Oestrogen has no short-term effect on intestinal strontium absorption in healthy postmenopausal women

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Summary

OBJECTIVE Impaired intestinal calcium absorption in postmenopausal women is often indirectly linked to decreased serum $1,25(\text{OH})_2\text{D}$ or to intestinal resistance to its action rather than directly to low circulating oestrogen levels following the menopause. The purpose of this clinical study was to investigate the short-term effect of oral 17β -oestradiol on intestinal calcium absorption, with strontium as a marker.

DESIGN AND PATIENTS Twenty-five healthy postmenopausal women participated in this randomised double blind placebo controlled clinical trial. Twelve women received oestradiol therapy (2 mg/day) and thirteen placebo for 2 months. Fractional strontium absorption (Fc_{240}) was assessed at baseline and after 2 months of oestradiol/placebo therapy.

RESULTS Intestinal strontium absorption (Fc_{240}) was unchanged after treatment with 17β -oestradiol (10.1 ± 5.0 vs. 10.2 ± 3.8 (%)). Serum total calcitriol ($1,25(\text{OH})_2\text{D}$) was unchanged after treatment with placebo (88 ± 22 vs. 79 ± 21 (pmol/l)) but increased after treatment with oestradiol (88 ± 30 vs. 116 ± 33 (pmol/l); $P < 0.005$). Serum vitamin D binding protein (DBP) increased after oestradiol but not after placebo treatment. The free serum $1,25(\text{OH})_2\text{D}$ index was calculated. This index did not change after oestrogen therapy (1.6 ± 0.5 vs. 1.8 ± 0.5).

CONCLUSION In healthy postmenopausal women, short-term suppletion with exogenous oral oestrogen

did not influence intestinal calcium absorption as measured by the strontium absorption test.

It is well known that ovarian hormone deficiency is a major risk factor for osteoporosis in postmenopausal women (Prince, 1994). Postmenopausal osteoporosis is often associated with intestinal malabsorption of calcium (Gallagher *et al.*, 1979). Impaired calcium absorption is believed to aggravate a negative calcium balance and contribute to bone loss in postmenopausal women (Gallagher *et al.*, 1980; Heaney *et al.*, 1978).

Gastrointestinal absorption of calcium is a sum of two processes: a saturable (active) and a non-saturable (passive) one (Blanchard & Aeschlimann, 1989). Passive absorption is a function of ingested calcium load and is neither saturable nor subject to regulation in human (Barger-Lux & Heaney, 1995). The active component of intestinal calcium absorption appears to be under relatively rigid hormonal control in which $1,25(\text{OH})_2\text{D}$ plays a dominant role.

There is a considerable uncertainty about the underlying cause of calcium malabsorption in postmenopausal women. It has often been attributed to decreased serum $1,25(\text{OH})_2$ vitamin D levels (Gallagher, 1990) and to intestinal resistance to its action (Gennari *et al.*, 1990), which could lead to a reduction of active intestinal calcium absorption (Francis *et al.*, 1984; Morris *et al.*, 1991). Recent *in vitro* and *in vivo* data showed that rat intestinal mucosa contained oestrogen receptor immunoactivity and responded to estradiol (Arjmandi *et al.*, 1993, 1994). These findings suggest that oestrogen may have a physiological role in the regulation of intestinal calcium absorption and that its deficiency in postmenopausal osteoporosis may directly result in intestinal calcium malabsorption. Therefore, the purpose of our study was to examine the effect of oestrogen on intestinal calcium absorption in healthy postmenopausal women.

Three methods are available for the measurement of intestinal calcium absorption in human: a full calcium balance which requires a stay of at least 2 weeks in a metabolic unit and is impractical for routine use; a double isotope procedure, with two either stable or radioactive calcium isotopes, which has a number of technical difficulties and implies a delay of several weeks before yielding a result; and a single isotope procedure, which is the simplest, cheapest and quickest method. For clinical use a simple, easy to perform intestinal absorption test with minor discomfort for the patient is preferred and thus

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suitable for large-scale application and frequently repeated tests within one person.

Nonradioactive stable strontium has been used as a tracer for calcium, to measure intestinal calcium absorption. A close correlation between the absorption of strontium and ^{45}Ca has been reported in normal subjects and in patients with various disorders of calcium metabolism (Reid *et al.*, 1986). A simple clinical test for estimating intestinal calcium absorption using stable strontium was elaborated in our group (Sips *et al.*, 1996). Using this test in healthy male volunteers, we found that the true bioavailability of strontium correlated best with the fractional absorption of strontium at 240 minutes (Fc_{240}) after oral intake of strontium.

Fractional absorption of strontium (Fc_{240}) is based on extracellular fluid volume. Usually, 15% of the body weight is used as an estimation of the extracellular volume (ECV) to calculate fractional absorption (Civitelli *et al.*, 1988; Need *et al.*, 1987; Gennari *et al.*, 1997). However, hormonal intervention may change the ECV and can thus result in an apparent change in intestinal absorption. Therefore, we assessed the ECV at the basal level and after oestrogen intervention and used the ECV, instead of 15% of body weight, for calculating the Fc_{240} of strontium. This gives us the opportunity to correct for a changing volume of ECV when necessary.

This adapted test was used to investigate the effect of oestrogen on intestinal strontium absorption in healthy postmenopausal women.

