Transdermal estrogen replacement does not increase calcitonin secretory reserve in postmenopausal women

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Abstract. The effect of transdermal estrogen replacement on ionized calcium and calcitonin levels was examined in 15 postmenopausal women. Following baseline measurement of calcitonin and ionized calcium in the fasting state, the effect of calcium infusion on calcitonin levels was studied. Estrogen replacement resulted in a fall in baseline ionized calcium, however, the rate of rise of calcium was the same before and after estrogen administration. Thus the time at which a particular calcium concentration was attained was later after the commencement of the calcium infusion following estrogen replacement. Although there was no detectable difference in baseline calcitonin concentrations (pre-estrogen, 2.4±0.4; post-estrogen, 2.1±0.4 pmol/l), following estrogen replacement the time at which a particular calcitonin concentration was attained was later after the commencement of the calcium infusion, reflecting the slower attainment of a particular calcium concentration (p=0.014 by ANOVA). Analysis of total calcitonin production by area under the curve, however, did not show a significant difference before and after estrogen replacement (643±184 and 407±115 pmol/l⁻¹100 min⁻¹, respectively). When the calcitonin response to calcium infusion was compared at the same calcium concentration, estrogen status had no effect on the relationship. We conclude that transdermal estrogen replacement has no effect on calcitonin secretory reserve in postmenopausal women and does not alter the relationship between elevated calcium and calcitonin levels. We cannot exclude an effect of estrogen on baseline calcitonin levels as the calcium concentration was lower but the calcitonin levels not different.

The mechanism by which estrogen therapy reduces bone resorption in postmenopausal women remains unclear. One potential mechanism which has been proposed is the stimulatory effect of estrogen on calcitonin secretion (1), a hormone which has been shown to act on bone to inhibit osteoclast function and bone resorption (2). Consistent with the hypothesis that the bone loss is associated with estrogen deficiency and mediated by calcitonin deficiency, calcitonin levels are reported to be lower in women than men (3-5) and to rise less after a calcium stimulus than in men (4,5). In some studies calcitonin secretion in response to a calcium stimulus has been shown to decrease with age in men and women (5,6) and after the menopause in women (7). There have been reports of lower calcitonin levels in osteoporotic women compared to normal postmenopausal women suggesting a role for calcitonin deficiency in the pathogenesis of osteoporosis (8,9), but this remains controversial with other studies showing no difference (10) or higher calcitonin levels in osteoporosis (11,12).

The effect of oral estrogen replacement on calcitonin secretion is controversial with three similar studies from the same group showing a rise in calcitonin levels (1,18,14) despite a fall in plasma calcium while other studies show lower or unchanged calcitonin levels after estrogen therapy (15-18). Transdermal estrogen replacement is a more physiological route of administration than oral estrogen replacement and different hormonal effects between the two have been noted. Transdermal estrogen replacement causes a rise in intact parathyroid hormone but not calcitriol or vitamin D binding protein levels (19), unlike oral estrogen replace-
ment which is reported to increase calcitriol and vitamin D binding protein levels (18). The present study examines the effect of transdermal estrogen therapy on the calcitonin response to a slow intravenous calcium challenge.

Subjects and Methods

Fifteen post menopausal women were recruited for the study. Their mean age was 44 years (range 37-54). Nine were studied one month after oophorectomy and hysterectomy. The remainder were studied 1-12 years after natural menopause. For the 24 h prior to the study the patients kept a food record. From 22.00 h on the night prior to the test they were allowed water only by mouth. At 09.00 h on the day of the test they were admitted to the research unit and a fasting morning urine sample was collected. Venous cannulae were placed in both arms, one was used for blood sampling at 10-min intervals, the other for infusing calcium gluconate (0.1 mmol·kg⁻¹·h⁻¹) until the blood ionized calcium reached 1.5 mmol/l. The calcium infusion was commenced after taking 3 baseline blood samples 10 min apart. At the end of the test the subjects were given a transdermal estrogen preparation (Estraderm®, Ciba Geigy, Basel, Switzerland) in the dose of a 100 μg patch changed every 3.5 days. One month to 6 weeks after the first study they underwent a second study identical to the first. They were instructed to eat exactly the same diet for the 24 h prior to the second study as they had consumed for the 24 h prior to the first study. The protocol was approved by the Human Ethics Committee of the Massachusetts General Hospital and informed consent obtained. The parathyroid hormone responses have been reported separately (19).

Assays

Ionized calcium was measured on heparinized venous blood using a Nova 2 ionized calcium electrode at the pH of the blood sample. Samples were kept on ice and measured within 5 min of collection. Immunoreactive calcitonin was measured by radioimmunoassay using an assay which has been previously described (12). In brief, the assay was performed on unextracted plasma using antiserum kindly supplied by Dr C J Hillyard, Hammersmith Hospital (London, UK). The antiserum cross-reacted with fragment 17-28 of human calcitonin and also had some C-terminal (29-32) specificity. Intra- and inter-assay coefficient of variation at 3 pmol/l are 15 and 16%, respectively. The smallest quantity detectable as defined by Ekins (20) was 0.6 pmol/l. For the purposes of analysis, when the calcitonin levels were undetectable they were ascribed a value equal to the smallest quantity detectable in that assay. Samples from the same subject were measured in the same assay. Estradiol-17β and FSH were measured by radioimmunoassay (21,22).

