British Journal of Obstetrics and Gynaecology
November 2000, Vol 107, pp. 1401–1406

Combined oral oestradiol valerate-norethisterone treatment over three years in postmenopausal women: correlation between oestrogen levels and bone mineral density sites

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Objective To compare trabecular and compact bone response and relationship to oestrogen status using continuous oestradiol valerate 2 mg and norethisterone 0·7 mg daily as hormone replacement and to determine the therapeutic range of 17 beta-oestradiol.

Design Open label trial.

Setting Independent endocrine clinic

Sample One hundred and thirty-one patients were compared at point of entry and at 36 months.

Methods Postmenopausal women were assessed using a Lunar dual photon and single photon bone scanner, and bone mineral density of the lumbar spine, right hip and left forearm were annually correlated with 17 beta-oestradiol and oestrone levels over three years. Total alkaline phosphatase was compared between improvers and decliners of bone mineral density.

Results Significant improvement in bone mineral density ($P < 0.0001$) occurred at all sites except the left forearm, where bone loss was prevented. There was no correlation between oestrogen levels and bone mineral density improvements at hip sites. However, in the lumbar spine larger improvements in bone mineral density occurred in women with 17 beta-oestradiol levels > 185 pmol/L compared with those below, which were statistically significant for those with 17 beta-oestradiol levels > 248 pmol/L. Bone turnover, as quanitifed by total alkaline phosphatase measurements, was suppressed in most patients, but there were no differences in the mean alkaline phosphatase levels between the best improvers and worst decliners for lumbar spine bone mineral density. Improvers had an age mean of 5·21 years greater than decliners ($P = 0.01$) and a mean duration difference since the menopause of 5·1 years compared with decliners ($P = 0.007$).

Conclusion This combined continuous preparation of hormone replacement therapy improves not only trabecular bone but prevents compact bone loss, and the data suggest that the therapeutic range of 17 beta-oestradiol is between 200 pmol/L and 350 pmol/L.

INTRODUCTION

Improvement in bone mineral density is an important and well established indication for oestrogen therapy, and if monthly bleeding can be avoided, acceptability and compliance are likely to be greater[1,2]. We have previously shown[3] that a combination of 2 mg of oestradiol valerate and 0·7 mg of norethisterone given continuously leads to amenorrhoea in the majority of women, eliminates or markedly alleviates menopausal symptoms in all women, and is not associated with abnormalities of endometrial histology. This paper reports the effect of this preparation on trabecular and compact bone, in order to clarify the differential effect on different bone sites and to attempt to correlate such changes with oestrogen status.

Bone mineral density in some patients is not improved or even continues to decrease despite hormone replacement treatment. The question arises whether there could be a critical level for gain in bone mineral density by the prevailing oestrogen status of the patient measured by 17 beta-oestradiol or its metabolite oestrone. It is also important to know the level of 17 beta-oestradiol in patients on hormone replacement treatment as an index of compliance, and thus we correlated 17 beta-oestradiol and oestrone levels with bone mineral density of the right hip and lumbar spine. Serum alkaline phosphatase is used as a readily available surrogate measure of bone turnover[4] and it has

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been suggested that bone markers can be an index of improvement in bone mineral density although more recent work suggests greater variability when bone mineral density response to hormone replacement treatment is monitored[5]. We compared the best improvers in bone mineral density with the worst decliners to see if there was a difference in their alkaline phosphatase response.

METHODS

A total of 243 postmenopausal women who had not received hormone replacement treatment within the last three months and who currently had symptoms requiring therapy were recruited to this single-centre study. Postmenopause was defined as > 6 months since the last spontaneous menstrual period with follicle stimulating hormone levels > 40 IU/L. Those who had stopped hormone replacement treatment between three and six months previously were required to have a clear history of menopausal symptoms. Patients who had a current serious medical illness, undiagnosed vaginal bleeding, previous hysterectomy, malignant disease or history of thromboembolism were excluded. Concomitant therapy, if any, was recorded throughout the study.

