J. Steroid Biochem. Molec. Biol. Vol. 37, No. 3, pp. 451–455, 1990
Printed in Great Britain. 

0960-0760/90 $3.00 + 0.00


# CALCITONIN, ESTROGENS AND THE BONE

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**Summary**—Estrogen deficiency following natural or surgical menopause, is thought to be the main factor leading to postmenopausal bone loss. Furthermore, after estrogen failure a significant reduction of intestinal calcium absorption and a negativization of calcium balance has been observed. The mechanism of estrogen effect on skeletal tissue is not yet fully elucidated. Recently, specific receptors for estrogens in osteoblastic cells have been described; however their low density does not give a full explanation about their functional role. Therefore estrogens act, at least in part, indirectly through calciotropic hormones. In order to further elucidate this issue, we performed some studies in postmenopausal osteoporotic patients and in fertile oophorectomized women. In the first double blind placebo controlled study, after a 1-yr estrogen treatment period we observed an increase in bone mineral content in the hormone-treated patients. Furthermore, in all treated patients an improvement of intestinal calcium absorption was detected, while 1,25-dihydroxy-vitamin D serum levels did not show significant changes. To further analyse the relationship between estrogens (E) and calcitonin (CT) in postmenopausal osteoporosis, we performed a double blind placebo controlled study to evaluate the effects of 1-yr estro–progestative treatment on CT secretory reserve, evaluated by calcium infusion test. Blood levels of CT showed a progressive increase during the study period in the hormone-treated group, with a significant increase in the CT response to calcium stimulation test, suggesting a modulation of CT secretion by E. Recently, we performed two studies in fertile oophorectomized women. In the first, we followed longitudinally 24 fertile women for 1 yr. In these patients we measured, before and after oophorectomy, biochemical indexes of bone metabolism and bone mass. During the observation period a significant increase in bone resorption and a significant drop in intestinal calcium absorption was observed. In the second study, performed on 14 women before and 6 months after oophorectomy, a treatment with conjugated estrogens allowed the correction of the primary intestinal defect responsible for the reduced calcium absorption.

In conclusion, cumulative results of our studies suggest that: (a) estrogen deficiency represents the main pathogenetic factor in postmenopausal osteoporosis; (b) estrogen replacement therapy is effective in the prevention of rapid bone loss that occurs after menopause; (c) estrogens regulate CT secretion in postmenopausal women, so that CT may be considered a mediator of estrogen action on bone; (d) oophorectomy causes and early increase of bone resorption, and a later increase of bone formation; (e) oophorectomy causes a precocious impairment of intestinal calcium absorption, independent of vitamin D metabolism; (f) calcium malabsorption appears to be "resistant" to the action of vitamin D metabolites; and (g) in oophorectomized patients estrogen treatment protects both bone and intestine.

It is well known that involutional osteoporosis has a multifactorial pathogenesis. Since loss of ovarian function, both natural and surgical, is followed by an enhanced bone loss, it is postulated that estrogen deficiency is the main pathogenetic factor for postmenopausal bone loss. In fact, after estrogen failure a significant reduction of intestinal calcium absorption and a negativization of calcium balance has been observed [1, 2].

The causal role of estrogen lack is demonstrated by the finding of a higher incidence of vertebral crush fractures in women compared to age-matched men and by the rapid and increased loss of trabecular bone characteristic of the first years following menopause and oophorectomy [3, 4]. On the other hand, estrogen replacement therapy is effective in the prevention of the rapid bone loss that occurs after natural and surgical menopause [5–9]. The mechanism of estrogen effect on skeletal tissue is still far from being elucidated. Recently specific receptors for estrogens in osteoblastic cells have been described by two groups [10, 11], however their low density raises some questions about their physiological and functional role.

*Proceedings of the XIV Meeting of the International Study Group for Steroid Hormones*, Rome, Italy, 30 November–2 December 1989.

