Gynecol Endocrinol 2003;17:463–470

# Five-year changes in bone density, and their relationship to plasma estradiol and pretreatment bone density, in an older population of postmenopausal women using long-term estradiol implants

*A. Vashisht and J. W. W. Studd*

Academic Department of Obstetrics and Gynaecology, Chelsea and Westminster Hospital, London, UK

Key words: BONE MINERAL DENSITY, HRT, IMPLANTS, OLDER WOMEN, PLASMA ESTRADIOL



## ABSTRACT

*The aim of this study was to observe whether bone mineral density (BMD) improves over 5 years in older women using estradiol implants. A total of 18 women were selected who had commenced hormone replacement therapy (HRT) around the age of 60 years. The median age was 60.9 years (range 59.7–63.2 years). Each woman had a pretreatment bone scan and then received 6-monthly subcutaneous 50 mg estradiol implants. Twelve untreated women were also selected who had had bone scans at baseline and after 5 years. A comparison of the changes in BMD between treated and untreated women was made using the Wilcoxon rank-sum test. All changes at the hip and spine were statistically significant improvements from baseline in the estradiol-treated group. After 5 years of treatment, the estradiol-treated group had significantly improved bone mineral densities compared with the untreated group. At the spine, the plasma estradiol concentration is statistically significantly correlated with the 5-year increase in bone density (*$r = 0.717$, $p = 0.004$*). There was found to be an inverse relationship between the percentage increase in BMD over the 5-year period and initial bone density (*$r = -0.635$, $p < 0.005$*). Thus estrogen is seen to have the effect of improving bone density in older women over 5 years of treatment. The increase in vertebral bone density is most marked in those women with the highest plasma estradiol levels and the lowest pretreatment bone density.*

## INTRODUCTION

Estrogen therapy is beneficial in the prevention and treatment of early postmenopausal bone loss[1,2], and the improvement in bone mineral density (BMD) leads to a reduction in fracture rates[3]. Without treatment, fracture rates increase almost exponentially beyond the menopause[4], and are associated with significant morbidity and mortality, as well as being an enormous consumer of health expenditure.

Correspondence: Professor J. W. W. Studd, Lister Hospital, Chelsea Bridge Road, London SW1W 8RH, UK

What is less clear is the effect of long-tem use of hormone replacement therapy (HRT) on bone mass. This is a fundamental unresolved issue that will help to determine the value and timing of prolonged estrogen replacement in postmenopausal women. Most studies have focussed on younger postmenopausal women with short follow-up times, and there is very little information on the effects of estrogen on the older postmenopausal woman. Very few studies have shown bone mass measurements in a population over a prolonged period of time, and none have looked at the effects of higher-dose estradiol therapy. Furthermore, the vast majority of studies have failed to correlate the skeletal response with plasma estradiol levels, as nearly all of the earlier studies have considered the effects of conjugated equine estrogens.

Early studies suggested that bone loss arrests in the elderly, but these reports were limited by their study design and by the use of small numbers[5]. More recent and larger-scale studies have shown a continued inverse relationship between age and bone mass, with bone loss continuing into the ninth decade[6]. The timing of estrogen therapy for optimal osteoprotection is debatable. It appears that one of the most important factors in maintaining BMD is current use of estrogen replacement therapy, irrespective of the age at which it is commenced. Thus it may never be too late to commence estrogen therapy for bone protection. In fact, the incremental rise in bone density in response to estrogens in the older age group is greater than that in the more recently menopausal woman[7,8]. Unfortunately, uptake of HRT is low among the elderly[9], and continuation with standard regimes of hormone replacement is poor, even in women with known low bone density[10]. The need for effective and well tolerated interventions is of paramount importance for long-term protection of bone, as once estrogen therapy is stopped, bone loss may resume at an accelerated rate[11]. Oral prescriptions of HRT are most commonly used, because of their low cost and their convenience and effectiveness. It has been shown that the minimum effective dose for preserving bone mass is 1–2 mg of estradiol[12]. However, problems of reduced bioavailability and variable absorption mean that standard doses of oral therapy can be inadequate for symptom relief and skeletal protection, which in turn may affect continuation with therapy. One of the most effective ways of maintaining compliance with HRT in postmenopausal women has been shown to be with subcutaneous estradiol implants. These have the advantage of avoiding the first-pass effect of hepatic metabolism, resulting in a physiological estradiol/estrone ratio and higher estradiol levels. It has been suggested that a plasma estradiol level of at least 300 pmol/l is required to prevent bone decline[13], and that higher levels may produce improvements in bone density. However, it is not clear whether estradiol concentrations are related to bone density changes over a prolonged period of therapy. If this were to be the case, it would be appropriate to continue monitoring estradiol concentrations during treatment to ensure an appropriate skeletal response.