Study design

This was an open label study. Patients acted as their own baseline control and a comparative group was unnecessary since the long duration of the study and the known efficacy of hormone replacement treatment made a placebo study unwarranted. It has, furthermore, been well established that in the absence of hormone replacement treatment most patients will continue to lose bone mineral density[6 8]. Patients received continuously one tablet a day of 17 beta-oestradiol valerate (2 mg) and norethisterone (0·7 mg). All patients gave written informed consent. The study protocol was approved by the medical ethics committee to the Endocrine Centre and ethical approval to continue the study was obtained for each year of the study.

Assessments

Baseline and annual bone mineral density measurements were taken by the same radiographer using a Lunar SP2 (Lunar Corporation, California, USA) single photon bone densitometer for distal left forearm and a Lunar DP3 dual photon bone densitometer for the lumbar spine and right hip by a standard technique[9]. Body mass index adjustment is made automatically in the dual photon technique. Calibration was performed daily using a phantom to correct for any effect of isotope decay using the dual photon method. A subset of approximately 35% of women had bone mineral density measured at the left forearm, while all women had bone mineral density measured at hip and spine. Right hip measurements were at femoral neck, greater trochanter and Ward's triangle and spine measurements were the mean of L2-L4. At the same time interval serum estimations of 17 beta-oestradiol, oestrone and total alkaline phosphatase were made using a Sorin radio-immunoassay technique and Boehringer Mannheim analyser, respectively. Women were encouraged to take tablets generally before breakfast and blood samples were drawn between 9 a.m. and 12 noon. Samples were assayed in monthly batches.

Statistical analysis

The analysis policy was determined before the start of the study, and for bone mineral density and laboratory parameters it was per-protocol patients. For evaluation of changes *versus* baseline both paired *t* tests and the Wilcoxon matched-pairs signed-ranks test were applied. Both *t* test and Wilcoxon tests were computed by the Stata Statistical System (version 5·0, Computing Resource Centre, Texas). For regression analysis, Pearson's correlation co-efficient method was used. For comparison of the means of sub-groups defined by the quartiles, Bonferroni tests were applied. Comparisons of the variance of such sub-groups were made by the Bartlett's test.

RESULTS

Two hundred and six women of the 243 assessed entered the study at baseline. One hundred and thirty-one were evaluable for bone mineral density at the end of three years. At the start of the study, mean age (SD) was 55·7 (6·2) (range 41–86 years). Pre-treatment mean weight (SD) was 65·6 kg (10·1) compared with 66·5 kg (10·1) at the end of year three ($P = 0·0124$).

Table 1 summarises the values in bone mineral density at baseline and at three years. The percentage age-matched improvement is more striking than absolute values as it takes into account the age-adjusted decline that would have occurred in bone mineral density three years later. Left distal forearm showed no loss in absolute value — measured in g/cm^2 — but no gain either. All other sites showed highly statistically significant improvements.

Bone mineral density gains appear to be related to the degree of trabecular bone present. Percentage improvements for absolute values (percentage age-matched differences) were: forearm 0% (2·4); femoral neck 2·3% (5); Ward's triangle 2·8% (8); femoral greater trochanter 8·3% (10·2); and lumbar spine 6·0% (8·6).

Table 1. Effect of continuous oestradiol valerate/norethisterone on bone mineral density over 3 years.

	Left forearm ($n = 45$)		Femoral neck ($n = 130$)		Ward's triangle ($n = 130$)		Femoral greater trochanter ($n = 130$)		Lumbar spine L2–L4 ($n = 131$)	
	g/cm^2	% age-matched	g/cm^2	% age-matched	g/cm^2	% age-matched	g/cm^2	% age-matched	g/cm^2	% age-matched
Baseline	0·30	86·7	0·86	107·1	0·72	101·8	0·72	102·9	1·17	104·7
36 months	0·30	89·1	0·88	112·1	0·74	109·8	0·78	113·1	1·24	113·3
P	0·06	0·046	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001

The cumulative percentage age-matched improvement is shown in Table 2 for the hip and spine. The changes were all highly statistically significantly improved against baseline ($P < 0·0001$). All absolute values were also significantly improved against baseline except for the femoral neck at year one ($P = 0·17$). The percentage of patients improving was broadly related to the degree of trabecular bone: forearm 54%; femoral neck 58%; Ward's triangle 62·3%; lumbar spine 80·9%; and greater trochanter 89·2%.

