Introduction

Osteoporosis is often considered to be a type of bone atrophy. The events which occur in bone at the time of menopause include a generalized increase in its metabolic activity. Agents including parathyroid hormone, thyroid hormone, interleukin-1 (IL-1), IL-6, IL-11, tumor necrosis factors, and epidermal growth factor, increase osteoclast activation; on the other hand, gonadal steroids are important inhibitors of activation, and their deficiency after menopause causes an increase in activation frequency. Pastoureau et al and Thompson et al studied ovariectomized ewes and baboons and indicated a 2- to 3-fold increase in the metabolic activity of bone surfaces. The increased activity of bone due to estrogen deficiency is predominantly due to an increase in the incidence of bone remodeling units. Estradiol inhibits IL-6 production and estrogen deficiency may in this way stimulate osteoclast activation. Estrogen deficiency related to menopause is clinically associated with a significant loss of bone mineral density (BMD). In postmenopausal women, hormone therapy (HT) has been shown to effectively protect and augment existing bone mass.
Transdermal $17\beta$-estradiol for preventing bone loss and reduce the incidence of fracture of the vertebrae and hip.6–9

Orally administered estrogen, the most frequently used replacement therapy, can stimulate the synthesis of hepatic proteins and increase the circulating levels of hormone-binding globulins and renin substrate.10,11 This effect has been attributed to the first-pass hepatic metabolism of the large bolus of estrogen that is absorbed by the intestine and delivered to the liver after an oral dose. Transdermal administration of estrogen avoids the first-pass hepatic effect and delivers estradiol to the general venous circulation at a continuous rate.11,12 Our laboratory has demonstrated previously that an increased serum estradiol ($E_2$) slope 2 hours after applying $E_2$ gel 2.5 g/day in Asian women allowed the elevation of the serum $E_2$ concentration to continue for 10 hours, with a peak estradiol level of approximately 557 pg/mL at 6 hours and a return to pretreatment level in 16 hours.13

It is generally accepted that a daily oral dose of 0.625 mg of conjugated equine estrogen or 1–2 mg $17\beta$-estradiol is needed to prevent postmenopausal bone loss.14–16 However, recent studies have indicated that lower doses of estrogen may be effective for maintaining bone mass.17–19 This study investigated the efficacy of 3 doses of a 25-day transdermal $17\beta$-estradiol delivery system for the prevention of bone loss in postmenopausal women.

### Methods

#### Subjects
This prospective study covered 120 postmenopausal women who were randomized and divided into 4 groups. There were no significant differences in baseline demographic or clinical characteristics including age, body mass index, smoking, physical activity and BMD among the 4 groups (Table 1). All participants received calcium carbonate 500 mg/day of elemental calcium. Group 1 comprised 30 women who received $E_2$ gel (Besins-lscovesco, Paris, France; 0.6 mg $17\beta$-estradiol/1 g) 1.25 g daily (containing 0.75 mg estradiol) from day 1 to day 25 each month. It was applied to the skin on both arms, preferably after washing in the evening. Thirty women in group 2 were treated with $E_2$ gel at 2.5 g (Besins-lscovesco; 1.5 mg $17\beta$-estradiol), used as above. Thirty women in group 3 received $E_2$ gel at 5.0 g (Besins-lscovesco; 3 mg $17\beta$-estradiol). Medroxyprogesterone acetate (Provera®, Upjohn, USA) 10 mg/day from cycle day 14 to 25 was given for every natural postmenopausal woman who received the $E_2$ gel treatment. Group 4 was composed of 30 women who used estriol (Ovestin, Organon, The Netherlands) 2 mg/day, and served as controls. Estriol can be a safe and effective alternative in the relief of climacteric symptoms in postmenopausal women but it cannot prevent bone loss.20

#### Quantitative computed tomography (QCT)
BMD was evaluated by 1 QCT at baseline (before treatment), then at 6-month intervals thereafter for 1 year. QCT was implemented on Somaton DRH

