The impact of subcutaneous oestradiol implants on biochemical markers of bone turnover and bone mineral density in postmenopausal women

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Objective To evaluate the anabolic effect of oestrogen on bone by comparing the response of markers of bone formation (and resorption) and bone mineral density (BMD) to subcutaneous oestradiol implants.

Design One year double-blind placebo controlled randomised study.

Setting Clinical research unit within a teaching hospital.

Population Twenty-one hysterectomised postmenopausal women were randomised to 25 mg oestradiol implants at baseline and at six months or to have a sham procedure at baseline and six months.

Methods BMD and quantitative ultrasound (QUS) were assessed at baseline and one year. Bone alkaline phosphatase (bone ALP), procollagen type I N-terminal propeptide (PINP), osteocalcin (OC), free deoxypyridinoline (iFDPD), N-telopeptide of type I collagen (NTX), serum oestradiol and intact parathyroid hormone (PTH) were measured at baseline, 4, 8, 12 and 24 weeks.

Main outcome measures Percentage change markers of bone turnover and PTH and change in oestradiol levels over first six months and percentage of changes in DXA and QUS over one year.

Results PINP, bone ALP and OC increased by 28%, 7% and 9%, respectively ($P < 0.01$) during the first four weeks of treatment and then decreased significantly. Lumbar spine (LS) and total hip (TH) BMD increased by 5.4% and 6.0% ($P < 0.001$), respectively, and femoral neck (FN) BMD by 3.7% ($P < 0.05$) during the first year of treatment compared with control subjects. The peak serum oestradiol level was achieved four weeks after implant insertion. Mean PTH levels increased significantly in subjects receiving subcutaneous oestradiol.

Conclusion Subcutaneous oestrogen exerted an apparent anabolic effect on bone, which was initially reflected by an increase in bone formation markers and later by a large increase in BMD.

INTRODUCTION

Previous non-randomised studies have suggested that oestradiol given as subcutaneous implant might be anabolic to bone[1,2]. In these studies, long term treatment resulted in significantly higher bone mineral density (BMD) than that in age matched controls. For example, Naessen et al.[2] described an increase of 20–25% in distal radius, lumbar spine (LS) and femoral neck (FN) BMD in postmenopausal subjects that received oestradiol implants for a mean period of 16 years. Wahab et al.[1] reported an increase of 40% and 45% in FN and LS BMD, respectively, in 12 oophorectomised postmenopausal women treated with subcutaneous oestradiol during a similar period. Tobias and Compston[3]

have suggested that osteoblast activity may be increased as a result of prolonged exposure to high doses of oestrogen as observed with oestradiol implants. However, there is little data on the effect of subcutaneous oestradiol implants on markers of bone turnover.

There is evidence, from animal studies, that oestradiol may be anabolic to bone, especially in the short term[4]. Khastgir et al.[5] investigated the effect of hormone replacement therapy (HRT) on bone histomorphometry and BMD in 22 postmenopausal women with low bone density and history of osteoporotic fractures receiving 75 mg oestradiol implants for six years. Bone cancellous volume and wall thickness, two parameters of bone formation, increased by 80% and 25%, respectively. LS BMD increased by 27% and FN BMD increased by 12%.

An increase in bone formation occurs soon after starting oestrogen therapy and this has been observed in clinical studies using transdermal patches of oestradiol[6], intranasal oestradiol[7] but not with oral oestrogens[8,9]. In this regard, we have recently shown in a prospective randomised study that postmenopausal women receiving intermittent transdermal oestradiol had a significant increase in markers of bone formation during the first four weeks of treatment[10].

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It was of interest to test the stimulatory effect of subcutaneous oestradiol implants on bone and the duration of the anabolic effect by means of markers of bone turnover. The aims of the present study were (1) to evaluate the consistency of the anabolic effect of oestrogen by comparing the response of markers of bone formation and resorption to a subcutaneous oestradiol implant and (2) to assess the effect of subcutaneous oestradiol on BMD by means of DXA and ultrasound measurements.