The changes in concentration of serum oestrone and 17 beta-oestradiol as indicators of oestrogen status (Table 3) showed a strong correlation with each other ($r = 0·69$; $P < 0·0001$) and marked improvement in levels at each later time point compared with baseline ($P < 0·0001$). However, analysis of absolute values of bone mineral density at year three showed that there was only a low and nonsignificant correlation between oestrone or 17 beta-oestradiol (baseline to year three differences) and bone mineral density improvement at hip sites, although femoral neck density and 17 beta-oestradiol approach probability ($P = 0·07$). There is a weak but statistically significant correlation with improvement in spinal bone mineral density ($P = < 0·001$) and higher levels of oestrone and 17 beta-oestradiol. These correlations are shown in Tables 4 and 5.

The medians and quartiles for oestrone and 17 beta-oestradiol were calculated to determine whether there was a relationship between the actual levels of oestrone and 17 beta-oestradiol and bone mineral density improvement.

For oestrone, the median was 1700 pmol/L (lower quartile 1100 pmol/L; upper quartile 2200 pmol/L) which values were used to define four groups. Table 6 shows the mean changes in absolute bone mineral density from baseline to year three, and overall *P* value (analysis of variance) only indicated a statistically significant relationship with oestrone level for bone mineral density improvements in spine. Bonferroni tests to compare subgroups showed that the two highest oestrone subgroups had significantly greater improvements in absolute bone mineral density for spine than the lowest

Table 2. Cumulative bone mineral density percentage age-matched improvement from baseline. Values are given as %.

	Year 1	Year 2	Year 3
Femoral neck	2·1	4·3	5·0
Ward's triangle	3·4	5·7	8·0
Femoral trochanter	4·0	6·5	10·2
Spine (L2-L4)	4·3	6·9	8·6

Table 3. Effect of 2 mg oral oestradiol valerate with 0·7 mg norethisterone on oestradiol and oestrone levels. Values are given as mean (SD).

Time	Oestrone (pg/ml)	Oestradiol (pg/ml)
Baseline	162·6 (61·3)	85·5 (72·2)
6 months	1568 (763·7)	278·6 (135·8)
12 months	1623 (779·3)	246·2 (102·0)
24 months	1764 (811·0)	272·0 (122·7)
36 months	1678 (749·8)	262·6 (123·8)
P (*t*)	< 0·0001	< 0·0001
P (W)	< 0·0001	< 0·0001

P values indicate significance between baseline and each subsequent visit.

Table 4. Correlation between oestrone and bone mineral density improvements (difference from baseline to year 3). Values are given as %, unless otherwise indicated.

	Femoral neck	Ward's triangle	Greater trochanter	Spine L2-L4
R^2	1·37	0·06	0·45	11·6
P	0·18	0·93	0·45	< 0·0001

Table 5. Correlation between 17β-oestradiol and bone mineral density improvements (differences from baseline to year 3). Values are given as %, unless otherwise indicated.

	Femoral neck	Ward's triangle	Greater trochanter	Spine L2-L4
R^2	2·56	0·51	1·55	13·2
P	0·07	0·42	0·16	< 0·0001

Table 6. Changes in absolute bone mineral density in g/cm^2 from baseline to year 3 by oestrone subgroups defined by median and quartiles. Values are given as g/cm^2, unless otherwise indicated. Q = quartile.