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Therefore, the hypothesis that estrogens act on bone, at least in part, indirectly still holds true. With regard to the indirect effect, two hypotheses have been postulated: (1) increase of 1,25-dihydroxyvitamin $D_3$ plasma levels; (2) increased secretion of calcitonin, the hormone that inhibits osteoclastic bone resorption.

A malabsorption of calcium has been frequently documented in postmenopausal osteoporotic patients. Nevertheless, the measurements of circulating levels of vitamin D metabolites in these patients have given conflicting results, in some reports they have been found to be low [12], but more frequently they are normal [13, 14].

Recently we have conducted a double blind placebo controlled study in order to assess the effect of 1 yr estrogen therapy on bone mass, intestinal calcium absorption and vitamin D metabolism in postmenopausal osteoporotic patients. We observed an increase in bone mineral content (BMC), measured by dual photon absorptiometry, both in the lumbar spine and in the femoral shaft, although the changes were more evident at the former site, predominantly composed by trabecular bone. Biochemical estimates of bone metabolism showed a decrease in urinary hydroxyproline/creatinine ratio and serum osteocalcin. In all treated patients an improvement of intestinal calcium absorption, measured by the $^{47}Ca$ oral test, was detected, while $1,25(OH)_2D$ showed a slight increase [15]. This study demonstrates that 1 yr treatment with estrogen improves BMC in postmenopausal osteoporotic patients and that vitamin D metabolites do not seem to play an important role in the mediation of estrogen action on bone. The slight increase of $1,25(OH)_2D$ levels observed after 1 yr treatment, apparently explains the positive effect of the hormonal therapy on calcium absorption. However, the estrogen-induced rise of $1,25(OH)_2D$ plasma levels has been found to be due to an increase of serum vitamin D binding protein (DBP), so that the free $1,25(OH)_2D$ is unchanged by the hormonal therapy in postmenopausal osteoporosis (PMO) [16, 17]. Therefore, the increase of $1,25(OH)_2D$ observed in our patients probably reflects only the effect of estrogens on DBP rather than a real action on vitamin D metabolism.

As it concerns the second hypothesis on the indirect effect of estrogen action on bone tissue, some authors have proposed calcitonin (CT) as a mediator of this effect. Clinical studies demonstrate that basal levels of CT decrease with age and appear to be lower in women than in men [18, 19] and lower still in postmenopausal women [20, 21]. Furthermore, a significant fall in CT levels follows surgical menopause [22, 23]; and estrogen treatment increases CT secretion in postmenopausal women [15, 24]. However, other investigators found no differences in CT levels between normal and osteoporotic women [25–27], and no effect of estrogen–progesterone treatment on the hormone plasma levels was observed in oophorectomized women [28]. To further analyse the interrelationship between estrogens and CT in PMO, we performed a double blind placebo controlled study to evaluate the effects of 1 yr estroprogestatives treatment on CT secretory reserve, evaluated by calcium infusion test, in postmenopausal women. A progressive and significant increase of BMC of the lumbar spine was observed in the hormones treated women, whereas the placebo treated group continued to lose a significant amount of bone mineral. Blood levels of CT showed a progressive increase during the study period in the hormone-treated group, reaching a plateau after nine months (Fig. 1). Hormonal therapy also significantly improved the CT response to calcium stimulation test (Fig. 2). This study suggests that estrogens regulate CT secretion in postmenopausal women; thus CT may be considered a mediator of estrogen action on bone. Recently, Reginster *et al.* [29] measured the metabolic clearance rate and production rate of CT in pre- and postmenopausal women, including osteoporotics. In healthy postmenopausal women the production rates were reduced, albeit not significantly, whereas osteoporotic patients presented a highly significant reduction of calcitonin production rate.

