				
Mean wall thickness (μm)	31.20 (28.65–34.05)	38.30 (35.20–41.45)	5.90 (2.02–8.10)	0.0001
Osteoid volume (%)	1.17 (0.69–1.98)	0.12 (0.07–0.31)	−0.89 (−1.91 to −0.21)	0.0004
Osteoid thickness (μm)	8.18 (6.04–9.15)	10.13 (7.82–12.40)	1.07 (−1.19 to 3.33)	0.0995
Osteoid surface (%)	5.07 (4.21–9.61)	3.76 (2.51–7.49)	−2.16 (−2.46 to −0.07)	0.0312
Eroded surface (%)	2.58 (1.19–3.74)	6.19 (4.37–8.63)	3.95 (1.18–4.68)	0.0008
Dynamic parameters				
Mineralizing surface (%)	2.76 (0.92–4.85)	2.67 (1.01–3.16)	−0.40 (−1.77 to 1.32)	0.5016
Mineral apposition rate (μm/day)	0.68 (0.51–0.90)	0.73 (0.50–0.85)	0.095 (−0.17 to 0.17)	0.8313
Adjusted appositional rate (μm/day)	0.30 (0.16–0.40)	0.47 (0.16–0.57)	0.07 (−0.02 to 0.24)	0.0980
Bone formation rate ($\times 10^{-2}$ $\mu m^3/\mu m^2$/day)	1.80 (0.43–2.92)	1.28 (0.48–1.97)	−0.13 (−0.83 to 1.13)	0.9826
Activation frequency (y − 1)	0.18 (0.04–0.34)	0.14 (0.05–0.23)	−0.03 (−0.09 to 0.03)	0.4777
Formation period (days)	93.55 (80.17–248.25)	81.00 (65.80–307.00)	−3.50 (−35.10 to 35.50)	0.7960
Active formation period (days)	47.66 (36.82–64.02)	51.14 (42.34–120.80)	1.16 (−10.74 to 32.10)	0.4265

[a] Median (interquartile range).

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a significant increase in wall thickness with a median percentage increase (95% CI) of 18.4 (5.3–28.5). There was a trend toward increase in mineral apposition rate, adjusted apositional rate, and active formation period, but with the small numbers of patients studied, these did not reach statistical significance. Similarly, there was a nonsignificant decrease in the formation period. There was a significant decrease in osteoid volume and osteoid surface without any change in osteoid thickness and increase in eroded surface. In those women whose dose of estradiol implant was reduced to 25 mg 1 yr before biopsy, a similar trend in the results was observed but with a slightly lower degree of change than the rest, who continued on the higher dose (75 mg). The changes in histomorphometric parameters were similar whether or not these three women are included in the analysis. There were no differences in the results between women with intact uteri taking progesterone supplements and hysterectomized women on unopposed ERT.

At the time of final bone biopsy the mean serum estradiol level was 1077 pmol/L (range, 180-2568), and serum FSH was 3.7 IU/L (range, 1–23.7). Serum estradiol levels in three women on 25-mg estradiol implants were 180, 281, and 287 pmol/L. Serum estradiol levels correlated directly with posttherapy levels of cancellous bone volume ($P = 0.002$; r = 0.618), trabecular number ($P = 0.005$; r = 0.574), and wall thickness ($P = 0.046$; r = 0.475), but inversely with trabecular separation ($P = 0.003$; r = −0.600), termini ($P = 0.018$; r = −0.501), and the ratio of terminus to nodes ($P = 0.019$; r = −0.495). None of the other histomorphometric parameters had a significant correlation with serum estradiol levels. Multiple regression analysis confirmed an independent influence of serum estradiol level on the posttherapy levels of cancellous bone volume, trabecular number, trabecular separation, and termini, without any significant effect of age, interval since menopause, BMI, or pretherapy level of the respective histomorphometric parameters (Table 2).