METHODS

Twenty-one postmenopausal women who had a previous hysterectomy were enrolled in the study. Ten women had had both ovaries removed, eight women had retained at least one ovary and in the three remaining women it was not possible to determine if their ovaries had been removed. The menopausal status of all the women was confirmed by serum oestradiol levels (<133 pmol/L) and follicle-stimulating hormone (FSH, >30 iu/L). The age range was 49–76 years (mean 65.5, SD 7.3 years). The mean time since hysterectomy was 14 years (range 2–38 years). None of the subjects had had malignant disease and all diagnoses were confirmed by pathology reports. Women were excluded if they had diseases or had taken drugs known to affect bone metabolism, had ever had an oestradiol implant or used oral or transdermal HRT within three months of enrolling in the study. All women had a physical examination before entering the study. We were not able to establish years since menopause in eight subjects. Five subjects were still having regular menstrual periods at the time of surgery and retained at least one ovary at surgery. For three subjects, it was not possible to establish their menstrual status at the time of surgery or the number of ovaries removed, if any, at surgery. The study was approved by the North Sheffield Research Ethics Committee. Written informed consent was obtained from each subject.

This was a randomised controlled double-blind study. Subjects in both groups made a total of nine visits to the Osteoporosis Centre at weeks: −1, 0, 4, 8, 12, 24, 25, 48 and 49. At the initial visit (week −1), oestradiol and FSH were measured to ensure that the subjects fulfilled the study entry criteria and a clinical examination performed. At week 0, subjects were randomised to the treatment ($n = 10$) or control ($n = 11$) group using a block or restricted procedure on a 1:1 basis. Power calculations based on a previous study[6] required seven subjects to be treated to show a 21% increase in procollagen type I N-terminal propeptide (PINP) in the first four weeks of treatment for 80% power at $P < 0.05$. BMD was measured by dual energy X-ray absorptiometry (DXA) and quantitative ultrasound (QUS) at weeks −1, 0, 48 and 49 in all subjects. Blood and urine samples were taken at each visit up to 25 weeks for the measurement of biochemical markers of bone turnover, oestradiol and parathyroid hormone (PTH). Subjects complete as Women's Health Questionnaire (WHQ)[11] at baseline and at the end of the study. The questionnaire comprised 35 questions divided into nine factors. The factors were as follows, with the number of questions in each factor in parentheses: depressed mood (7), somatic symptoms (7), memory/concentration (3), vasomotor symptoms (2), anxiety/fears (4), sexual behaviour (3), sleep problems (3), menstrual symptoms (4) and attractiveness (2).

The initial implant procedures were carried out at week 0 immediately after blood and urine samples had been collected and DXA and QUS measurements made. The treatment group received a 25 mg oestradiol implant (Organon, Dublin, Ireland) that was inserted subcutaneously beneath the skin of the abdomen using a trochar and was replaced by a new implant after six months. The control group had a sham procedure (i.e. insertion of trochar but no insertion of implants). In all cases, this procedure was performed under local anaesthetic (2–4 mL 1% lignocaine) and the trochar inserted via a 0.5 cm incision made in the right iliac fossa at the level of the pubic hair line. The skin was closed with a Steristrip or with 1 vicryl suture if required.

All samples were collected between 8:30 and 9:30 a.m. after an overnight fast. Blood samples were allowed to clot at room temperature for 30 minutes. They were then centrifuged at $2000 \times g$ for 10 minutes and serum stored at $-70°C$. Second morning void urine samples were stored at $-20°C$.

All samples were run in duplicate with samples of the same individual in the same analytical batch.

Serum PINP was measured by radio-immunoassay (Orion Diagnostica, Espoo, Finland). Intra- and inter-assay coefficients of variation (CV) were 5% and 10%, respectively. Bone alkaline phosphatase (bone ALP) was measured by ELISA, (Alkphase B, Metra Biosystems, Mountain View, CA, USA). Intra- and inter-assay CVs were 5% and 3%, respectively. Serum osteocalcin (OC) was measured by IRMA (ELSA-OSTEO, CIS Biointernational, Gif-Sur Yvette, France). Intra- and inter-assay CVs were 2% and 9%, respectively. Urinary free deoxypyridinoline (iFDPD) was measured by ELISA (Pyrilinks D, Metra Biosystems). Intra- and inter-assay CVs were 5% and 7%, respectively. Urinary cross-linked N-telopeptides of type I collagen (NTX) was measured by ELISA (Osteomark, Ostex International, Seattle, WA, USA). Intra- and inter-assay CVs were 6% and 9%, respectively. iFDPD and NTX were expressed as a ratio to urinary creatinine, which was measured by Vitros dry slide chemistry (Ortho-Clinical Diagnostics, Chalfont St Giles, UK). Serum oestradiol was measured by Elecsys 2010 automated immunoanalyser (Roche Diagnostics, Mannheim, Germany). The limit of detection was 18 pmol/L. Intra-assay CV was 10.2% at postmenopausal concentrations and the inter-assay CV was 7.9%.