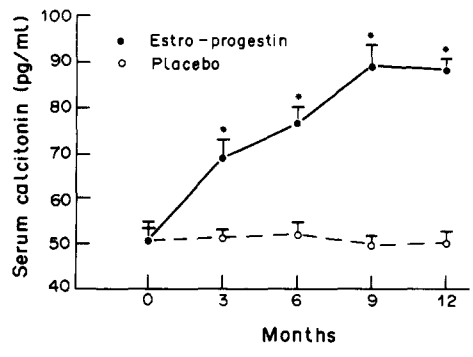


Fig. 1. Effect of 1 yr treatment with either estrogen–progesterone or placebo on basal serum calcitonin in postmenopausal osteoporotic women. Asterisks indicate a significant difference vs time 0 at 95% confidence limit (Scheffe's multiple range test).

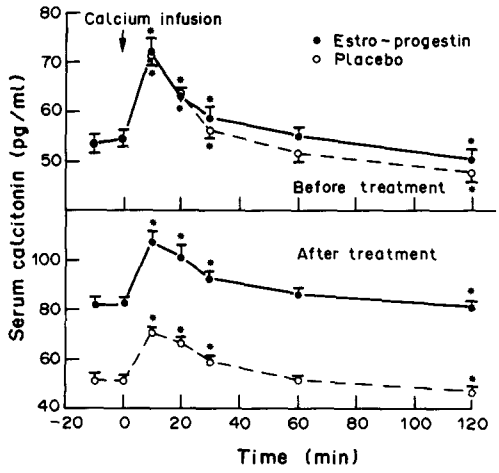


Fig. 2. Serum calcitonin during a calcium infusion test in postmenopausal osteoporotic women before (upper panel) and after (lower panel) the treatment period. Asterisks indicate a significant difference vs time 0 at 95% confidence limit (Scheffe's multiple range test). The effect of the treatments on serum calcitonin variance was assessed by two-factor ANOVA.

Since there was a strong relationship between estrone circulating levels and CT production rates, calcitonin secretory capacity appears to be modulated by estrogens.

The acute estrogen failure due to ovariectomy is known to induce a rapid phase of bone loss, which can be improved by estrogen replacement therapy [4, 5, 7]. The early effects of ovarian failure on bone remodeling have not yet been fully elucidated.

To address this issue we have recently followed longitudinally for 24 fertile women (age 28–52 yr) 1 yr who underwent the surgical removal of both ovaries [30]. In these patients we have measured before and after 10, 20, 30 days and 2, 6 and 12 months the serum levels of calcium, iPTH, iCT, $1,25(OH)_2D_3$, osteocalcin and the urinary excretion of hydroxyproline. Furthermore, the intestinal calcium absorption was measured before and after 2, 6 and 12 months by the $^{47}Ca$ oral test. The BMC of the lumbar spine and femoral shaft was measured before and after 2, 6 and 12 months by dual photon absorptiometry.

The serum calcium levels did not show significant changes, but a trend in increase was evident immediately after OOX. The circulating levels of the two main calciotropic hormones, the iPTH and the iCT, did not change significantly. The urinary hydroxyproline/creatinine ratio dramatically increased during the first 2 months, reaching a plateau maintained until the end of the study; on the other hand, the serum osteocalcin levels showed a later and progressive significant increase until the end of the study. The intestinal radiocalcium absorption, expressed as the circulating fraction of the absorbed dose, was significantly depressed only after 1 month; the malabsorption of calcium also persisted after 2, 6 and 12 months. At the same time no significant variations of the main metabolite of vitamin D were detected.

The BMC decreased both at lumbar spine and femoral diaphysis sites. The bone loss was more precocious and more evident at the trabecular bone level.

The analysis of variance demonstrated that, beside the changes in the female hormones, the drop in calcium absorption and the increase in HOP were highly significant.

These results are in keeping with similar findings reported in other recent studies [31, 32].

More recently, we have performed another study on 14 women before and after 6 months from ovariectomy.

After operation, the patients were randomly assigned to a treatment with placebo or conjugated estrogens (Premarin, 0.625 mg/day). The intestinal calcium absorption was measured before and after 6 months, according to a double radiocalcium oral test: the first performed before and the second after 7 days of treatment with $1,25(OH)_2D_3$ (1 mcg/day). The plasma levels of 1,25 were measured as well.