Our open-plan longitudinal study aims to assess the increase in bone mass in older women receiving estradiol implant therapy over a period of 5 years.

## MATERIAL AND METHODS

We selected older women who had commenced hormone replacement therapy with estradiol implants around the age of 60 years. All women had 6-monthly implants of 50 mg estradiol (Organon Laboratories Ltd, Cambridge, UK) inserted into the subcutaneous fat of the anterior abdominal wall or thigh under local anesthesia. Those with an intact uterus received oral medroxyprogesterone acetate therapy 5 mg (Upjohn Ltd, Crawley, UK) daily for 10 days each calendar month. None of the women were receiving oral bisphosphonates, fluoride, vitamin D or testosterone, and women with any medical conditions known to affect bone metabolism were excluded from the study. As a reference group, we recruited 12 women who had baseline BMD scans, and repeat scans after 5 years during which they received no medical treatment. There was no parallel group taking oral estrogen included, as the long-term compliance with oral regimes is low.

At baseline, lumbar spine and proximal femur BMD was measured using a Hologic 1000 QDR dual-energy X-ray absorptiometry (DEXA) scanner (Hologic Waltham, MA, USA). These were reassessed after 5 years. The equipment was standardized daily using a spine phantom. 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 annually, and the mean coefficients of variation were 0.97% at the lumbar spine and 1.19% at the proximal femur. There were no major repairs

to the DEXA scanner over the study period. Serum estradiol levels were obtained using an automated enzyme-linked immunosorbent assay (ELISA) using ES700 kits (Roche Diagnostics Ltd, Lewes, UK). The inter-assay 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.

BMD results are presented as absolute values ($g/cm^2$) and also as the standard deviation above or below the mean result of young female adults (*T*-score). Our aim was to observe the pattern of BMD changes with time, both as an absolute value and as a percentage difference. The change in BMD was correlated with plasma estradiol and initial bone density. Their changes in bone density were compared with the reference group who had taken no active treatment.

Data were analyzed using the Statistical Package for Social Sciences (SPSS) package. The Wilcoxon matched-pairs signed-rank test was used to compare actual and percentage BMD measurements over time. A comparison of changes in BMD between treated and untreated women was also made using the Wilcoxon rank-sum test. The Spearman correlation coefficient was used to examine the relationship between hormone levels and changes in bone density.

## RESULTS

The median age of our treated population was 60.9 years (range 59.7–63.2 years) at the commencement of therapy. In total, 18 women were found. The median age of our reference group was 54.2 years (47.1–59.7 years).

Table 1 shows the changes in BMD after 5 years in estrogen-treated women and untreated women. After 5 years there was an increase of 18.5% (interquartile range (IQR), 14.2–29.4) in spinal BMD in treated women, compared with a decrease of 4.9% (IQR, 3.5–8.7) in the reference group. At the neck of femur in treated women there was a BMD increase of 9.4% (IQR, 6.3–16.3) after 5 years, compared with a decrease of −3.3% (IQR, 0.6–9.2) in the reference group. There was a highly statistically significant

**Table 1** Changes in vertebral and hip bone mineral density (BMD) after 5 years in treated and untreated women (median (IQR) shown); *Wilcoxon matched pair significance