Table 1. General characteristics of both study groups.

	E$_2$ implant group	Control group
Number of subjects	10	11
Age (years)†	64 (60–75)	67 (49–76)
Height (m)†	1.60 (1.49–1.67)	1.58 (1.48–1.64)
Weight (kg)†	77 (62–100)	76 (55–89)
BMI (kg/m²)†	30.0 (20.3–38.4)	30.7 (21.2–35.0)
Years since hysterectomy†	13 (4–30)	15 (2–38)
LS BMD (g/cm²)*	0.97 [0.15]	0.96 [0.13]
FN BMD (g/cm²)*	0.77 [0.08]	0.74 [0.08]
TH BMD (g/cm²)*	0.91 [0.11]	0.94 [0.13]
Achilles SOS (m/s)*	1543 [22]	1550 [37]
Achilles BUA (dB/MHz)*	108 [73]	108 [10]
Achilles stiffness*	84.4 [10.0]	86.4 [16.1]
Sahara SOS (m/s)*	1545 [33]	1543 [32]
Sahara BUA (dB/MHz)*	64.5 [10.3]	65.6 [10.2]
Sahara QUI*	84.9 [12.3]	84.4 [20.5]

* Figures in brackets indicate SD.
† Figures in parentheses indicate ranges.

Intact PTH was measured by IRMA INTACT PTH-parathyroid hormone (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Intra- and inter-assay CVs were 9% and 6%, respectively.

BMD was assessed using a Hologic QDR 4500 A (Hologic, Waltham, MA, USA) at baseline and one year at the LS, FN and total hip (TH). All measurements were performed in duplicate, one week apart. Short term CV, calculated from the duplicate baseline measurements, for each site were LS 1.9%, FN 1.7% and TH 2.1%.

Ultrasound measurements of the calcaneous were performed using Lunar Achilles+ (Lunar, Madison, WI, USA) and Hologic Sahara (Hologic) devices at baseline and one year. All measurements were performed in duplicate, one week apart. Short term CVs, calculated from the duplicate baseline measurements, for measurements made on the Lunar Achilles+ were broadband ultrasound attenuation (BUA) 3.5%, SOS 0.6% and stiffness index 3.2%. Short term CVs, calculated from the duplicate baseline measurements, for measurements made on the Hologic Sahara were BUA 4.2%, speed of sound (SOS) 0.3% and quantitative ultrasound index (QUI) 2.3%.

Statistical analysis

All statistical analyses were performed employing the software Statgraphics versions 3 and 4 (STSC, Rockville, MD, USA).

Baseline measurements of bone markers, oestradiol and PTH in the two groups were compared using two-sample t test. The changes in concentration of markers of bone turnover and PTH were expressed as a percentage of the mean of the two baseline measurements (weeks −1 and 0).

The percentage changes at six months were calculated from the mean of measurements at 24 and 25 weeks. Two-way ANOVA with Scheffé correction was used analyse the change in biochemical indices, oestradiol and PTH, over the first 24 weeks of the study. The responses of DXA and QUS were calculated as the difference between the mean one year measurement (weeks 48 and 49) and the mean baseline measurement (weeks −1 and 0) expressed as a percentage of the mean baseline measurement and compared using two-sample t tests.

Factor scores for the WHQ were calculated according to Hunter[11]. A positive response to a question scored 1 and a negative response scored 0. The scores for all questions in each factor were summed and divided by the number of questions in each factor to give a factor score. The mean changes in factor scores between baseline and week 24 in the treated and control groups were compared by t test.

RESULTS

There were no significant differences between the treatment group and controls in age, height, weight, body mass index (BMI), years since hysterectomy, LS, FN and TH BMD and QUS (Table 1).

Most of the women in the treatment group reported mastalgia over a 10 day period following insertion of the oestradiol implant. Most women in the treatment group also reported relief from hot flushes. This was reflected in the results of the WHQ. The women in the treatment group showed a decrease in factor score (i.e. an improvement of symptoms) of borderline significance in vasomotor symptoms ($P = 0.07$) and sleep problems ($P = 0.06$). There was no significant change in other factors. Two women in the treatment group withdrew from the study after six months because of the mastalgia (i.e. did not have second subcutaneous implant). Two women in the control group also withdrew after six months, one because she developed severe migraine and the other because she required a hip replacement due to osteoarthritis.

