The Effects of Plasma Estradiol Levels on Increases in Vertebral and Femoral Bone Density Following Therapy With Estradiol and Estradiol With Testosterone Implants

TIMOTHY GARNETT, MBBS, JOHN STUDD, MD, FRCOG, NEALE WATSON, MBBS, MICHAEL SAVVAS, MRCOG, AND ANDREW LEATHER, MBBS

Percutaneous estradiol (E2) implants effectively preserve bone density in postmenopausal women. However, these implants are often given with testosterone, which may itself have an anabolic effect on bone. To determine whether testosterone confers any additional bone-sparing effect, we studied 50 postmenopausal women randomly allocated to receive E2 (75 mg) alone or with testosterone (100 mg) every 6 months for 1 year. Women with an intact uterus received cyclic norethindrone (5 mg) for 10 days of each calendar month. Twenty-five untreated women were recruited to act as a reference group. Bone density was measured at the lumbar spine and proximal femur by dual x-ray densitometry. By 1 year, bone density at the lumbar spine had fallen by 1.8% in the reference group. In the women treated with E2 alone, it increased significantly by 7.8% ($P < .0001$) and in those receiving E2 with testosterone, it increased by 6.3% ($P < .0001$). At the femoral neck, bone density decreased by 3% in the controls and increased by approximately 4% in both treated groups ($P < .0001$). The increase in bone density at these sites was unrelated to the woman's chronological age, menopausal age, or initial bone density. However, it correlated significantly with the serum E2 levels attained after 1 year of therapy. In no treated patients did bone density decrease significantly. These data show that testosterone confers no additional bone-sparing effect in postmenopausal women. *(Obstet Gynecol 1992;79:968–72)*

Estrogens given in sufficient doses prevent postmenopausal bone loss and reduce subsequent rates of osteoporotic fracture.[1–5] Most published studies have involved oral estrogens and have measured bone density in the peripheral skeleton. Some have examined the effects of hormone replacement therapy on the lumbar spine, but only one has investigated prospectively the effects of estrogens on the femoral neck and none involved estradiol (E2) and testosterone implants.[6]

Estradiol implants, a convenient form of estrogen replacement therapy, are widely used for many severe problems of the climacteric[7] and have been shown in cross-sectional studies to prevent postmenopausal bone loss.[8] There is a suggestion that E2 implants are more effective than oral therapy.[8] To determine whether testosterone confers any additional bone-sparing effect, we conducted an alternating study of postmenopausal women receiving E2 implants either alone or with testosterone. These subjects were compared with a group of healthy untreated postmenopausal women.

Materials and Methods

Seventy-five healthy postmenopausal women were recruited from our menopause clinics. All were at least 1 year postmenopausal as judged by duration of amenorrhea, or by menopausal symptoms in 16 who had previously had a hysterectomy with conservation of ovarian tissue. Fifty women requested hormone replacement therapy and were alternately allocated to E2 implants either alone or with testosterone. Twenty-five women chose not to receive hormone replacement therapy and acted as a reference group. No patients were taking any medication known to affect bone metabolism or had any condition likely to affect bone density. We excluded women with excessive cigarette smoking (greater than 20/day) or excessive alcohol consumption (greater than 300 g/week). The percent-

From The Menopause Clinic, King's College Hospital, Denmark Hill, London; and the Fertility and Endocrinology Centre, Lister Hospital, London, United Kingdom.

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ages of women who had breast-fed or used oral contraceptives (OCs) for greater than 1 year were recorded. Subjects returned to the clinic at 6-month intervals and bone scans were performed at the Lister Hospital, Chelsea, at baseline and after 1 year of therapy.

Hormone implants (Organon, Cambridge, UK) were placed in the subcutaneous fat of the anterior abdominal wall under local anesthesia every 6 months.[9] The estrogen-only group received 75 mg (one 50-mg and one 25-mg pellet). The E2 and testosterone group also received testosterone, 100 mg, at the same site. Women with an intact uterus received norethindrone acetate, 5 mg/day for the first 10 days of each calendar month.[10]

Height, weight, alcohol consumption, cigarette use, frequency of exercise, parity, and previous OC use were recorded at the start of the study. Full blood count, liver function tests, and electrolyte measurements were performed to exclude hepatic and renal impairment. At the baseline and 1-year visits, blood samples were taken for full blood count, calcium, phosphate, alkaline phosphatase, FSH, E2, and testosterone measurements.

Bone density was measured by quantitative digital radiography (Hologic, Waltham, MA). The precision, determined by scanning a spine phantom daily, was 0.87% during the study. Bone density was measured at the second and third lumbar vertebrae, femoral neck, Ward triangle, and trochanteric and intertrochanteric regions. Values are expressed in grams of hydroxyapatite (gHa)/cm^2.

Two-tailed unpaired Student t tests and Mann-Whitney U tests were used to compare differences between the groups. A two-tailed paired Student t test was used to compare the differences between means at baseline and 1 year. Bone density changes were evaluated both as absolute values and as percentage changes calculated from the individual values at baseline.

Results

The baseline demographic data (Table 1) revealed adequate matching of the three study groups. The reference group was slightly older and of lower parity, and had a higher alcohol consumption than the treated groups.