With regard to the bone mineral density of lumbar spine, at the end of the study a significant decrease in placebo-treated subjects was documented; while no significant change was observed in estrogen-treated patients (Fig. 3). After 6 months, the basal intestinal absorption of radiocalcium decreased significantly in placebo- but not in estrogen-treated patients. No significant changes in serum $1,25(OH)_2D_3$ were observed in placebo-treated patients, while a slight but significant increase was documented in estrogen-treated patients.

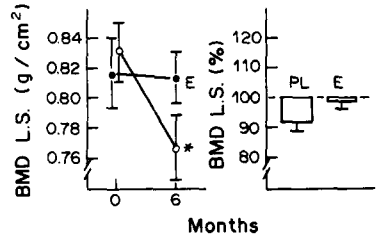


Fig. 3. Effects of 1 yr treatment with conjugated estrogens (E) or placebo (PL) on the bone mineral density of the lumbar spine in 14 ovariectomized. Asterisk indicates a significant difference ($P < 0.05$) vs time 0 (Student's paired $t$-test). (Values expressed as Mean ± SEM).

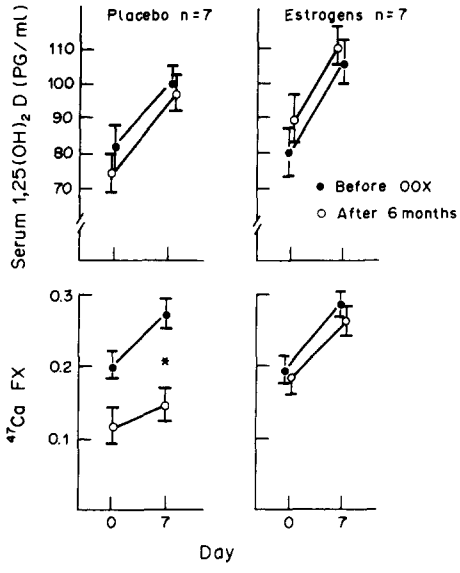


Fig. 4. Intestinal absorption of radiocalcium and circulating levels of 1,25-dihydroxycholecalciferol in ovariectomized women treated for six months with conjugated estrogens (on the right) or placebo (on the left). A double radiocalcium oral test was performed before the operation and at the end of treatments in order to evaluate the stimulating effect of 7-days treatment with $1,25(OH)_2D_3$ (1 mcg/day). The intestinal absorption of radiocalcium has been expressed as the circulating fraction of the absorbed dose ($^{47}Ca$ fx).

The $1,25(OH)_2D_3$ test before ovariectomy provoked a significant increase in intestinal absorption of radiocalcium in all patients.

After 6 months, at the second 1,25 test, the increase in calcium absorption resulted significantly lower in placebo group when compared to estrogen-treated women (Fig. 4). The plasma levels of $1,25(OH)_2D_3$ increased after $1,25(OH)_2D_3$ administration to the same extent before and after 6 months in both groups.

Our data suggest that estrogens might correct a primary intestinal defect responsible for the reduced calcium absorption. In fact, a deficiency of $1,25(OH)_2D_3$ receptors in the gut has been shown in *in vivo* and *in vitro* studies [33, 34]. Furthermore, other investigators showed, in elderly women, a resistance of calcium malabsorption to the action of vitamin D metabolites [35].

In conclusion, cumulative results obtained in our studies suggest that:

—Estrogen deficiency represents the main pathogenetic factor in postmenopausal osteoporosis.
—Estrogen replacement therapy is effective in the prevention of rapid bone loss that occurs after menopause.
—Estrogens regulate CT secretion in postmenopausal women, so that CT may be considered a mediator of estrogen action on bone.
—OOX causes an early increase of bone resorption, and a later increase of bone formation.
—OOX causes a precocious impairment of intestinal calcium absorption, independent of vitamin D metabolism.
—Calcium malabsorption appears to be "resistant" to the action of vitamin D metabolites.
—In OOX patients estrogen treatment protects both bone and intestine.

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