---

**Table 1.** Baseline characteristics of study population

<table>
<thead>
<tr>
<th></th>
<th>Estriol (mg/d)</th>
<th>Oestrogel® (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>1.25</td>
</tr>
<tr>
<td>Subjects (n)</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>49.1</td>
<td>51.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54.2</td>
<td>54.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>156.6</td>
<td>153.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1</td>
<td>23.3</td>
</tr>
<tr>
<td>Menopause type (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Surgical</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Spine BMD (g/cm³)</td>
<td>113.2 ± 24.1</td>
<td>113.3 ± 21.3</td>
</tr>
</tbody>
</table>

*BMI = body mass index; BMD = bone mineral density.*
Scanners (Siemens, Erlangen, Germany), with a single dual-energy approach. The scanner was equipped with an option for rapid kilovolt peak (KVP) switching and calibrated every day. The voltage was switched between 125 and 85 KVP from pulse to pulse, which allowed necessary data to be obtained at 2 KVP values. The BMD was measured by representative volume from T12 to L3. The calcium and soft tissue equivalent-density images were scaled in milligrams per milliliter.

**Statistical analysis**

The results of baseline demographic characteristics and participant-related data between E2 gel and estriol groups were presented as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) test. The paired-sample t test was used to examine BMD in each group before and after treatment. The independent-sample t test was used to examine BMD difference between each group at same time. The changes in BMD were analyzed as follows. For treatment effects, ANOVA for repeated measures was used to examine the changes in the E2 gel and estriol groups independently. When patients were separated into surgical and natural menopause groups, we used Kruskal–Wallis test to examine the changes in BMD. A post hoc test (Games–Howell) was used to examine the level of significance of each E2 gel group compared to the control group. Statistical analysis was performed using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). All analyses were 2-sided and performed at a p < 0.05 level of significance.

**Results**

A total of 120 women were enrolled and 82 (68%) completed 1 year of treatment. Twenty-three women did not complete the study because of discontinuing the HT, 10 were lost to follow-up or relocated and 5 had mild adverse events. There were no significant differences in baseline demographic or clinical characteristics among the 4 groups (Table 1).

As shown in Table 2, all 3 treatment groups showed increases from baseline in the mean BMD of the lumbar spine, as detected by QCT at 6- and 12-month intervals; however, the control group experienced a decrease in bone mass. In the stratified subgroups of the natural and surgical menopausal patients, the result in surgical menopause groups was quite different; decreased BMD was noted in the 2.5 mg group as compared to the control group. Although the HT, 10 were lost to follow-up or relocated and 5 had mild adverse events. There were no significant differences in baseline demographic or clinical characteristics among the 4 groups (Table 1).

As shown in Table 2, all 3 treatment groups showed increases from baseline in the mean BMD of the lumbar spine, as detected by QCT at 6- and 12-month intervals; however, the control group experienced a decrease in bone mass. In the stratified subgroups of the natural and surgical menopausal patients, the result in surgical menopause groups was quite different; decreased BMD was noted in the 2.5 mg group as compared to the increased BMD in the other 2 groups. Significant bone loss occurred at 12 months after estriol treatment (p = 0.001).

Figure 1 shows the mean percentage of change, from baseline, in the BMD of the lumbar spine after 6 and 12 months of treatment. All 3 estrogen treatment groups showed significant increases in lumbar spine BMD after 6 months of treatment (p < 0.05), except the subjects in the E2 gel 1.25 g/day group at the 6-month interval, in which a minimal increase was seen (p = 0.232). There were significant changes between the bone mass decrements in the treatment groups and the control group at the 6-month period (p = 0.032) after 12 months of treatment (p = 0.004) (Table 2).