| | *Baseline* | *5 years* | *p-value** | *Percentage 5-year change* | *Absolute 5-year change* |
|---|---|---|---|---|---|
| *Lumbar spine* | | | | | |
| Treated ($n$ = 18) | | | | | |
| BMD ($g/cm^2$) | 0.714 (0.614–0.940) | 0.873 (0.742–1.119) | 0.0002 | 18.5 (14.2–29.4) | 0.141 (0.114–0.188) |
| *T*-score (SD) | −3.16 (−4.03 to −1.19) | −1.88 (−2.86–0.57) | | | |
| Untreated ($n$ = 12) | | | | | |
| BMD ($g/cm^2$) | 0.886 (0.768–0.970) | 0.77 (0.738–0.913) | 0.002 | −4.9 (−8.7 to −3.5) | −0.044 (−0.089 to −0.027) |
| *T*-score (SD) | −1.54 (−2.54 to −0.70) | −2.53 (−2.81 to −1.20) | | | |
| *Proximal femur* | | | | | |
| Treated ($n$ = 18) | | | | | |
| BMD ($g/cm^2$) | 0.594 (0.554–0.668) | 0.69 (0.606–0.750) | 0.0004 | 9.4 (6.3–16.3) | 0.049 (0.037–0.101) |
| *T*-score (SD) | −2.3 (−2.66 to −1.64) | −1.44 (−2.19 to −0.89) | | | |
| Untreated ($n$ = 12) | | | | | |
| BMD ($g/cm^2$) | 0.689 (0.585–0.799) | 0.627 (0.574–0.774) | 0.008 | −3.3 (−9.2 to −0.6) | −0.023 (−0.066 to −0.004) |
| *T*-score (SD) | −1.45 (−2.38 to −0.45) | −2 (−2.48 to −0.67) | | | |

difference between estrogen-treated women and untreated women, in terms of both absolute and percentage change in BMD at the lumbar spine and neck of femur ($p < 0.0001$). Figures 1 and 2 show the change in BMD after 5 years in the two groups at the neck of femur and at the lumbar spine. The absolute changes after 5 years at the neck of femur and lumbar spine are shown in both estrogen-treated and nontreated women (Figure 3). There was no significant difference in baseline BMD at the spine or hip for our study and reference populations, although the latter group was significantly younger. Furthermore, there was no correlation between age and change in BMD over 5 years at either site or in either treated or untreated groups.

The median estradiol level was 660 pmol/l (range 263–1278 pmol/l). There was a positive association between plasma estradiol concentration and increase in BMD after 5 years at both the spine and the hip. At the spine, the estradiol concentration was statistically significantly correlated both with the 5-year increase in BMD ($r = 0.717$, $p = 0.004$) and with the 5-year percentage change in BMD ($r = 0.61$, $p = 0.021$). At the hip, the correlation was not statistically significant for either 5-year increase in BMD ($r = 0.27$, $p = 0.35$) or 5-year percentage increase ($r = 0.36$, $p = 0.20$) (Figure 4).

There was found to be an inverse relationship between increase in BMD over the 5-year period and initial bone density. This was shown at the lumbar spine and hip, and was found to exist for both percentage change in bone density and actual change in bone density, although statistical significance is only achieved at the lumbar spine ($r = -0.635$, $p < 0.005$) (Figure 5).

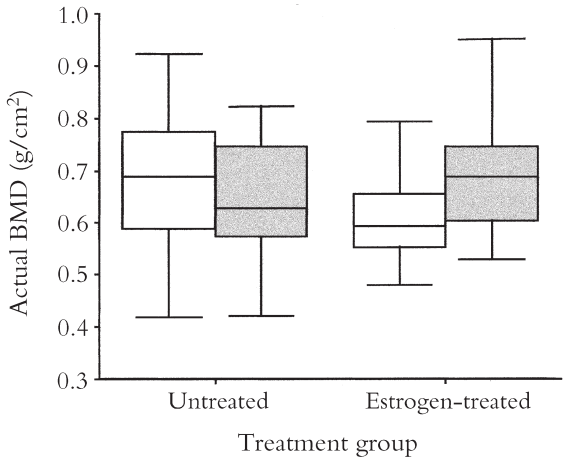


**Figure 1** Five-year hip bone mineral density (BMD) according to treatment group (median, interquartile range and total range are shown). ▭, Baseline BMD; ▨, 5-year BMD

## DISCUSSION

Defining an upper age limit for the effectiveness of estrogen as an osteoprotective agent appears to be incorrect. The Rancho Bernardo study[14] looked at the effect of the timing of initiation of postmenopausal estrogen therapy on BMD. It was concluded that estrogen treatment started after the age of 60 years offered nearly the same bone-conserving