Table 2 lists the changes in bone density expressed as both absolute values and percentage changes. At the lumbar spine (mean L2, L3), bone density decreased by an average of 1.8% in the reference group and increased by an average of 7.8 and 6.3% in the E2 and E2 with testosterone groups, respectively ($P <$ .0001). At the femoral neck, bone density decreased by 3% in the reference group and increased by approximately 4% with E2 with or without testosterone ($P <$ .0001). At the Ward triangle, density increased by 7.3% in the estrogen group and 5.6% in those treated with E2 and testosterone, whereas it decreased by 3.5% in the reference group ($P <$ .0001). There were no significant differences in bone density at any of the sites measured between women receiving E2 alone and those receiving E2 with testosterone. No treated subjects had a significant bone loss (more than twice the measurement precision) at either the spine or femoral neck at 1 year, but three in each treated group showed a small but nonsignificant decrease at both sites.

Table 1. Baseline Demographic Data

	Reference (N = 25)	E2 only (N = 25)	E2 with testosterone (N = 25)
Age (y)	56.6* ± 8.6	54.3 ± 6.9	53.8 ± 8.4
Years since menopause	7.2* (9–47)	5.6 (1–25)	5.4 (1–29)
Height (cm)	164 ± 6.5	165 ± 6.4	161 ± 6.2
Weight (kg)	67.6 ± 12.4	64.6 ± 8.01	62.0 ± 8.2
Parity	1.44 ± 0.5	1.96 ± 0.4	2.24 ± 0.5
Hysterectomy	16%	24%	28%
Breast-feeding†	32%	28%	36%
Previous oral contraception‡	24%	32%	32%
Alcohol (>100 g/wk)	52%	40%	44%
Smoker	28%	24%	24%
Regular exercise	60%*	36%	32%

E2 = estradiol.
Data are presented as mean ± SD, mean (range), or percent.
* $P <$ .005 *vs* both treated groups.
† Percent greater than 2 months of breast-feeding.
‡ Percent greater than 2 months of oral contraception.

Table 3 presents the biochemical and hormonal data. There were no changes in the reference group, but significant decreases were noted in serum calcium, phosphate, and alkaline phosphatase in the treated women. The FSH level decreased significantly in both treated groups, to 13.3 IU/L with estrogen only and 9.8 IU/L with E2 and testosterone. This was accompanied by a significant increase in serum E2, to a mean of 471 pmol/L in the E2 group and 490 pmol/L in those treated with E2 and testosterone. Serum testosterone increased in those treated with testosterone implants.

Linear regression analysis revealed no correlation between the increase in bone density achieved in either treated group and the chronological age, menopausal age, or initial bone density. There was, however, a significant positive correlation between serum E2 levels achieved after 1 year of therapy and the increase in bone density at the lumbar spine (r = 0.34, $P <$ .01), femoral neck (r = 0.25, $P <$ .05), and Ward triangle (r = 0.25, $P <$ .05).

Table 2. Changes in Bone Density

Density (gHa/cm²)	Reference	E2	E2 with testosterone
Lumbar spine (L2, L3)			
Baseline	0.996 (0.670–1.470)	0.900 (0.606–1.343)	0.941 (0.519–1.308)
1 y	0.978 (0.665–1.495)	0.970 (0.689–1.451)	1.000 (0.561–1.360)
% change	−1.8	+7.8	+6.3
Proximal femur (femoral neck)			
Baseline	0.780 (0.394–1.015)	0.709 (0.548–1.001)	0.747 (0.498–0.962)
1 y	0.757 (0.359–1.001)	0.737 (0.579–1.079)	0.778 (0.532–1.013)
% change	−3.0	+4.0	+4.2
Trochanteric			
Baseline	0.683 (0.438–0.843)	0.593 (0.456–0.802)	0.636 (0.390–0.837)
1 y	0.661 (0.421–0.831)	0.627 (0.476–0.791)	0.674 (0.369–0.975)
% change	−3.2	+6.1	+5.6
Intertrochanteric			
Baseline	1.109 (0.527–1.436)	0.992 (0.754–1.314)	1.039 (0.630–1.313)
1 y	1.056 (0.480–1.402)	1.005 (0.738–1.159)	1.072 (0.667–1.350)
% change	−4.8	+1.8	+3.7
Total			
Baseline	0.951 (0.480–1.209)	0.854 (0.668–1.139)	0.882 (0.523–1.142)
1 y	0.904 (0.444–1.198)	0.879 (0.673–1.190)	0.930 (0.553–1.180)
% change	−5.0	+3.3	+6.0
Ward triangle			
Baseline	0.571 (0.237–0.852)	0.503 (0.343–0.868)	0.558 (0.263–0.824)
1 y	0.556 (0.153–0.849)	0.534 (0.375–0.833)	0.587 (0.283–0.865)
% change	−3.5	+7.3	+5.6

gHa = grams of hydroxyapatite; E2 = estradiol.