Table 2. Mean [SEM] baseline values of biochemical markers of bone turnover and hormones. BCE = bone collagen equivalents.

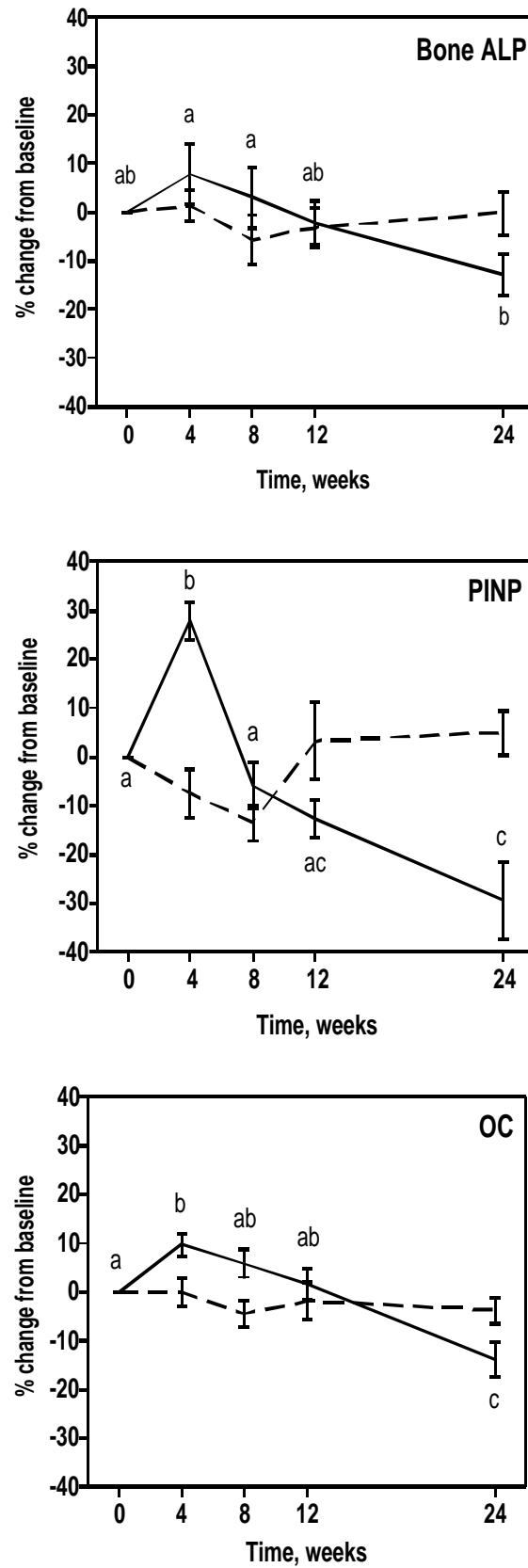|	E$_2$ implant group	Control group
Number of subjects	10	11
Bone ALP (iu/L)	23.4 [1.8]	23.0 [1.7]
PINP (µg/L)	48.3 [7.6]	49.5 [6.2]
OC (µg/L)	29.1 [3.9]	30.3 [1.4]
NTX (nmol BCE/mmol)	58.4 [7.7]	58.8 [6.5]
iFDPD (nmol/mmol)	7.8 [0.6]	7.9 [0.4]
E$_2$ (pmol/L)	67.4 [3.0]	65.6 [3.4]
PTH (pmol/L)	32.6 [5.4]	35.5 [4.7]

Fig. 1. Bone ALP, PINP and OC percentage change in the treatment group (solid line) and control group (broken line) over 24 weeks. There was a significant effect of time only in the treatment group for all markers (two-way ANOVA, $P < 0.01$). Time points designated with different letters were significantly different from each other at $P < 0.05$ (Scheffé test).

There was no significant difference between serum concentrations of markers of bone formation in the treatment and control groups at baseline (Table 2). There was a significant change in mean concentrations of all markers of bone formation over 24 weeks ($P < 0.01$). All formation markers increased during the first four weeks of treatment. The greatest increase was PINP [mean (SEM) = 28% (4)]. Bone ALP and OC exhibited small increases of 8% (6) and 10% (2), respectively (Fig. 1). By 24 weeks, all formation markers had decreased and once more PINP showed the largest decrease of 29% (8). The response at

24 weeks of OC was significantly related to age ($r = 0.81$, $P < 0.01$). The response of PINP was related to age ($r = 0.61$), but this was of borderline significance ($P = 0.06$), but there was no significant relationship between response of bone ALP and age. Response was not related to baseline levels for any of the formation markers. The control group displayed no change in markers of bone formation during the study (Fig. 1).

