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Comparison of effects of estriol on bone mineral density of vertebrae between elderly and postmenopausal women

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Abstract: To compare the efficacy of estriol (E_3) for postmenopausal and senile osteoporosis, we administered orally 1 g/day calcium lactate alone (control groups) or with 2 mg/day estriol (E_3 groups) to 20 postmenopausal women aged 50–65 years and 29 elderly women aged 70–84 years, and determined their bone mineral density (BMD) of the lumbar vertebrae AP scan by dual-energy X-ray absorptiometory. Of 41 subjects who completed 10 months of treatment, 8 postmenopausal women and 12 elderly women in the E_3 groups showed a significant ($P < .05$) increase in BMD, 5.59% ± 4.79% and 3.83% ± 7.90% of the respective basal values, while 10 postmenopausal women and 11 elderly women in the control groups showed a decrease in BMD, −4.02% ± 7.00% and −3.26% ± 4.60% of the respective basal values, after 10 months. On the other hand, genital bleeding as a side effect of E_3 occurred in 6 elderly subjects at this dose. Moreover, decrease in serum level of corrected calcium was seen only in the elderly women receiving E_3. Although a lower dosage of E_3 may be recommended for elderly subjects, these observations suggest, first, that hormone replacement therapy with E_3 has efficacy for involutional osteoporosis, and, second, that the bones in elderly women also maintain responsiveness to E_3.

Key words: estriol, elderly women, postmenopausal women, osteoporosis, hormone replacement therapy

Introduction

Osteoporosis not only reduces the quality of life (QOL) but also presents serious clinical and social problems in Japan [1]. Postmenopausal osteoporosis is caused mainly by a decrease in estrogen resulting from hypofunction of the ovaries, and senile osteoporosis is caused mainly by bone senescence, which is aggravated in the elderly [2]. The effectiveness of hormone replacement therapy (HRT) using estrogens for osteoporosis in postmenopausal women is widely acknowledged [3]. It has also been clarified that estrogen replacement therapy (ERT) inhibits bone loss and reduces the fracture rate [4,5]. The age of the patient at the start of administration is important in maximizing its effectiveness while minimizing its adverse effects such as genital bleeding and carcinogenesis. Estriol (E_3) is a sex hormone with a very weak stimulating effect on the growth of the endometrium. However, its effect on bone mineral density (BMD) has not been fully evaluated. E_3 as well as E_2 improved the BMD of osteoporosis model rats. In this study, we examined the effects of E_3 on BMD of the lumbar vertebrae in elderly and postmenopausal women.

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Subjects and methods

Subjects

The subjects were 49 outpatients of our hospital, consisting of 20 women aged 50–65 years (mean ± SD, 57.6 ± 4.5 years) who had undergone natural menopause (postmenopausal group) and 29 elderly women aged 70–84 years (75.0 ± 3.5 years) (elderly group). All patients in the postmenopausal, and elderly groups showed decreased mean BMD of the lumbar vertebrae (L2–L4) measured by dual-energy X-ray absorptiometry (DXA; Lunar Radiation, Madison, WI, USA) of more than 1 SD and 2 SD, respectively, compared to the mean value of Japanese young adult women [6]. The variation in BMD measurements in each vertebra after five repositionings was less than 0.8%. Patients who had significant chronic diseases affecting BMD such as Cushing syndrome, diabetes mellitus, hyperthyroidism, and hyperparathyroidism, or were receiving medications known to affect BMD, including steroid hormones or thiazide diuretics, were excluded from the study. Patients with marked scoliosis

and vertebral fracture(s) were excluded from the DXA measurement. None of the subjects had a history of fracture, thrombophlebitis, or pulmonary embolism, and none had an estrogen-dependent tumor, renal dysfunction, heart disease, hypercalcemia, or renal calculus. None of the subjects had a habit of drinking, smoking, or prior HRT use, and the subjects were required to take drugs for treatment of osteoporosis or drugs that could affect the metabolism of female sex hormones for at least 2 months before the start of the study and during the study period. None of the subjects have undergone ergotherapy during the study. The details of this study and possible side effects of the drugs used were explained to all subjects, and their written consent was obtained.