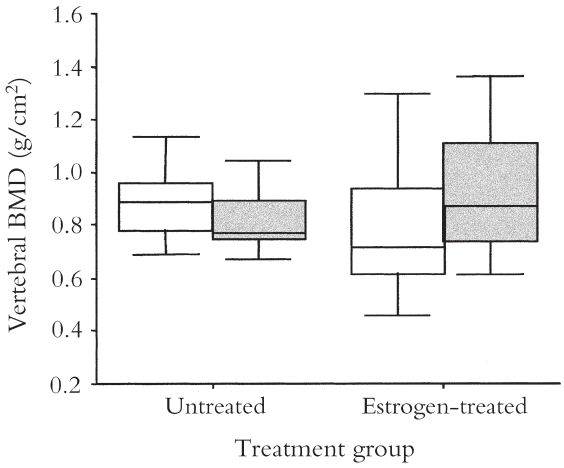


**Figure 2** Five-year vertebral bone mineral density (BMD) according to treatment group. ▭, Baseline BMD; ▨, 5-year BMD

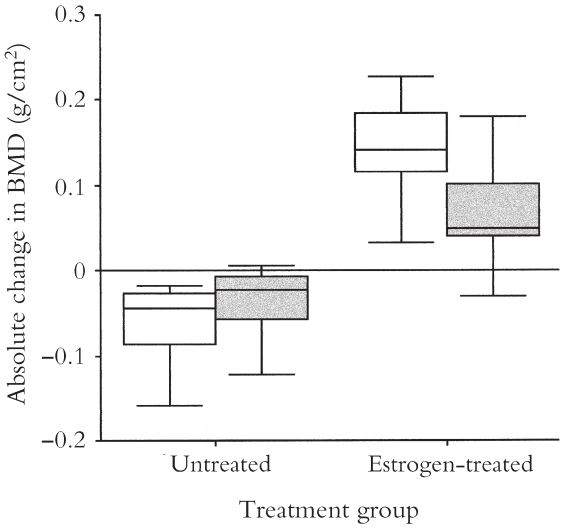


**Figure 3** Five-year absolute change in bone mineral density (BMD) at hip and spine. ▭, Lumbar spine; ▨, neck of femur

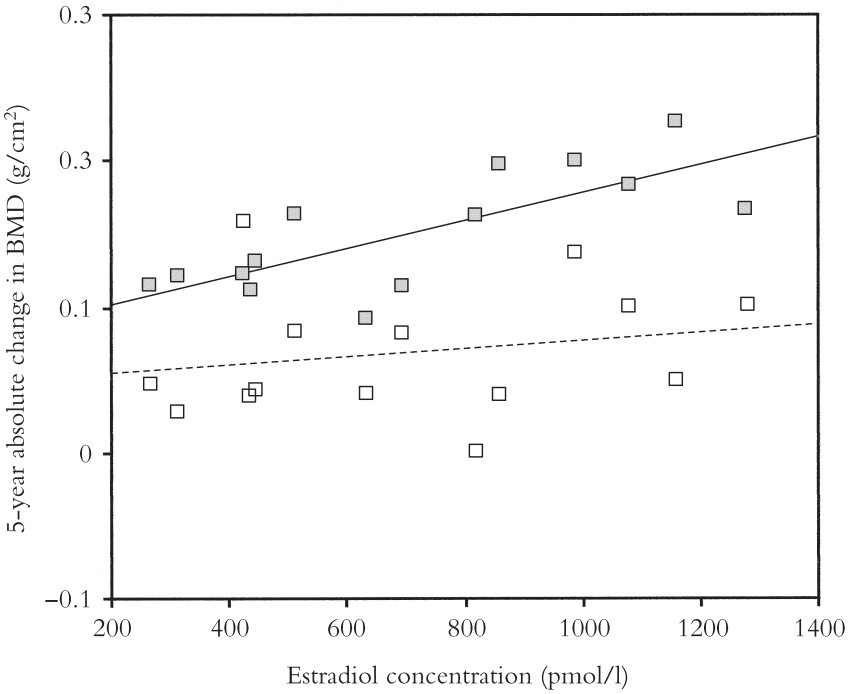


**Figure 4** Five-year absolute change in bone mineral density (BMD) at hip and spine vs. estradiol concentration (pmol/l). ▨——, Lumbar spine; □- - - -, neck of femur