There was no significant difference between levels of markers of bone resorption in the treatment and control groups at baseline (Table 2). In the treatment group, there
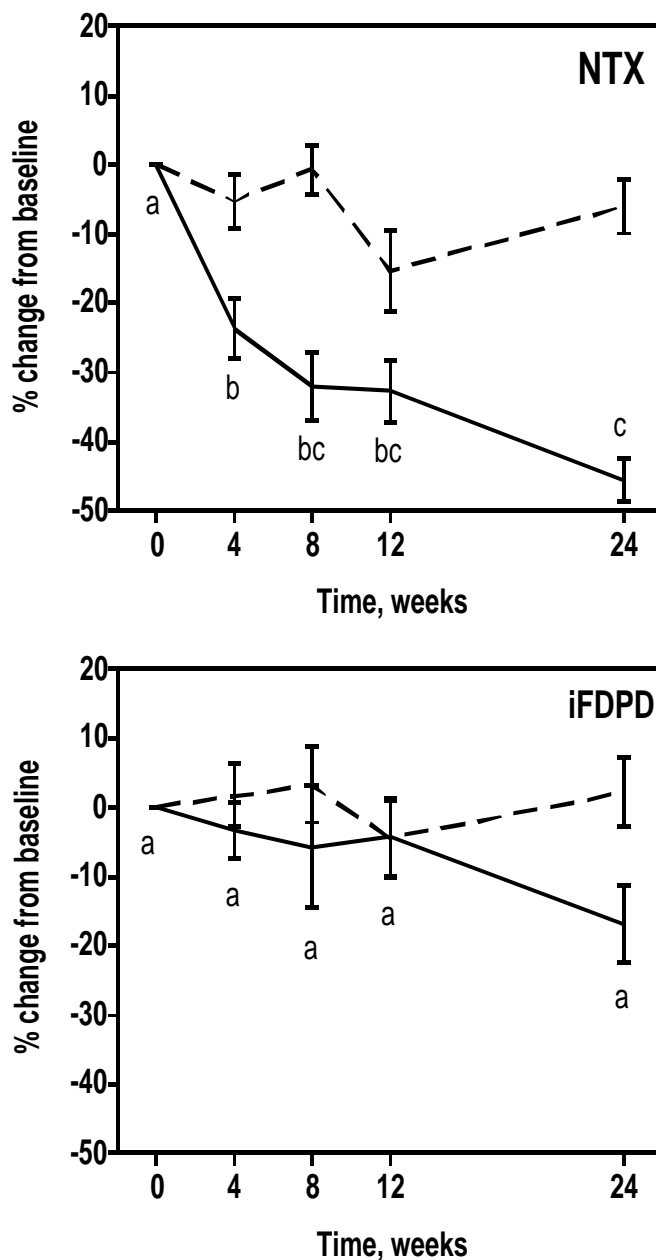

Fig. 2. NTX and iFDPD percentage change in the treatment group (solid line) and control group (broken line) over 24 weeks. NTX showed a significant effect of time in the treatment group only ($P < 0.01$, two-way ANOVA). iFDPD exhibited no significant effect of time ($P > 0.05$). Time points designated with different letters were significantly different from each other at $P < 0.05$ (Scheffé test).

Table 3. Serum oestradiol mean [SEM] levels in the treatment and control groups at different time points throughout the study.

	Oestradiol (pmol/L)				
	Baseline	Week 4	Week 8	Week 12	Week 24
HRT	67.4 [3.0]	271.0 [23.3]*	260.4 [23.7]*	246.4 [17.9]*	211.9 [14.8]*
No HRT	65.6 [3.4]	64.3 [4.2]	63.2 [4.3]	66.0 [3.7]	66.2 [4.3]

* $P < 0.05$, two-way ANOVA with Scheffé correction.

was a significant decrease in NTX ($P < 0.01$) throughout the first 24 weeks of the study. At 24 weeks, mean NTX had decreased by 46% (3) (Fig. 2). iFDPD also decreased throughout the first 24 weeks of the study but this was not statistically significant. At 24 weeks, mean iFDPD had only decreased by 17% (6) (Fig. 2). The response at 24 weeks of NTX and iFDPD was significantly related to age ($r = 0.76$, $P < 0.01$ and $r = 0.74$, $P < 0.05$, respectively). Response was not related to baseline levels for either of the resorption markers. In the control group, no significant changes were observed in either markers of bone resorption throughout the study (Fig. 2).