Methods

The patients in the postmenopausal and elderly groups were each divided into an estrogen group (E group) and a control group (C group) using the envelope method, comprising 10 each in E and C groups from the postmenopausal group, and 15 in E group and 14 in C group from the elderly group. In E group, 2 mg/day E_3 (the E_3 is a chemically synthesized compound and does not include E_1 and E_2 with purity >99%) and 1 g/day calcium lactate (184 mg/day calcium; for Japanese people the average calcium intake is only about 600 mg/day, so we compensated for the shortage of calcium) were orally administered. In C group, the same amount of calcium lactate alone was administered. For the 10-month administration period, the drugs were taken every day and the compliance of these drugs was checked every month. BMD of the L2–L4 lumbar vertebrae was measured by antero pasterior (AP) scan by DXA three times, before administration, and after 5 and 10 months of administration. The body mass index (BMI) was assessed as reported elsewhere [7]. The presence of fractures was examined by radiography of the lateral lumbar vertebrae. Before and after the study, examination of endometrial cytology was performed with the patients' consent.

Blood samples were obtained from the subjects early in the morning after an overnight fast. Serum levels of calcium (Ca), inorganic phosphate (Pi), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), and albumin were measured by a multichannel autoanalyzer (Hitachi, Tokyo, Japan). Serum corrected Ca level was determined according to the method of Payne et al. [8]. Complications in the patients including genital bleeding were monitored every month. All data are presented as mean ± SD. Statistical analysis among the three groups was performed by one-way analysis of variance (ANOVA). Comparisons of the parameters before and after administration in each group and the rates of changes of BMD were examined using Student's paired *t*-test, and comparisons between E and C groups were performed using Student's unpaired *t*-test. *P* values less than .05 were regarded as significant.

Results

Subjects

Of the 49 patients, 5 patients in E group (3 in the elderly group and 2 in the postmenopausal group) and 3 in C group (all in the elderly group) discontinued the study during the administration period. The reasons for discontinuation in E group were genital bleeding (3 patients in the elderly group), epigastralgia (1 patient in the postmenopausal group), and failure to come to hospital (1 patient in the postmenopausal group). In C group, 3 patients changed their residence or stopped coming to hospital. In the 41 combined patients excluding 8 who dropped out, there were no significant differences in the clinical background and biochemical measurements between E and C groups before the start of administration (Table 1). The percent BMD before administration with respect to the mean BMD value of Japanese young adult women [6] was 74% (−2.0 SD) in E group and 76% (−1.9 SD) in C group of the postmenopausal group, and 63% (−2.9 SD) and 69% (−2.4 SD) in the elderly E and C groups, respectively.

Changes in BMD

The rate of increase of BMD after 5 and 10 months of administration with respect the basal values is shown in Fig. 1. Changes in the bone area on the images obtained at BMD measurement were within ±5% in all patients. In the postmenopausal E group, the rate of increase of BMD was 2.39% ± 9.03% after 5 months and 5.59% ± 4.79% ($P < .05$) after 10 months of administration. The postmenopausal C group showed a slight but nonsignificant decrease, −1.66% ± 2.50% and −4.02% ± 7.00% after 5 and 10 months, respectively. There was a significant difference ($P < .05$) in the rate of increase of BMD after 10 months between E and C groups in the postmenopausal women. On the other hand, in the elderly E group, the rate of increase was 2.32% ± 4.47% after 5 months and 3.83% ± 7.90% ($P < .05$) after 10 months; in the elderly C group, it was −2.50% ± 2.25% and −3.26% ± 4.60% ($P < .05$) after 5 and 10 months, respectively. There were significant differences ($P < .01$) in the rates of increase of BMD after both 5 and 10 months between the E and C groups of the elderly women. However, there were no significant differences

Table 1. Clinical and biochemical characteristics of postmenopausal and elderly women administered 2 mg/day estriol (E_3) and 1 g/day calcium lactate (E groups) or 1 g/day calcium lactate alone (C groups)