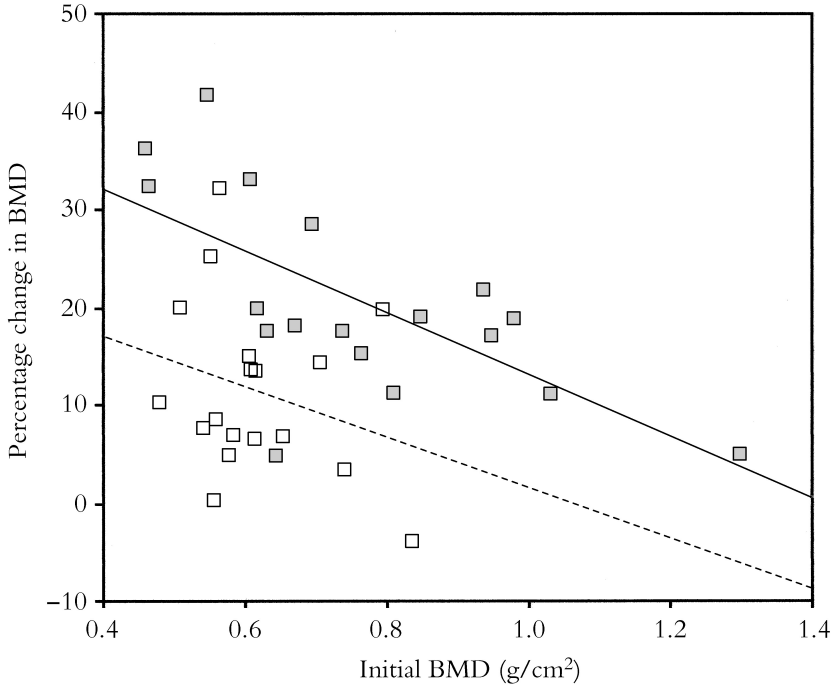


**Figure 5** Five-year percentage increases in bone mineral density (BMD) vs. initial BMD at the hip and spine. ▨——, Lumbar spine; □- - - -, neck of femur

benefit as initiating estrogen in the menopausal period. It actually appears that older women achieve the largest increases in BMD on initiation of HRT. Holland *et al.*[8], using a higher dose of estradiol implants in women over the age of 60 years with an initial low BMD, found a 12.6% median increase in lumbar spine BMD and a 5.2% increase at the hip after 1 year. Other studies, such as that by

Lindsay *et al.*[2,7] using oral estrogens, have found that the most pronounced increase in bone mass is noted in women furthest from the menopause. Women with a low initial BMD have been noted to show the greatest improvements in bone density on commencing estrogen therapy[7,8,15].

There has been a great deal of uncertainty as to whether there is a continued effect of estrogen on bone density. Bone remodeling space accounts for about 6–8% of skeletal volume[16], and represents the area of bone resorbed by the osteoclasts, but not that formed by the osteoblasts. It has been suggested that closure of this remodeling space demarcates the limit of effectiveness of estrogen therapy, and that this occurs over a finite time span. Some authors have proposed that the bone-preserving effects of estrogens might decline with time as age-related bone loss supercedes[17,18].

Eiken *et al.*[19] showed that after 10 years of treatment with oral HRT, there was a substantial increase in lumbar spine BMD. They concluded that long-term HRT exerts a continuous effect against bone loss in postmenopausal women. Using low-dose estradiol implants, Naessen *et al.*[20] showed a significant increase in bone density with increasing duration of treatment (up to 30 years) without any inevitable age-related bone loss, although the cross-sectional design of the study precluded any definite conclusions. Unfortunately, it is not possible to see how the BMD of these and other study populations[21] had changed over time, and rather than showing a dynamic representation of estrogen's effects on bone with time, it merely provides a snapshot. Our study shows an increase in vertebral BMD with time over the 5-year period. All increases were statistically significant compared with baseline values. At the lumbar spine, there was a median increase of 18.5% after 5 years of therapy. This compares with a loss of 4.9% in our reference group. The improvement in vertebral BMD is reflected by an increase in both actual change in BMD ($g/cm^2$) and percentage change in original BMD. At the neck of femur, after 5 years of treatment, there was a median increase of 9.4% in estrogen-treated women, compared with a median loss of 3.3% in the untreated women.

Clearly, closure of the bone remodeling space does not entirely account for estrogen's action on bone. Indeed, Khastgir *et al.*[22] provided histological evidence for an anabolic effect of estrogen on bone in older osteoporotic postmenopausal women over a time period of 6 years. Cancellous bone structure in women who have been on long-term estradiol implants has been shown to be remarkably similar to that of premenopausal women[23]. These findings lend support to the theory of a continuing anabolic effect of estrogen in the preservation of bone density.