There was no significant difference between serum concentration of oestradiol or PTH in the treatment and control groups at baseline. The mean (SEM) serum oestradiol concentration in the treatment group increased fourfold by week 4 of treatment, 271 pmol/L (24), and remained significantly elevated at 24 weeks. Mean concentrations in the control group remained unchanged compared with baseline over 24 weeks (Table 3).

The mean baseline PTH concentration was 33 pg/mL (5) in the treatment group and 36 pg/mL (5) in the control group. Mean PTH concentration increased by up to 50% at week 8 in the treatment group and remained steady from week 12 to 24. The control group showed slight increase at week 8, which was not significant, and remained steady at baseline concentrations up to 24 weeks (Fig. 3).

The mean difference between the treated and control groups in percentage change of BMD at the LS, FN and TH was 5.2%, 3.7% and 6.0%, respectively, after one year (Table 4). There was no relationship between change in BMD and age at any site. With regard to ultrasound measurements, stiffness index measured by the Lunar Achilles+ showed a significant mean difference in percentage change of 4.3% after one year. None of the variables measured by Hologic Sahara differed significantly between the two groups (Table 4).

DISCUSSION

We have observed a clear increase in markers of bone formation during the first four weeks of treatment with
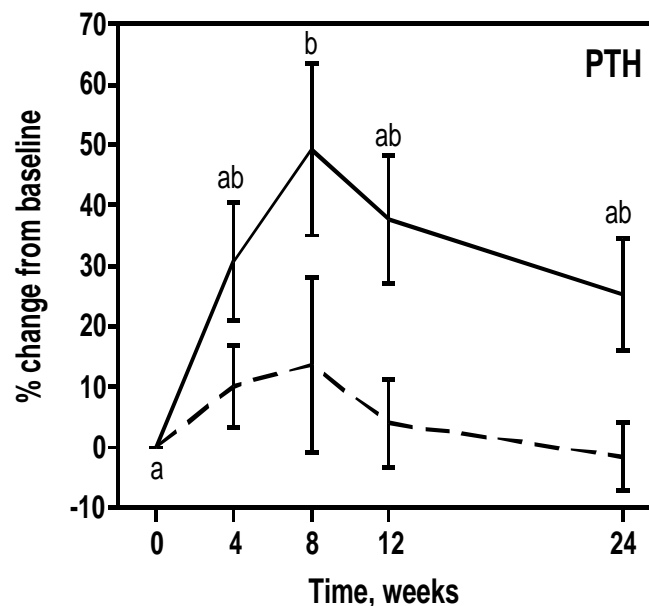

Fig. 3. PTH mean percentage change over 24 weeks in both study groups. There was a significant effect of time in the treatment group only ($P < 0.01$, two-way ANOVA). Time points designated with different letters were significantly different from each other at $P < 0.05$ (Scheffé test).

Table 4. Change in BMD after one year: comparison of two groups (*t* test).

	Mean % change		Mean difference (95% CI)
	HRT implant (*n* = 8)	Controls (*n* = 9)	
LS BMD	5.84***	0.64	5.20 (2.76, 7.63)
FN BMD	3.24*	−0.42	3.67 (0.61, 6.74)
TH BMD	3.69***	−2.32	6.01 (3.11, 8.91)
Achilles SOS	0.12	−0.15	0.27 (−0.41, 0.94)
Achilles BUA	4.37	0.68	3.69 (−0.13, 7.52)
Achilles stiffness	4.34**	−0.15	4.49 (1.65, 7.3)
Sahara QUI	−0.24	2.25	−2.49 (−7.12, 2.15)
Sahara SOS	−0.09	0.13	−0.22 (−0.64, 0.19)
Sahara BUA	1.01	4.81	−3.80 (−10.2, 2.65)