Neither age nor the interval since menopause correlated with any histomorphometric parameters either before or after therapy. Similarly, height, weight, and BMI had no relationship with pre- or posttherapy histomorphometric results. However, the changes in all structural and static parameters correlated inversely with their respective pretherapy results: cancellous bone volume ($P = 0.005$; r = −0.577), trabecular thickness ($P = 0.002$; r = −0.618), trabecular number ($P < 0.001$; r = −0.703), trabecular separation ($P < 0.001$; r = −0.801), termini ($P < 0.001$; r = −0.777), nodes ($P = 0.004$; r = −0.583), osteoid volume ($P < 0.001$; r = −0.775), osteoid thickness ($P = 0.006$; r = −0.605), osteoid surface ($P = 0.002$; r = −0.620), eroded surface ($P = 0.001$; r = −0.653), and wall thickness ($P < 0.001$; r = −0.763).

TABLE 2. Multiple regression analysis to identify the independent influence of serum oestradiol levels on the changes in bone histomorphometric parameters after 6 yr of sc oestradiol replacement

Posttherapy results	Serum oestradiol level (pmol/L)			
	r^2	Regression coefficient	(SE)	*P*
Cancellous bone volume	0.5497	0.0053	(0.0014)	0.0014
Trabecular thickness	0.4462	0.1198	(0.0087)	0.1854
Trabecular separation	0.8359	−0.1676	(0.0693)	0.0265
Trabecular number	0.6197	2.4988	(8.8110)	0.0110
Termini	0.8111	−2.5922	(1.3427)	0.0694
Mean wall thickness	0.4536	6.5651	(0.0017)	0.7087
Osteoid volume	0.9903	1.0092	(1.2914)	0.4101
Osteoid surface	0.1155	−0.0020	(0.0027)	0.4817
Eroded surface	0.1874	−0.0014	(0.0018)	0.4414

The BMD showed a significant improvement in every woman both at the lumbar spine and proximal femur. The median percentage rise at the lumbar spine was 31.4 (95% CI, 17.2–40.9), and that at the proximal femur was 15.1 (95% CI, 9.6–23.6). Both T and Z scores improved from osteoporotic levels before therapy to normal levels 6 yr after therapy (Table 3). The increase in BMD at both sites correlated directly with the changes in cancellous bone volume (lumbar spine: $P = 0.02$, r = 0.492; proximal femur: $P = 0.041$, r = 0.438), but not with any other histomorphometric parameter.

Discussion

This is the first longitudinal study providing histomorphometric evidence for an increase in cancellous bone volume, confirming the anabolic effect of ERT on human skeleton. Such a large rise cannot be explained by the expected mode of E_2 action in reducing the bone turnover and remodeling space, which occupies only 6–8% of bone volume (23, 24). The increase in wall thickness indicates increased filling of the bone remodeling unit and provides evidence for an anabolic effect of ERT due to increased bone formation at a cellular level. The accompanying improvement in cancellous structure and connectivity also suggests possible improvement of the architectural changes associated with osteoporosis.

The bone loss associated with E_2 deficiency is generally attributed to increased bone resorption and increased bone turnover. These processes are particularly striking in primary hyperparathyroidism, but cancellous bone is actually better preserved and increased bone volume is not uncommonly observed (25). This is because, under normal circumstances, bone formation is coupled to bone resorption and bone balance is maintained. Therefore, defective bone formation must contribute to the mechanism by which E_2 deficiency bone loss occurs. A decrease in wall thickness has been observed in postmenopausal osteoporosis (26, 27). Unlike the lack of positive effect of short-term or oral ERT on wall thickness, we found that 6 yr of sc E_2 implants increased wall thickness. Similar findings have recently been reported in a cross-sectional study of postmenopausal women on long-term E_2 implant therapy (17). We did not measure resorption depth in this group of patients; there is no agreed method of measurement for this parameter (20, 28–30), and widely different values have been found in humans. Nevertheless, the increased wall thickness represents net bone gain at individual bone remodeling units.