Oestrone levels by subgroups (pmol/L)	Femoral neck	Ward's triangle	Greater trochanter	Spine
Above Q3: > 2200	0·025	0·032	0·066	0·096
Median to Q3: 1700 to 2199	0·025	0·023	0·060	0·075
Q2 to median: 1100 to 1699	0·014	0·031	0·066	0·066
Overall Q1: < 1100	0·005	0·025	0·058	0·030
Overall *P*	0·40	0·94	0·91	0·004

subgroup (P = 0·003 for lowest *versus* highest subgroup; P = 0·048 for lowest *versus* second highest).

For 17 beta-oestradiol, the median was 248 pmol/L (lower quartile 185 pmol/L, upper quartile 335 pmol/L) which was used to define four groups. Table 7 shows an identical pattern of results as were found with oestrone. Bonferroni tests were also similar and significant for the same subgroups (P = 0·004 for lowest *versus* highest; P = 0·036 for lowest *versus* second highest).

Total alkaline phosphatase declined significantly from baseline in most patients on each annual testing (baseline: 81·9 IU/L; 12 months: 59·2 IU/L; 36 months: 54·9 IU/L; P < 0·0001). However, this change as a reflection of decline in bone turnover was not significantly related to improvement in bone mineral density. Comparisons of the best improvers of lumbar spine bone mineral density (16·6% to 23·1%; n = 14) to the worst decliners (–2·4% to –8·3%; n = 15) revealed little difference in mean alkaline phosphatase (SD): 56·5 (17·9) to 56·0 (13·4).

Furthermore, we examined whether age at entry and duration since the last menstrual period affected the outcome of treatment in these improvers and decliners. There was a mean difference for improvers compared with decliners of 5·21 years (CI 1·32–9·11) which was significant at P = 0·01 using the t test. There was also a mean difference of 5·1 years of duration since the menopause in improvers compared with decliners. This was highly significant (P = 0·007; CI 1·5–8·7) using the two sample Wilcoxon rank-sum test.

DISCUSSION

Apart from the improvement in bone mineral density expected from this treatment combination, the differential effect on trabecular and compact bone is now clearly established. Mainly trabecular sites showed 8% or more age-matched improvements, whereas compact bone (forearm) showed only 2·4% and no absolute improvement in bone mineral density. Mixed bone (femoral neck) had an intermediate improvement of 5%. Norethisterone is a powerful progestogen and can add to this effect, but whether it has an influence on compact bone is still not clear[10,11]. In the absence of a comparative group without norethisterone component we were unable to determine if the progestogen had an additional effect. If it had, one might have expected a more significant result in the forearm. It may therefore be safely concluded that the progestogenic effect is unlikely to be on compact bone. The improvements in trabecular bone are consistent with the effect of oestrogen alone and any progestogenic effect appears small, if at all present.

It was suggested that the degree of oestrogen status of the patient will further improve bone mineral density. There is evidence that the potent oestrogen ethinyloestradiol in the contraceptive pill adds to bone mineral density in premenopausal women[12,13], but there is more controversy about whether very high 17 beta-oestradiol levels in postmenopausal women will produce additional improvement in bone mineral density compared with physiological replacement seen

Table 7. Changes in absolute bone mineral density in g/cm^2 from baseline to year 3 by 17β-oestradiol subgroups defined by median and quartiles. Values are given as g/cm^2, unless otherwise indicated. Q = quartile.

17β-oestradiol levels by subgroups (pmol/L)	Femoral neck	Ward's triangle	Greater trochanter	Spine
Above Q3: > 335	0·022	0·029	0·070	0·089
Median to Q3: 248–334	0·027	0·029	0·069	0·077
Q2 to median: 185–247	0·015	0·025	0·052	0·067
Overall Q1: < 184	0·004	0·025	0·060	0·028
Overall *P*	0·36	0·67	0·99	0·004