**Table 2.** Bone mass revolution after treatment with Oestrogel® and estriol

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Pretreatment</th>
<th>Post-treatment</th>
<th>Change (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6 mo</td>
<td>12 mo</td>
<td></td>
</tr>
<tr>
<td>Oestrogel® 1.25 g/d (n = 20)</td>
<td>113.3 ± 21.3</td>
<td>113.7 ± 24.2</td>
<td>118.7 ± 22.0</td>
<td>4.82</td>
</tr>
<tr>
<td>Surgical menopause (n = 7)</td>
<td>115.3 ± 14.6</td>
<td>114.7 ± 20.4</td>
<td>126.8 ± 10.0</td>
<td>9.99</td>
</tr>
<tr>
<td>Natural menopause (n = 13)</td>
<td>112.2 ± 24.7</td>
<td>113.3 ± 27.0</td>
<td>115.6 ± 24.7</td>
<td>3.09</td>
</tr>
<tr>
<td>Oestrogel® 2.5 g/d (n = 21)</td>
<td>111.2 ± 31.4</td>
<td>115.6 ± 31.4</td>
<td>114.2 ± 30.9</td>
<td>2.72</td>
</tr>
<tr>
<td>Surgical menopause (n = 7)</td>
<td>115.6 ± 23.0</td>
<td>124.7 ± 23.5</td>
<td>125.1 ± 21.6</td>
<td>8.19</td>
</tr>
<tr>
<td>Natural menopause (n = 14)</td>
<td>109.0 ± 35.4</td>
<td>110.3 ± 35.0</td>
<td>108.8 ± 34.0</td>
<td>-0.19</td>
</tr>
<tr>
<td>Oestrogel® 5.0 g/d (n = 20)</td>
<td>94.9 ± 31.1</td>
<td>105.6 ± 31.0</td>
<td>103.0 ± 31.4</td>
<td>8.69</td>
</tr>
<tr>
<td>Surgical menopause (n = 4)</td>
<td>80.5 ± 26.4</td>
<td>86.2 ± 8.8</td>
<td>94.1 ± 8.4</td>
<td>13.21</td>
</tr>
<tr>
<td>Natural menopause (n = 16)</td>
<td>98.5 ± 31.9</td>
<td>109.8 ± 32.6</td>
<td>106.1 ± 34.4</td>
<td>7.76</td>
</tr>
<tr>
<td>Estriol 2 mg/d (n = 21)</td>
<td>113.2 ± 24.1</td>
<td>114.0 ± 27.2</td>
<td>108.6 ± 25.8</td>
<td>-1.44</td>
</tr>
<tr>
<td>Surgical menopause (n = 6)</td>
<td>106.3 ± 33.2</td>
<td>106.5 ± 41.6</td>
<td>98.5 ± 34.2</td>
<td>-7.40</td>
</tr>
<tr>
<td>Natural menopause (n = 15)</td>
<td>116.0 ± 20.2</td>
<td>116.7 ± 22.0</td>
<td>112.6 ± 21.7</td>
<td>-2.91</td>
</tr>
</tbody>
</table>

*Change (%) = BMD after 12-month treatment − baseline BMD/baseline BMD × 100%; †12-month post-treatment of Oestrogel® versus estriol 2 mg/day; ‡12-month post-treatment of Oestrogel® versus estriol in surgical menopausal women; §12-month post-treatment of Oestrogel® versus estriol in natural menopausal women.

**Figure 1.** Mean percentage of change in bone mineral density among the 4 groups (control, E2 gel 1.25 g/day, E2 gel 2.5 g/day, and E2 gel 5.0 g/day) compared to baseline.
Transdermal 17β-estradiol for preventing bone loss

Figure 2 shows that the increments of lumbar spine BMD in the E2 gel 2.5 g/day groups were more prominent in surgical menopausal women \( (p=0.019) \) than in the natural menopause groups \( (p=0.080) \) compared with the decrements in the control group after 12 months of treatment. The difference may be due to case variation. No statistical significance was found in the E2 gel 1.25 g/day groups, with \( p=0.167 \) and \( p=0.157 \) for oophorectomized and intact ovary women, respectively. Significant difference was observed in both

Figure 1. Mean percentage change from baseline for bone mineral density of the lumbar spine with active treatment of Oestrogel® and estriol.