Discussion

This study demonstrates that E2 implants increase the bone density of postmenopausal women regardless of chronological age, menopausal age, or initial bone density. Increases at both the spine and femoral neck were significantly correlated with the serum E2 levels achieved after 12 months of therapy.

Table 3. Biochemical and Endocrinologic Changes

	Reference	E2	E2 with testosterone
FSH (IU/L)			
Baseline	55.5 ± 22.7	64.5 ± 28.7	60.5 ± 24.5
1 y	49.3 ± 29.2	13.3* ± 17.7	9.8* ± 9.3
E2 (pmol/L)			
Baseline	89.5 ± 28.7	79.2 ± 32.1	125.9 ± 65.2
1 y	101.1 ± 35.4	471.3* ± 148.3	489.8* ± 289.8
Testosterone (mg/L)			
Baseline	0.942 ± 0.51	0.809 ± 0.71	0.837 ± 0.49
1 y	0.931 ± 0.50	0.885 ± 0.39	1.686† ± 2.0
Calcium (mmol/L)			
Baseline	2.40 ± 0.07	2.39 ± 0.08	2.42 ± 0.12
1 y	2.39 ± 0.08	2.36‡ ± 0.08	2.33‡ ± 0.09
Phosphate (nmol/L)			
Baseline	1.09 ± 0.12	1.11 ± 0.13	1.15 ± 0.15
1 y	1.10 ± 0.13	1.06‡ ± 0.14	0.96‡ ± 0.16
Alkaline phosphatase (IU/L)			
Baseline	84.3 ± 28.1	85.6 ± 29.3	78.0 ± 23.4
1 y	61.5‡ ± 26.1	66.5‡ ± 16.0	61.1‡ ± 14.1

E2 = estradiol.
Data are presented as mean ± SD.
* $P < .001$ *vs* baseline.
† $P < .001$ *vs* baseline.
‡ $P < .05$ *vs* baseline.

It has been claimed that the minimum effective dose of estrogen required to conserve bone density is 0.625 mg conjugated equine estrogens, but the same study showed that a significant proportion of patients still lost bone at this low dose.[11] A number of prospective studies using a variety of oral and percutaneous estrogens have demonstrated a small increase in bone density with estrogen therapy.[1–5,12–14] This has usually been regarded as a transient effect caused by a reduction in activation frequency and thereby remodeling space. The remodeling space, the amount of bone removed by osteoclasts but not yet replaced by osteoblasts, is 6–8% of the skeletal volume at any given time, and estrogens reduce the number of remodeling processes by 50% in trabecular bone.[15] This reduces the remodeling space by the same proportion and results in a reversible bone gain of 3–4%. However, it cannot explain the magnitude of the increases in bone density at the lumbar spine seen in the present study.

Bone consists largely of collagen matrix, and in vitro studies have shown a direct effect of osteoblasts on collagen synthesis after stimulation with physiologic levels of E2.[16] Earlier work from our clinic demonstrated the profound effect of estrogens on skin colla-

gen synthesis in postmenopausal women.[17] Both skin and bone consist largely of type I collagen, and some of the effects of estrogens on bone density may be mediated by collagen synthesis, resulting in a significant increase in bone strength. This has major consequences for older women with established osteoporosis who may benefit from hormone replacement therapy even many years after menopause.

Testosterone conferred no additional bone-sparing effect in this study. Although testosterone is often given with E2 implants because of a beneficial effect on libido, depression, and general well-being,[18–20] it would seem that it has no extra value in bone-sparing ability.

A confounding variable in these patients was the use of progestogens in those with an intact uterus. However, women with hysterectomies were distributed equally between the two treated groups, so any additional effect of progestogens on bone density would not be apparent.

Three subjects in each treatment group lost bone, although in none was this statistically significant. This contrasts with the response to oral therapy and transdermal patches, which cause significant bone loss in some treated patients, particularly at the femoral neck.[6,11] Thus, patients treated with implants can be reassured that the therapy will have a beneficial effect on bone density. This is important information for those with no access to bone density measurements.

A number of epidemiologic studies have demonstrated a loss of bone at the proximal femur at menopause in addition to age-related loss,[21,22] and an association between low bone density and an increased incidence of subsequent osteoporotic fractures.[23] Consequently, any therapy that preserves bone density at these sites will lessen the risks of such fractures. An association between estrogen use and a reduction in the incidence of hip fractures has also been demonstrated.[24,25] It has been presumed that this effect results from the increase in femoral neck bone density with estrogen therapy, a theory that is reinforced by cross-sectional data.[8,26] A substantial increase in spinal and hip bone density has been reported using dual-photon technology, but this is the first prospective study using the new technique of quantitative digital radiography to confirm these effects and one of the few studies to examine the effects of estrogens at the femoral neck.[6] Estradiol implants increase bone density at the lumbar spine and femoral neck in postmenopausal women in proportion to the serum E2 levels obtained during therapy. Because higher E2 levels occur with percutaneous E2 implants than with oral estrogens,[27] there may be an advantage in this route of administration. The addition of testosterone confers no additional bone-sparing effect.

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Address reprint requests to:
John Studd, MD, FRCOG
Dulwich Hospital
East Dulwich Grove
London SE22 8PT
United Kingdom

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