Skeletal responses to estrogen are known to depend on the mode of administration of estradiol. Implant therapy in particular produces higher estradiol levels than other preparations, and this is associated with the greatest therapeutic effect in the skeleton[13,24]. Supraphysiological levels of estradiol may be reached (tachyphylaxis effect[25,26]), although no harmful effects are noted with repeated 6-monthly implants. Studd *et al.*[27] showed a significant correlation between the percentage increase in vertebral bone density and the plasma estradiol levels achieved after 1 year of treatment with hormone implants. This introduced the view that the greater effectiveness of implant therapy compared with oral therapy in improving bone density is a result of the higher serum estradiol concentrations achieved. Indeed, in women who have previously been on long-term oral estrogens, changing to implant therapy causes a substantial increase in bone density. In such a group of women, Savvas *et al.*[28] found increases of 5.7% at the lumbar spine and 5.2% at the neck of femur after 1 year. Conversely, those women who remained on oral therapy showed no significant further improvement in bone density.

We have shown that after 5 years of estrogen therapy, the estradiol concentration remains related to increases in BMD at the lumbar spine. This is of particular clinical importance when monitoring the skeletal response to HRT. However, these findings were not obtained at the hip. Whether these disparate findings are due to our small population, or whether there are intrinsically different processes involved in long-term bone remodeling at the two sites, is unclear. Certainly we know that there are different skeletal responses to estrogen at different sites of the body, reflecting varying rates of bone remodeling.

The decision as to whether to continue HRT is a complex one based on a careful assessment of the individual risks and benefits of therapy. It has been suggested that exposure to HRT for 5 years may increase the incidence of breast cancer by around two cases per 1000 women[29]. Our study suggests that estrogen may preserve bone mass over such a time period. The significance of this is not to be underestimated, as there is evidence of a rising incidence of

osteoporotic fractures[30], with associated morbidity and mortality.

The major limitation of this study is that our sample size is not large enough to give an assessment of changing osteoporotic fracture rates with time, which is the ultimate clinical criterion by which any interventional therapy is judged. Our reference group was significantly younger than the treated group, although there was no correlation between age and BMD changes over the 5-year period. It was deemed unethical to allow an older group of women with low bone density to be untreated for 5 years, and this is a factor that limits the prolonged prospective study of older women with low bone density. Notwithstanding, our results record the results over the study period for older women receiving one standard form of estradiol therapy.

## CONCLUSIONS

Our study shows that there is a place for estrogen replacement therapy in the older woman, as there is evidence of a significant improvement in bone mineral density while on therapy. In this study we have also been able to show that over 5 years these improvements at the spine are positively correlated with the plasma estradiol concentration and negatively correlated with initial bone density. Larger prospective studies with an equally long follow-up period are required in order to corroborate our findings, and in particular to assess clinical effectiveness in terms of fracture prevention. This may serve to assist physicians and patients alike in the decision as to whether to maintain long-term continuation with hormone replacement therapy.