	Postmenopausal women			Elderly women		
	E group (n = 8)	C group (n = 10)	Significance	E group (n = 12)	C group (n = 11)	Significance
Age (years)	57.4 ± 5.5	57.0 ± 3.8	NS	75.6 ± 3.3	73.4 ± 2.8	NS
Postmenopausal years	6.5 ± 6.9	7.4 ± 5.0	NS	26.2 ± 3.8	23.5 ± 5.4	NS
Height (m)	1.55 ± 0.06	1.54 ± 0.05	NS	1.46 ± 0.05	1.50 ± 0.08	NS
Weight (kg)	49.6 ± 7.3	50.3 ± 6.4	NS	44.9 ± 6.4	47.6 ± 7.6	NS
BMI (kg/m^2)	20.7 ± 2.3	21.2 ± 2.3	NS	20.9 ± 2.9	21.3 ± 2.7	NS
BMD (g/cm^2)	0.84 ± 0.16	0.86 ± 0.11	NS	0.70 ± 0.11	0.78 ± 0.08	NS
Serum levels						
ALP (U/l)	171 ± 79	126.3 ± 30	NS	190 ± 106	147 ± 32	NS
AST (U/l)	17.7 ± 6.0	15.0 ± 2.9	NS	14.6 ± 4.8	23.0 ± 18.3	NS
ALT (U/l)	16.7 ± 8.2	11.8 ± 5.4	NS	11.8 ± 5.0	19.6 ± 25.4	NS
γ-GTP (U/l)	29.7 ± 35.0	18.3 ± 9.0	NS	15.8 ± 10.2	18.5 ± 11.7	NS
Albumin (g/l)	41.6 ± 1.2	41.7 ± 15	NS	38.8 ± 4.5	39.0 ± 2.0	NS
Ca (mmol/l)	2.30 ± 0.10	2.30 ± 0.10	NS	2.20 ± 0.05	2.25 ± 0.10	NS
P (mmol/l)	1.19 ± 0.16	1.22 ± 0.16	NS	1.22 ± 0.19	1.19 ± 0.16	NS
Corrected Ca (mmol/l)	2.22 ± 0.10	2.21 ± 0.10	NS	2.26 ± 0.05	2.29 ± 0.10	NS
Habit						
Alcohol	No	No		No	No	
Smoking	No	No		No	No	
Prior HRT use	No	No		No	No	

BMI, body mass index; BMD, bone mineral density; ALP, alkaline leukocyte phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HRT, hormone replacement therapy; NS, not significant

in the rate of increase of BMD between the age groups in the E and C groups. There was no significant correlation between age and the rate of increase of the E and C groups.

Biochemical measurements

There were no significant changes in serum corrected Ca level in both the E and C groups in the postmenopausal women. On the other hand, in the elderly women, there was a significant decrease in serum corrected Ca level after 10 months of administration compared with that before administration in E group, and it was also significantly lower than the serum corrected Ca measured after 10 months of administration in C group. There were no significant differences in the other clinical parameters between the E and C groups in both age groups.

Safety

Compression fracture of the lumbar vertebrae did not occur in any of the 41 patients during the test period. Endometrial examination revealed no abnormality. Clinical complications were observed in 7 patients in E group only (28%): genital bleeding in 6 patients (24%) and epigastralgia in 1 patient (4%). All patients who had genital bleeding were in the elderly group, and the bleeding occurred 19.8 ± 7.5 weeks (range, 11–29 weeks) after the start of E_3 administration. The degree of bleeding was moderate in 1 patient and mild in the others. Three of the patients who had genital bleeding discontinued the trial at their own wish as described earlier.

Discussion

The discoveries of estrogen receptors in osteoblasts [9,10] and osteoclasts [11] have attracted attention to its direct actions on bone. E_3, referred to as a terminal metabolite or as an impending estrogen, was also revealed to have a role in the metabolic responses in estrogen receptor-containing target tissues [12]. Estrogens are known to suppress bone resorption at the time of accelerated bone turnover in the early stage after menopause and in elderly women, and to suppress reduction of bone mass, resulting in maintenance of or an increase in BMD [13]. Therefore, estrogen is clinically effective for patients at the early stage after menopause in particular. On the other hand, in elderly patients, bone turnover is thought to be relatively reduced, resulting in slow reduction of BMD [14]. The main cause of the reduction of BMD at these ages is most likely