Figure 2. Mean percentage change from baseline for bone mineral density of the lumbar spine with 12-month treatment of Oestrogel® and estriol in surgical and natural menopause women.
groups \( p=0.013 \) and \( p=0.028 \), respectively) in the E2 gel 5 g/day groups, which obtained substantial increases in BMD, compared with the decrements in the control groups.

**Discussion**

In the present study, we confirmed the efficacy of the lower-dose transdermal delivery of 17\( \beta \)-estradiol for preventing postmenopausal bone loss. In contrast to the control group (estradiol, 2 mg/day), there were increases in lumbar spine BMD, detected by QCT at 6 months in the 2.5 and 5.0 g/day groups, which were maintained to 12 months. In the 1.25 g/day group, there was a minimal increase in lumbar spine BMD at 6 months, in accordance with the report of Rigg's group that little difference had been achieved initially in the half-dose (estradiol, 0.025 mg/day) group, then a significant difference in lumbar spine BMD from a placebo occurred later.

The most widely prescribed doses of estrogen (0.625 mg/day of conjugated estrogen or 1 mg/day of 17\( \beta \)-estradiol orally, and 50 \( \mu \)g/day transdermally) have been believed to be at or near the minimal effective dose for preventing postmenopausal bone loss. However, an effective low dose of estrogen has always been a goal for investigators to pursue. Horsman et al treated 120 postmenopausal women with ethinyl estradiol and found no beneficial effects on BMD with daily doses of 25 or 15 \( \mu \)g. Lindsay et al observed that daily dose levels of less than 0.625 mg of conjugated equine estrogen were essentially ineffective in preventing bone loss. The most recent report showing no benefit from low-dose estrogen was a case-control study in which Michaelsson et al demonstrated that low doses of estrogen (conjugated estrogen <0.625 mg/day, estradiol <2 mg/day, ethinyl estradiol <10 \( \mu \)g/day, or transdermal estradiol <50 \( \mu \)g/day) had less effectiveness in preventing hip fractures in senile women (mean age, 72.5 \( \pm \) 6.8 years). These results might be due to the lower accuracy of older instrumentation, or be limited by confounding bias and low statistical power in the subgroup analyses. Most recent studies have shown the efficacy of low doses of estrogen. Genant et al demonstrated that 0.3 mg of conjugated estrogen was able to preserve BMD. Estradiol (0.5 mg) administered orally, as prescribed by Dr Ettinger, showed a preservation of BMD in the lumbar spine at 18 months. A 2-year prospective study of the Transdermal Estradiol Investigation Group has demonstrated that doses of transdermal E2 as low as 25 \( \mu \)g/day prevented osteoporosis in postmenopausal women.

Our current study reaffirmed the effective low dose concept for the prevention of postmenopausal bone loss; although the one-half dose maintained BMD at 6 months of active treatment, it did improve BMD significantly at 12 months.

Oophorectomy has a profound impact on women, especially premenopausal subjects. Since the ovary is the major site for the production of estradiol (90\%), testosterone (50\%) and androstenedione (50\%), naturally occurring menopause is associated with a marked decline in serum concentrations of estradiol and androstenedione, but not testosterone, because the postmenopausal ovary produces even more testosterone. The fact that postmenopausal ovaries continue to secrete testosterone was revealed by Judd et al. Ohta et al found that serum levels of estrone and androstenedione were significantly lower in oophorectomized women than in natural menopausal women. Laughlin et al demonstrated that among oophorectomized women, total and bioavailable testosterone levels were 40–50\% lower than those in intact women throughout the 50–89 year age range, while androstenedione levels decreased 27\% and sex-hormone binding globulin levels increased 30\%. Thus, the presence or absence of ovaries which produce testosterone or other unknown substances may play a crucial role in the subsequent decades of life in natural or surgical menopausal women.