## REFERENCES


1. Lindsay R, Hart DM, Forrest C, *et al.* Prevention of spinal osteoporosis in oophorectomised women. *Lancet* 1980;2:1151–4
2. Lufkin EG, Wahner HW, O'Fallon WM, *et al.* Treatment of postmenopausal osteoporosis with transdermal estrogen. *Ann Intern Med* 1992;117:1–9
3. Rossouw JE, Anderson GL, Prentice RL, *et al.* Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *J Am Med Assoc* 2002;288:321–33
4. Farmer ME, White LR, Brody JA, *et al.* Race and sex differences in hip fracture incidence. *Am J Public Health* 1984;74:1374–80
5. Hui SL, Epstein S, Johnston CC Jr. A prospective study of bone mass in patients with type I diabetes. *J Clin Endocrinol Metab* 1985;60:74–80
6. Steiger P, Cummings SR, Black DM, *et al.* Age-related decrements in bone mineral density in women over 65. *J Bone Miner Res* 1992;7:625–32
7. Lindsay R, Tohme JF. Estrogen treatment of patients with established postmenopausal osteoporosis. *Obstet Gynecol* 1990;76:290–5
8. Holland EFN, Leather AT, Studd JWW. Increase in bone mass of older postmenopausal women with low bone mineral density after one year of percutaneous oestradiol implants. *Br J Obstet Gynaecol* 1995;102:238–42
9. Handa VL, Landerman R, Hanlon JT, *et al.* Do older women use estrogen replacement? Data from the Duke Established Populations for Epidemiologic Studies of the Elderly (EPESE). *J Am Geriatr Soc* 1996;44:1–6
10. Ryan PJ, Harrison R, Blake GM, *et al.* Compliance with hormone replacement therapy (HRT) after screening for postmenopausal osteoporosis. *Br J Obstet Gynaecol* 1992;99:325–8
11. Lindsay R, Hart DM, MacLean A, *et al.* Bone response to termination of oestrogen treatment. *Lancet* 1978;1:1325–7
12. Christiansen C, Christiansen MS, Larsen N-E, *et al.* Pathophysiological mechanisms of estrogen effect on bone metabolism. Dose–response relationships in early postmenopausal women. *J Clin Endocrinol Metab* 1982;55:1124–30
13. Studd J, Holland E, Leather A, *et al.* The dose–response of percutaneous oestradiol implants on the skeletons of postmenopausal women. *Br J Obstet Gynaecol* 1994;101:787–91
14. Schneider DL, Barrett-Connor EL, Morton DJ. Timing of postmenopausal estrogen for optimal bone mineral density. The Rancho Bernardo Study. *J Am Med Assoc* 1997;277:543–7
15. Hillard TC, Whitcroft SJ, Marsh MS, *et al.* Long-term effects of transdermal and oral hormone replacement therapy on postmenopausal bone loss. *Osteoporos Int* 1994;4:341–8
16. Parfitt A. The physiological and clinical significance of bone histomorphometric data. In Recker RR, ed. *Bone Histomorphometry: Techniques and Interpretation*. Boca Raton, FL: CRC Press Inc. 1983:143–224

17. Quigley MET, Martin PL, Burnier AM, *et al*. Estrogen therapy arrests bone loss in elderly women. *Am J Obstet Gynecol* 1987;156:1516–23
18. Nordin BE, Horsman A, Crilly RG, *et al*. Treatment of spinal osteoporosis in postmenopausal women. *Br Med J* 1980;280:451–4
19. Eiken P, Kolthoff N, Nielsen SP. Effect of 10 years hormone replacement therapy on bone mineral content in postmenopausal women. *Bone* 1996; 19:191–3S
20. Naessen T, Persson I, Thor L, *et al*. Maintained bone density at advanced ages after long-term treatment with low-dose oestradiol implants. *Br J Obstet Gynaecol* 1993;100:454–9
21. Garnett T, Studd J, Watson N, *et al*. A cross-sectional study of the effects of long-term percutaneous hormone replacement therapy on bone density. *Obstet Gynecol* 1991;78:1002–7
22. Khastgir G, Studd J, Holland N, *et al*. Histomorphometric evidence of an anabolic effect of oestrogen on bone in older postmenopausal women. *Br J Obstet Gynaecol* 1998;105(Suppl.17):6
23. Vedi S, Purdie DW, Ballard P, *et al*. Bone remodeling and structure in postmenopausal women treated with long-term, high-dose estrogen therapy. *Osteoporos Int* 1999;10:52–8
24. Holland EFN, Leather AT, Studd JWW. The effect of 25 mg percutaneous estradiol implants on the bone mass of postmenopausal women. *Obstet Gynecol* 1994;83:43–6
25. Gangar K, Whitehead MI. Hormone implants and tachyphylaxis. *Br J Obstet Gynaecol* 1991;98:607–9
26. Garnett T, Studd JWW, Henderson A, *et al*. Hormone implants and tachyphylaxis. *Br J Obstet Gynaecol* 1990;97:917–21
27. Studd J, Savvas M, Watson N, *et al*. The relationship between plasma estradiol and the increase in bone density in postmenopausal women after treatment with subcutaneous hormone implants. *Am J Obstet Gynecol* 1990;163:1474–9
28. Savvas M, Studd JW, Norman S, *et al*. Increase in bone mass after one year of percutaneous oestradiol and testosterone implants in postmenopausal women who have previously received long-term oral oestrogens. *Br J Obstet Gynaecol* 1992;99:757–60
29. Anonymous. Breast cancer and hormone replacement therapy: collaborative re-analysis of data from 51 epidemiological studies of 52 705 women with breast cancer and 108 411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. *Lancet* 1997; 350:1047–59
30. Boyce WJ, Vessey MP. Rising incidence of fracture of the proximal femur. *Lancet* 1985;i:150–51