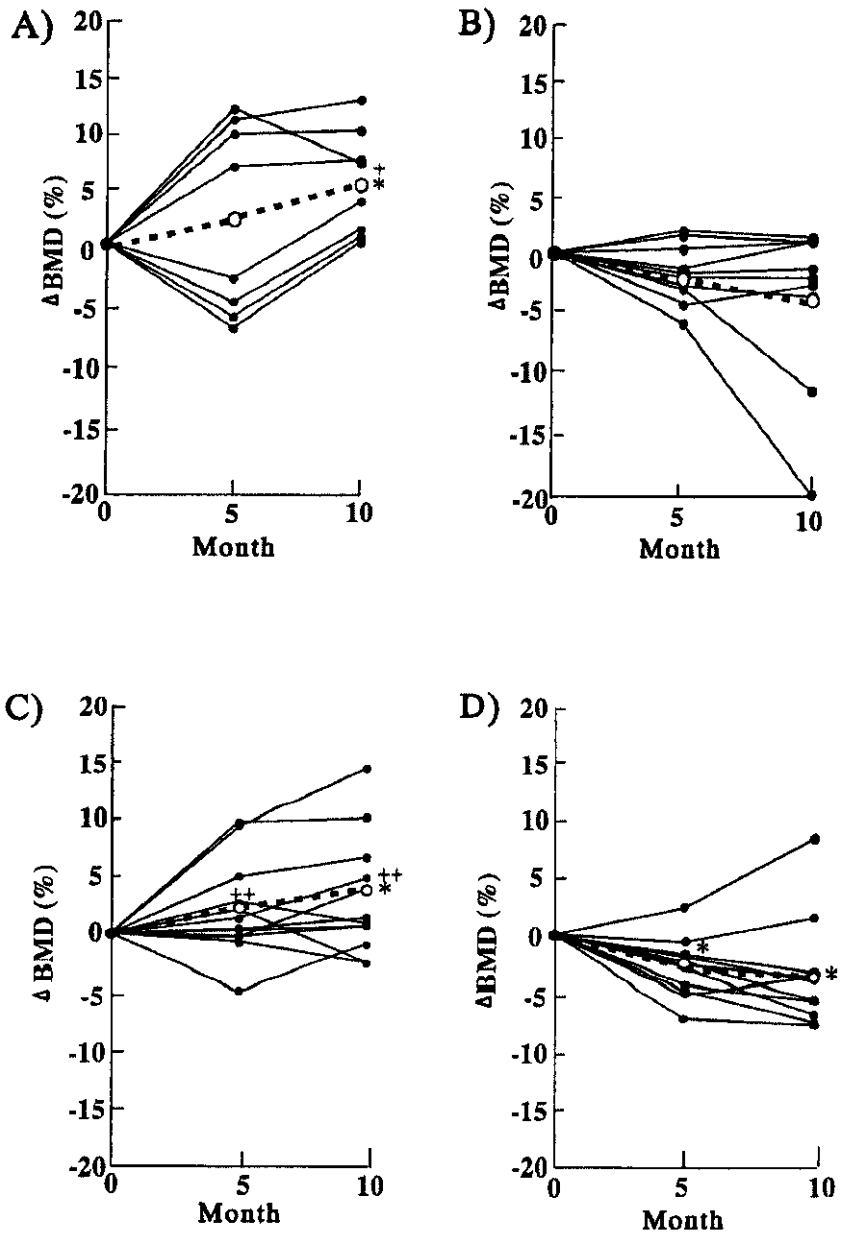

Fig. 1A–D. Comparison of rates of increase of bone mineral density (BMD) of lumbar vertebrae from basal values after 5 and 10 months of estriol (E_3) administration. **A** Postmenopausal E group; **B** postmenopausal C group; **C** elderly E group; **D** elderly C group. *Solid lines and circles*, individual data; *dotted lines and open circles*, mean data of each group. Data are mean ± SD. *, $P < .05$ *vs* respective basal values; ⁺, $P < .05$; ⁺⁺, $P < .01$ *vs* C group, at the same time

aging itself. Therefore, some investigations throw doubt on the involvement of estrogen in the reduction of bone density in elderly women [15].

In this study, the mean BMD value of L2–L4 was slightly but not significantly decreased in the postmenopausal women, and was significantly decreased in the elderly women who were administered calcium lactate alone during the 10-month period. On the other hand, in the patients administered E_3 and calcium lactate, BMD of the lumbar vertebrae increased during the 10 months of administration by 5.59% on average in the postmenopausal women and by 3.83% on average in the elderly women, the increase being significant in both groups. This result indicates two conclusions. First, E_3 is also effective when used as HRT for involutional osteoporosis. In basic studies of the effects of E_3 on bone, Atokins et al. [16] confirmed that parathyroid hormone- (PTH) induced bone resorption is suppressed by E_3 in mouse calvaria and found that its effect is equally strong as those of estrone (E_1) and estradiol (E_2). In clinical studies, Marslew et al. [17] reported that BMD of the lumbar vertebrae measured by DXA was

increased by 4%–5% in postmenopausal women (45–54 years) administered E_2 or E_2 valerate together with progestin during a 2-year period. Ettinger et al. [18] also found that BMD of the vertebral bones measured by quantitative computed tomography was increased by 2.3%–2.5% in postmenopausal women (60–85 years) administered with conjugated estrogen over a 1-year period. The rate of increase of BMD of the lumbar vertebrae in the postmenopausal women administered E_3 that was observed in this study is similar to the results of the foregoing reports. However, the actions of estrogen through receptors of the bone cells have been examined using mainly E_2, and a detailed comparison of E_3 and E_2 is yet to be performed.