This increased bone formation at a cellular level may be due to increased numbers of osteoblasts recruited to individual bone remodeling sites, increased activity or vigor of individual osteoblasts, and/or increased active lifespan of osteoblasts. The trend toward an increase in adjusted apositional rate suggests that increased activity of osteoblasts may contribute to increased bone formation and mineral-

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TABLE 3. Changes in BMD, T score, and Z score with sc oestradiol replacement therapy for 6 yr

	Pretherapy (n = 22)	Posttherapy[a] (n = 22)	Median difference (95% CI)	*P*
Lumbar spine				
BMD (gm/cm^2)	0.744 (0.649–0.798)	0.989 (0.846–1.055)	0.222 (0.171–0.281)	<0.0001
T score (SD)	−2.80 (−3.91 to −2.26)	−0.55 (−1.83 to 0.09)	2.05 (1.55–2.52)	<0.0001
Z score (SD)	−0.92 (−1.99 to −0.21)	0.97 (0.14–1.97)	2.06 (1.67–2.52)	<0.0001
Proximal femur				
BMD (gm/cm^2)	0.742 (0.683–0.786)	0.873 (0.800–0.917)	0.121 (0.088–0.166)	<0.0001
T score (SD)	−1.63 (−2.12 to −1.28)	−0.85 (−1.46 to −0.47)	0.72 (0.44–1.07)	<0.0001
Z score (SD)	−0.49 (−0.80 to −0.17)	0.54 (0.11–1.09)	1.01 (0.79–1.30)	<0.0001

[a] Median (interquartile range).

ization. The tendency to an increase in active formation period and a decrease in formation period suggests that the osteoblast also spends a greater proportion of its life span in an active state. A cellular mechanism for increased osteoblast life span and activity may be reduced apoptosis by E_2 (31, 32). Although these effects are likely to be mediated through the estrogen receptor that is present on osteoblasts and osteocytes (33, 34), we cannot exclude the possibility that the anabolic effect may be due to activation of the androgen receptor by the relatively high doses of estrogen (35). Unfortunately, the small numbers of women studied did not confer sufficient statistical power for significance. Sample sizes of 30 and 1000 would have been required to establish a difference of 50% with a power of 80% and probability of 5% for the adjusted appositional rate and active formation period, respectively. Nevertheless, the net increase in wall thickness and cancellous bone volume strongly implicates these mechanisms, which may be substantiated in larger studies. The lack of increase in labeled bone surface and of the calculated bone formation rate reflect the suppression of bone turnover, well-recognized with E_2 therapy, and, therefore, does not negate the stimulatory action that E_2 may also exert on osteoblasts.

In many of these patients, the posttreatment biopsy was taken from the ipsilateral iliac crest 6 yr after the pretreatment biopsy. This time interval was chosen to allow a sufficient length of time for most of the wall packets measured to be formed under the influence of estrogen treatment. It was also considered that the 6-yr interval would minimize or obviate the effect of a regional acceleratory phenomenon or repair of a bony defect. The lack of increase of labeled bone surface or woven bone lends support to this.

Physiological and supraphysiological doses of ERT have been shown to stimulate osteoblastic recruitment, leading to increased cancellous bone volume in animal models (4, 5, 36). There is also *in vitro* evidence that estradiol may stimulate osteoblast differentiation and function (2, 3). The standard dose of ERT commonly used results in relatively low serum estradiol levels, only reaching that of early or mid follicular range of a normal menstrual cycle (37). This may be sufficient to suppress bone resorption but is inadequate to stimulate bone formation and, therefore, merely serves to prevent bone loss. However, the sc route used in our study ensures complete compliance and enables a much higher estradiol level to be achieved, similar to those in the mid-luteal phase, and a more physiological estradiol to estrone ratio by avoiding the hepatic first-pass effect. All patients, except those who reduced the dose of estradiol implant, had serum estradiol levels in the mid-luteal range (450 pmol/L) or above. Although the dose of E_2 used was high, the side effects and dropout rate was similar to that of conventional hormone replacement therapy. The side effects of ERT, such as mastalgia and headaches, settled spontaneously in those who continued it long term. In those women with heavy withdrawal bleeding a reduction in dose solved the problem, and annual endometrial biopsies did not show hyperplasia or malignancy. However, the same dose resulted in a wide range of serum estradiol levels (15, 38), indicating a variation in the pharmacodynamics and possible cumulative effect after long-term use, which may explain differences in the skeletal response.