Both estrogens and androgens play an important role in increasing and maintaining BMD. The assumption that estrogens may play a more critical role than androgens in bone maturation and mineralization is partly derived from the studies of mutations in patients with defective estrogen receptors or mutated aromatase cytochrome P450 (CYP 19). In hypogonadal men and prostate cancer patients who had received androgen deprivation therapy, a marked decrease in bone density was observed; the BMD was modestly increased by testosterone replacement therapy. Cell culture studies using human osteoblast-like cells have shown specific androgen receptors, through which the effects of testosterone on bone may be mediated. Androgen may promote the proliferation and differentiation of osteoblasts, inhibit osteoclast recruitment or affect osteoblast-to-osteoclast signaling. The aromatization of testosterone to estradiol is another important way that androgens exert their action on the male skeleton. The same may be true for the female skeleton where androgens act. Recently, Arisaka et al demonstrated that girls with congenital adrenal hyperplasia had significantly higher BMD than girls with central precocious puberty. Their finding suggested that adrenal androgen, as well as estrogen, played an
important role in increasing BMD, and adrenal androgen might act on bone not only as androgen, but as estrogen after having been metabolized into an aromatized bone-active compound in the peripheral tissues, such as bone and fat. Therefore, androgens may have a more important role in increasing BMD than previously realized. The postmenopausal ovaries, where more androgens can be produced, may have some advantages that oophorectomized women do not have, thus, a difference in BMD ensues. In this study, we demonstrated a more rapid loss of bone minerals in oophorectomized women than in natural menopause women in the control group and a prominent response to estrogen replacement therapy in women without ovaries treated with E2 gel (Figure 2).

Several techniques are available for the clinical measurement of BMD. In QCT, a thin transverse slice through the body is imaged, and then the image can be quantitated to give a measure of volumetric BMD. Cancellous bone can be measured independently of the surrounding cortical bone, osteoarthritis and aortic calcification. Only dual-energy X-ray absorptiometry (DXA) or bone densitometry and QCT are able to measure BMD in the spine, where a 0:100 cortical to cancellous bone ratio can be taken by QCT, a 50:50 ratio by an anterior–posterior DXA view and a 10:90 ratio by a lateral DXA view, hence, the site of choice to measure responsiveness to therapy. However, the radiation dose of QCT is significantly higher than when using absorptiometric techniques, which limits the number of repeated measurements that are possible. Capital and running costs of QCT are so high that an installation solely for bone mineral measurement is not generally feasible. With DXA, a measurement of the density of the proximal femur is the most useful for predicting fractures and measurement of lumbar spine density is the most useful for monitoring therapy. However, QCT is more accurate than DXA. Quantitative ultrasonography (QUS) has not yet been extensively validated; the sensitivities and specificities of the QUS parameters are not high enough for QUS to be used as an alternative to DXA.

The limitations of this study include the following: lack of blinding; lack of true placebo control group; small numbers; short follow-up; lack of power to distinguish difference among doses; no measures of drug compliance. However, according to the results of this preliminary study, it is worthy to perform a larger, serious, randomized, double-blind, placebo-controlled trial.

The positive effects of HT on the signs and symptoms of postmenopausal estrogen deficiency and the long-term effects of reducing morbidity and mortality from cardiovascular disease and osteoporotic fracture are well known. However, most postmenopausal women do not initiate HT and of those who do, most discontinue therapy within 2 years, depriving themselves of the potential long-term benefits. Among the reasons cited for not starting or for discontinuing HT are the risk of cancer, presence of side-effects and presence of uterine bleeding. The challenge is to overcome the hesitations of women to continue long-term therapy with HT. A low-dose transdermal delivery system that prevents bone loss is an important option that may address the problem of long-term compliance by reducing dose-related adverse events or offering alternative delivery systems for postmenopausal women to choose with ease.

References