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

subcutaneous 17 β oestradiol. PINP displayed a mean increase of 28% at week 4, which is considerably greater than the response observed in a previous study using intermittent transdermal HRT[10]. The changes in bone ALP and OC are very much in line with those reported by Hannon *et al.*[6] although the response of PINP was much lower in that study. Serum PINP concentration is affected by the fact that this marker together with pro-collagen Type 1 c-terminal propeptide (PICP) reflects not only bone collagen synthesis but also synthesis in other sites, for example the skin. Brincat *et al.*[12] showed that the skin collagen content increased significantly among subjects receiving HRT (oestrogen and testosterone). However, type I collagen produced in bones probably exceeds that produced in the skin or other tissues. The increase in bone formation markers was transient. Subjects receiving subcutaneous HRT exhibited a decrease in bone formation markers after four weeks of treatment, reaching a nadir at 24 weeks. This observation has been pointed out in previous animal and clinical studies[13,6]. Intranasal oestradiol appears to have a similar effect on bone markers. One month treatment with 300 and 400 μg/day induced a significant increase in OC, bone ALP and PINP levels that were not detected after three months of treatment[7]. PINP also showed the largest decrease among bone formation markers in the treatment group after six months. This result is consistent with the literature[6,14]. Levels in the control group did not differ throughout the study.

We observed a significant response in bone resorption markers in the treatment group and especially for NTX. This has been previously observed with oral HRT[15] and with transdermal HRT[6]. The 17% decrease in iFDPD observed in our study is slightly lower compared with other studies[16,6].

Interestingly, we found a positive relationship between the response of PINP, OC, NTX and iFDPD to treatment at 24 weeks and age independent of baseline levels of markers.

In other words, markers decreased less in response to HRT in the older women. This would be in keeping with studies which showed that HRT does not decrease the rate of bone loss at the distal radius in women aged 71–80 years[17] and possibly does not have as great an impact on reducing the relative risk of non-vertebral fracture in women over 60 years of age as it does in younger women[18]. However, because of the size of our study, the relationship between marker response and age should be regarded with caution.

Subcutaneous HRT increased BMD at LS, FN and TH by 5.8%, 3.2% and 3.7%, respectively, at 1 year. Previous studies pointed out increases of 12% at LS and 5% at FN after one year with 75 mg implants[19]. Wahab *et al.*[1] reported a 30% increase in LS and hip compared with controls after 15 years of treatment with 100 mg implants. Our increases in BMD are very much in line with the only study using 25 mg implant for one year[20]. The advantage of our study is that at present it is the first randomised controlled study showing the effect of subcutaneous implants on bone mass.

The Lunar Achilles+ stiffness index was the only QUS variable to show significant change over one year. Beardsworth *et al.*[21], in a cross sectional study with long term users of oestradiol implants, found no difference in heel ultrasound measurements between treatment and control groups. In addition, there was no correlation between calcaneous ultrasound measurements and DXA measurements in the treatment group.

The increase of 4.3% ($P < 0.01$) in stiffness found in the treatment group in our study was higher than the 2.7% and 2.1% increase after the first and second year of treatment with calcitonin, respectively, as reported by Gonnelli *et al.*[22].

The lack of response of most QUS variables in this small study is not totally unexpected. The long term variability of QUS variables is two to three times that of DXA variables and as a consequence the period of monitoring probably needs to be longer to see a significant change in QUS

variables. In the present study, patients were only monitored for one year, which may not be sufficient to see significant changes[23].

Serum levels of oestradiol increased significantly in the treatment group, which is consistent with the literature[19]. Mean serum levels reached in our implant study were in line with a previous report using an equivalent dose[24] and much lower when compared with higher doses[24,25]. The peak serum oestradiol level was achieved between four and eight weeks after the implant insertion and this is similar to that found in other studies[24,26]. Serum PTH was increased in the HRT treated group, throughout the 24 weeks of the study but was highest at eight weeks. Increases in PTH in response to oral and transdermal HRT have been reported by others[27–29], although this is not a universal finding[30]. The increase of PTH in response to HRT is probably due to a decrease in serum calcium resulting from decreased bone resorption. We have shown in this study that during the first eight weeks of subcutaneous HRT not only was bone resorption suppressed but bone formation was stimulated resulting in an increase in net bone formation. This would result in an exaggerated decrease in serum calcium during the first eight weeks of treatment and lead to the peak in PTH levels, which we observed at eight weeks. In summary, 25 mg subcutaneous oestradiol implants resulted in an apparent anabolic effect on bone as assessed by bone turnover markers and BMD. This stimulatory effect could explain the large increases in bone density reported in previous studies.

Acknowledgements

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