Previous longitudinal studies on the effect of ERT in postmenopausal women with osteopenia or osteoporosis have failed to show an anabolic effect on bone within 2 yr. All except one study used oral or transdermal ERT, which results in a lower serum estradiol level and is less effective in improving BMD (9–12). The results of our current study and a recent cross-sectional study using similar sc E_2 implants (17) suggest that E_2 levels in the higher end of the physiological range are required for the anabolic effect of E_2. Some of our patients had supraphysiological levels of E_2 after 6 yr of ERT. High doses of E_2 in mice and birds are known to cause a dramatic increase in bone volume (39). Although some of the new bone formation is appositional, E_2 also induces *de novo* bone formation in these species (39). Despite absence of woven bone in any of the iliac bone biopsies, we cannot exclude the possibility that *de novo* new bone formation may have contributed to the substantial increase in bone volume observed in these women. The striking increase in cancellous bone volume, trabecular thickness, and connectivity is difficult to attribute to increased wall thickness alone. *De novo* lamellar bone formation on preexisting quiescent bone surface has been described with PTH treatment (40). Estrogen has been shown to induce osteoblast progenitors in bone marrow and lead to medullary bone formation in mice (41). Although this has not been demonstrated under physiological circumstances in humans, we cannot exclude this as a mechanism whereby high dose E_2 causes new bone formation.

The increase in cancellous bone volume and wall thickness are related to estradiol levels. It is unlikely that progesterone on its own stimulated bone formation or had a synergistic effect with estrogen, because women on these supplements did not show increased bone volume or wall thickness compared with those on estrogen alone. There is currently no evidence that progesterone at the doses commonly used with hormone replacement therapy exerts an anabolic effect on

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bone (42). Whether long-term use of E_2 is also required for the anabolic effect is unclear. It was estimated that 6 yr is required for a new steady state to be achieved, whereby nearly all bone packets measured would have been formed under the influence of E_2 replacement. It would also be of interest to know if the increase in bone volume and improvement in cancellous connectivity results in increase in mechanical strength and a reduced risk of osteoporotic fractures. The lack of height loss in these women after 6 yr is encouraging, but larger studies are required for confirmation.

The pretreatment bone volume, cancellous structure and connectivity, and wall thickness results in this study are similar to published reports on postmenopausal osteoporosis (26, 27), but after 6 yr of ERT the results were similar to those in normal postmenopausal women (43). Because these parameters are expected to decline with age (44–47), the positive changes shown in this study over a period of 6 yr suggests that ERT is capable of reversing the bone loss and disruption of bone architecture. The increase in cancellous bone volume is paralleled by the substantial rise in BMD, which exceeds that reported for long-term oral ERT (48–51).

Unlike other studies, and our own experience with 1 yr ERT (9–11), we did not find suppression in activation frequency despite a decrease in osteoid surface. This may reflect increased activation of new bone-forming sites with long-term sc E_2 after the initial suppression of bone resorption and turnover by E_2. The increase in eroded surface was an unexpected finding. This may be due to mild secondary hyperparathyroidism, sometimes observed in the elderly, since these women were not on calcium supplements, or alternatively may be due to observer variation, recognized to be problematic in assessment of eroded surface.

In conclusion, we have shown that 6 yr of treatment of osteoporotic postmenopausal women with estradiol implants that produce serum estradiol levels in the mid-luteal range cause a substantial increase in bone mass, measured by bone densitometry and bone histomorphometry. One of the main drawbacks of this study is the lack of control subjects. Nevertheless, the evidence that we present in our longitudinal study, together with similar findings in a cross-sectional study of women on long-term estradiol implants (17) demonstrate that E_2 is capable of exerting an anabolic effect in the human skeleton. The resultant substantial increase in bone volume and restoration of cancellous bone connectivity has important implications for the role of E_2 in the prevention and treatment of osteoporosis.

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