Data analysis

The data were examined using the SPSSPC statistical package. Baseline data were examined by paired Student’s t-test and results defined as mean ±SEM. Calcitonin response to calcium infusion before and after estrogen therapy was analysed by groupings the ionized calcium results at 0.05 mmol/l intervals and calculating the mean ±SEM for the corresponding calcitonin value. Differences between curves were analysed by two-way analysis of variance. Total calcitonin secretion was also calculated by area under the curve (AUC) and compared before and after estrogen therapy by Student’s paired t-test.

Results

Estrogen replacement resulted in a rise in estradiol-17β from 84±7 to 204±35 pmol/l and a fall in FSH from 128±13 to 51±5 U/l (p<0.001). The baseline concentration of ionized calcium decreased from 1.23±0.01 to 1.19±0.01 mmol/l (p<0.001) after estrogen therapy, and the baseline calcitonin levels before and after estrogen treatment were 2.4±0.4 and 2.1±0.4 pmol/l, respectively, which was not a significant change. Values were undetectable in 6 subjects. Analysis in only those subjects in whom

![Fig. 1](image-url)

The effect of a calcium infusion with time on plasma ionized calcium levels before and after transdermal estrogen replacement. Results are mean ± SEM for 15 subjects. Open symbols pre-estrogen, closed symbols post-estrogen. The calcium infusion was commenced at time zero. The two curves are significantly different (p<0.001) by two-way ANOVA.
there were detectable baseline levels of calcitonin also did not show any effect of estrogen. The calcitonin levels before and after estrogen in this group were 4.8±1.1 and 4.8±1.3 pmol/l, respectively.

The rate of increase in plasma ionized calcium during calcium infusion was not altered by estrogen therapy (Fig. 1). Because of the lower baseline calcium concentration after estrogen replacement there was a delay in attaining similar calcium concentrations. There was a concomitant statistically significant decrease by ANOVA (p=0.014) in the stimulated calcitonin levels with time after estrogen therapy (Fig. 2). Total calcitonin secretion calculated by area under the curve before and after estrogen therapy was not significantly different by two-tailed paired t-test (643±184 and 407±115 pmol·l\(^{-1}\)·100 min\(^{-1}\), respectively). When calcitonin levels were analysed with respect to ionized calcium levels, there was no difference in the shape of the curve before and after estrogen replacement (Fig. 5). Calcitonin levels were undetectable at any time point in 6 patients. Analysis of subjects in whom there were detectable levels of calcitonin did not show any effect of estrogen. There was no correlation between baseline calcitonin and estrogen levels or estrogen levels after estrogen replacement and change in basal calcitonin levels. There was also no correlation between the change in area under the curve with change in estrogen levels after estrogen replacement therapy.

**Discussion**

The lack of a stimulatory effect of estrogen therapy on baseline calcitonin levels in this study of healthy postmenopausal women supports some (15-18) but not all (1,13,14) previous reports. In one study an increase in basal calcitonin levels occurred only after 6 months of therapy (23) suggesting that estrogen may only have long-term effects on calcitonin secretion. Because the baseline ionized calcium concentrations were lower after estrogen it might be expected that the calcitonin levels should be lower, but we were not able to detect this. This study differs from previous ones by employing a longer calcium infusion technique with a slower rise in ionized calcium. Estrogen therapy in this study did not alter calcium-stimulated calcitonin levels. After estrogen therapy, the observed slight reduction in calcitonin with time noted by ANOVA analysis but not AUC analysis during the calcium infusion may have been the result of the lower baseline ionized calcium levels associated with estrogen therapy. Alternatively, the lack of difference in the AUC analysis supports the lack of effect of estradiol on calcitonin secretion. Certainly, calcitonin
levels compared to ionized calcium levels were the same before and after therapy. These results are in accord with some previous studies using short bolus dose calcium infusions (15-17), but not with another (24) which showed a stimulatory effect of oral estrogen administration on calcitonin secretion. A recent study by Reginster et al. (25) comparing the production rate of calcitonin in pre and postmenopausal women did not show any marked change in production rate with menopausal status. There did appear to be a positive correlation between plasma estrone and calcitonin production rate but this was not factored for body size. Calcitonin clearance rates were also not altered with menopause (25), indicating that the serum concentration of calcitonin is a reasonable indicator of production under these circumstances. Finally, evidence of raised basal levels of calcitonin in osteoporosis (11,12), makes a reduction in secretory reserve or production rate less likely in this condition.

It is clear that different researchers using different assay methodologies have different findings on the effects of estrogen on basal calcitonin levels and calcitonin secretory reserve. These varying results could be due to differences in calcitonin assay methodologies as some whole plasma calcitonin assays may cross-react with non-biologically active fragments, accounting for large variations in calcitonin levels reported in the literature. Our results agree with those studies based on assays using "extracted calcitonin" (16,17) and by the Immunonuclear Corporation (15) but not those based on other antibodies (1,24,25). The circulating levels of calcitonin measured in the "extracted calcitonin" assay and our assay appear to be lower than those reported by other groups (1,24,25) suggesting that one explanation for the discrepant results is that there is a factor cross-reacting in other calcitonin assays but not ours that is estrogen-dependent. If it exists, the biological activity of this factor remains to be determined, but in view of the evidence that the calcitonin-deficient state is not associated with accelerated bone loss (26) it is unlikely to have biological activity related to skeletal homeostasis.

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