Second, elderly women with suspected low bone turnover rates responded to E_3. From the viewpoint of fracture prevention, it is generally considered that HRT for involutional osteoporosis using estrogens should be started in the early stage of postmenopause (within 4 years after menopause) and continued for a long period [19]. However, Prestwood et al. [20] analyzed changes in the parameters of bone metabolism caused by short-term administration of conjugated estrogens to elderly women, and found that the bone remained responsive to estrogens even in the aged.

The results obtained in this study support the cited reports. In postmenopausal women the basal serum level of E_2 is significantly lower than during the early follicular phase of the cycle and the metabolic clearance rate of E_3 decreases only 13%; thus, the E_3 level in postmenopausal women should be low. Orally administered E_3 is rapidly absorbed, but elimination is also very rapid compared to other estrogens [21]. E_3 cannot be converted to E_2. Only 1%–2% of E_3 reaches the circulation, and 1% of serum E_3 bound to sex hormone-binding globulin (SHBG) [22]. SHBG was reported to be a powerful predictor of metacarpal bone loss in postmenopausal women, an effect that they attributed to its modulating effect on free E_2 levels [23], but it had very little effect on E_3. Although we did not record data on serum E_2, E_3, and SHBG levels in this study, we suggest that daily oral doses of E_3 will make E_3 a more potent hormone.

The differences between our estriol/BMD study and the negative study by Christiansen and Rodbro [24] concern the treatment for osteoporosis using E_3, the ages, and the races. The study showed that E_3 does not add a significantly beneficial effect of combined hormonal (E_2 + E_3) prophylaxis against early postmenopausal osteoporosis in Denmark, but we examined the effects of E_3 on BMD of the lumbar vertebrae in elderly and postmenopausal women in Japan. The aim of our study was to investigate the treatment of osteoporosis using only E_3. There were nonresponders to E_3 treatment in the early phase of our study, but E_3 has a beneficial effect on BMD of the lumbar vertebrae after 10 months. Nozaki et al. also reported that E_3 did not increase (n = 4) BMD after 6 months in postmenopausal women who had undergone natural menopause within 10 years [25].

The final aim of the treatment of osteoporosis is prevention of bone fractures. However, even in estrogen replacement therapy, which is considered most effective, prolonged administration of the hormone is required, and it is known that the decrease in BMD is accelerated if estrogen replacement therapy is discontinued [15]. Therefore, it is important to continue estrogen replacement therapy for a long period. One of the main causes of decrease in patient compliance when taking female sex hormones is genital bleeding caused by the growth of endometrium stimulated by estrogens. In long-term administration of estrogens, it is important to minimize genital bleeding, and the selection of medication and its regimen and dose should be carefully determined. E_3, which has a much weaker stimulating effect on endometrial growth than E_2 as described here, does not often cause genital bleeding [26].

In this study, genital bleeding was observed only in the elderly group, which may reflect the decreased rate of metabolism of the medication with aging. Therefore, when E_3 is to be administered to elderly patients, it is important to prevent genital bleeding by starting with a small dose of E_3 such as 0.5 or 1 mg/day, and then increase the dose according to the patient's condition. It is also necessary to examine the changes in BMD in patients administered E_3 for a period longer than 2 years. Therefore, E_3 is considered to be effective for long-term estrogen replacement therapy to maintain and improve the quality of life of elderly women as well as postmenopausal women. On the other hand, treatment with estrogen analogs (for example, tamoxifen) for osteoporosis was reported by Powles et al. [27]. Their study showed that tamoxifen treatment is associated with a significant loss of BMD in premenopausal women, whereas it prevents bone loss in postmenopausal women. Tamoxifen is important in chemoprevention of breast cancer, so we must soon compare it with E_3.

In the clinical parameters examined, there was a significant decrease in serum corrected Ca in the elderly E group, compared to that in the elderly C group, at 5 months after the start of administration and at the end of administration. It is reported that serum Ca in postmenopausal women significantly increases compared to that in premenopausal women, and is reduced to the premenopausal level by HRT [28]. In this study, a decrease in serum Ca was observed in the elderly group but not in the postmenopausal group. Although the reason for these results is not known, it is possible that the same amount of E_3 has stronger effects, including genital bleeding, in elderly women. The abruptly in-

creased serum E_3 level may cause genital bleeding to occur only in the elderly group. On the other hand, long-term estrogen replacement therapy is expected to be effective in the prevention and treatment of atherosclerosis as well as osteoporosis. A daily dose of less than 2 mg E_3 was considered to not prevent bone loss alone in postmenopausal and elderly women.

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