in standard oral and transdermal hormone replacement treatment. We examined this question retrospectively to determine whether a threshold for 17 beta-oestradiol (or oestrone) exists, which safeguards the patient from bone mineral density decline. One potential problem is that oestrogen status of the patient (oestrone and 17 beta-oestradiol levels) was determined only once at each time point. Oestrogen assessments were carried out mainly to ensure compliance, but results differed from year to year, being influenced by the time of sampling, time of tablet ingestion, missed days, smoking and possibly absorption or metabolism changes in some patients. There was no correlation between serum levels and hip bone mineral density improvement, but absolute bone mineral density improvement in lumbar spine showed a relationship with 17 beta-oestradiol levels. The subgroups above the median (i.e. > 248 pmol/L) showed significantly greater improvement compared with the lower subgroup. The second lowest subgroup had values in the range 183–247 pmol/L and had bone mineral density improvement approaching that of the two highest subgroups. Women in the highest subgroup showed little difference from the second highest which suggests that little is to be gained from 17 beta-oestradiol level greatly in excess of 335 pmol/L. We suggest therefore that the minimum target for 17 beta-oestradiol should be approximately 200 pmol/L and the general target should be in the range of 250–350 pmol/L.

By the same rationale a minimum target level for oestrone is 1,100 pmol/L and a desirable level is in the range of 1700 to 2200 pmol/L.

It is not clear why relationships for oestrone and 17 beta-oestradiol and bone mineral density at hip sites are not significant, especially at trabecular sites, but over a three year period our patients showed only small differences between oestrogen subgroups for absolute bone mineral density improvements at greater trochanter and Ward's triangle, and for femoral neck only the lowest subgroup in oestrogen status had a (nonsignificant) lesser improvement than other subgroups. This does not alter our view of the desirable level (for spine) of serum oestrone or 17 beta-oestradiol in the postmenopausal woman.

Bone turnover markers were suggested as a useful index for monitoring improvement in bone mineral density. Total alkaline phosphatase is an easily available index of bone turnover although more specific collagen markers may have an advantage. However, in this study no difference in alkaline phosphatase was found between the best improvers and the worst decliners in lumbar spine bone mineral density. We believe that comparing these two polar groups is strong evidence against the value of this marker in monitoring bone mineral density improvement. There appears to be no real substitute for the direct measurement of bone mineral density.

Despite adequate 17 beta-oestradiol or oestrone levels some women continued to lose bone mineral density, although we speculate probably not at the rate that would have occurred without hormone replacement treatment. Some of these women are likely to be smokers, but we did not attempt to correlate smoking habits with oestrogen levels. It may well be that, due to the effects of liver enzyme induction, higher doses of hormone replacement treatment may be required in these women. If adequate 17 beta-oestradiol levels (between 200–350 pmol/L) have been established and bone mineral density is declining, alternative strategies such as a diphosphonate or possibly osteoblast stimulation using sodium fluoride or 1,25-dihydroxy vitamin D should be considered. It is still not clear why some women should be poorly responsive and others gain over 20% bone mineral density in three years.

We could not confidently exclude differences in compliance or smoking between improvers and decliners, although 17-beta oestradiol levels were ≥ 200 pmol/L in both groups. It is also of interest that patients with the best improvement in bone mineral density had a significant higher mean age difference at entry compared with the worst decliners. Duration since the menopause was also greater for improvers compared with decliners. Thus in this study the age of entry and duration since the last menstrual period did appear to affect the outcome of treatment. This raises the interesting question whether the older the woman the more responsive she may be to hormone replacement treatment in terms of bone mineral density improvement.

We conclude that the combination of 17 beta-oestradiol valerate (2 mg) and norethisterone (0·7 mg) given daily and continuously over three years provides significant improvement in bone mineral density in most patients with physiological serum levels of 17 beta-oestradiol.

Acknowledgements

We would like to thank Professor K. D. MacRae, Professor of Medical Statistics at the European Institute of Health and Medical Sciences, University of Surrey, Guildford, for his help with all the statistical analyses.

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Accepted 3 May 2000