Materials and methods

Study population

Twenty-six healthy postmenopausal women participated in the study after written informed consent. Fifteen women endured hysterectomy. In the other women menopause was presumed to be present if the menstrual cycle had stopped for more than a year. Menopause was confirmed biochemically by low plasma oestradiol levels ($<95\text{ pmol/l}$) and by follicle-stimulating hormone (FSH) plasma levels exceeding 30 IU/l . Major exclusion criteria were: known or suspected oestrogen-dependent carcinoma, known thromboembolic disease, diabetes mellitus, a history of excessive alcohol ingestion, changes of body weight $>4\text{ kg}$ during the previous 4 weeks, intake of corticosteroids during the previous 6 months, intake of female sex hormones during the previous 3 months or any medication influencing calcium and bone homeostasis. Women with a serum creatinine $>100\text{ }\mu\text{mol/l}$, ALAT (liver enzyme) $>100\text{ U/l}$, alkaline phosphatase $>300\text{ U/l}$ were also excluded. One participant dropped out of the study because of a hospital admission for varicose vein treatment. Data in this study are therefore based on 25 women (mean age 52.9, range 48 to 57 years) randomly assigned to either 17β -oestradiol or placebo.

All subjects gave informed consent to participate in the study, which was performed in our outpatient clinic of the University Hospital Vrije Universiteit, Amsterdam. The study was approved by the ethics committee of our hospital. All procedures followed were in accordance with the Declaration of Helsinki.

Study design

All subjects came to the hospital after overnight fasting. Blood samples were obtained for the determination of plasma strontium, bromide, calcium, albumin, phosphate, serum $1,25(\text{OH})_2\text{D}$ and DBP. Because of the possibility that oestrogen could change the extracellular fluid volume (ECV), necessary for the calculation of the fractional absorption of strontium (Fc_{240}), the ECV was assessed by means of an intravenous injection (in 10 minutes) of sodium bromide (1 ml/kg of an aqueous 5% sodium bromide solution, which is equivalent to $38.8\text{ mg Br}^-/\text{kg}$). After administration of sodium bromide subjects received a 200 ml test solution, which contained 5.0 mmol SrCl_2 in the middle of a standardized meal, which consisted of 150g Fortipudding with vanilla flavour (Nutricia, Zoetermeer, The Netherlands). Both the pudding and the test solution were consumed in approximately 3 to 4 minutes. The pudding was a semi-solid ready to eat standardized nutritional supplement. Four hours after ingestion of strontium a blood sample was withdrawn for the determination of plasma bromide and strontium. Blood samples were centrifuged at $1500g$ for 10 min. Subsequently, plasma was separated and stored at -20°C until analysis. All plasma samples were analysed for strontium by graphite furnace atomic absorption spectrophotometry (Barto *et al.*, 1995) and for bromide by ion-exchange chromatography (Miller *et al.*, 1989, with modifications). Solutions of NaBr and $\text{SrCl}_2.6\text{H}_2\text{O}$ (p.a., Merck, Amsterdam, The Netherlands) were prepared by the Pharmacy Department of the University Hospital Vrije Universiteit.

After this test, participants were randomised to either treatment with 17β -oestradiol, 2 mg orally a day (Zumenon, Solvay Pharma, Weesp, The Netherlands) or placebo for 2 months. They had to take this medication in the morning before breakfast. The strontium absorption test was repeated at the end of the study i.e. 2 months after the first test.

Methods

Serum FSH was measured by a luminescence immunometric assay (Amerlite, Amersham, UK). Intra- and inter-assay coefficients of variation were 5% and 7%, respectively. Serum oestradiol was measured by a double radioimmunoassay (RIA) (Sorin Biomedica, Saluggia, Italy). Intra- and inter-assay coefficients of variations were 4.5% and 10.5%, respectively.

Table 1 Characteristics of the participating women. Values are expressed as mean $\pm$ SD.

Parameter	Placebo (<i>n</i> = 13)	Oestrogen (<i>n</i> = 12)
Age (years)	52.9 $\pm$ 2.8	53.4 $\pm$ 3.4
Weight (kg)	70.9 $\pm$ 11.1	75.2 $\pm$ 12.5
Height (m)	1.67 $\pm$ 0.04	1.65 $\pm$ 0.09
ECV (l)	16.7 $\pm$ 3.5	16.0 $\pm$ 1.9
FSH (U/l)	59.8 $\pm$ 21.6	55.2 $\pm$ 20.3
Oestradiol (pmol/l)	26.1 $\pm$ 8.6	39.9 $\pm$ 24.3
Albumin (g/l)	41 $\pm$ 2	41 $\pm$ 2
Total protein (g/l)	70 $\pm$ 4	69 $\pm$ 4
Calcium (mmol/l)	2.32 $\pm$ 0.06	2.30 $\pm$ 0.07
Phosphate (mmol/l)	0.94 $\pm$ 0.14	0.97 $\pm$ 0.17
25(OH)D ₃ (nmol/l)	71 $\pm$ 19	61 $\pm$ 21
1,25(OH) ₂ D (pmol/l)	88 $\pm$ 22	88 $\pm$ 30
DBP (mg/l)	325 $\pm$ 54	310 $\pm$ 45
Free 1,25(OH) ₂ D index	1.6 $\pm$ 0.5	1.6 $\pm$ 0.5

None of the differences between groups is significant.

Serum 1,25(OH)₂D was measured by radioimmunoassay after immune-extraction (IDS, Tyne and Wear, UK). Intra- and inter-assay coefficients of variation were 6.3% and 9.7%, respectively. Serum vitamin D-binding protein (DBP) was determined by Bouillon *et al.* (1977), and the free serum index of 1,25(OH)₂D was calculated as the molar ratio of 1,25(OH)₂D concentration to DBP concentration (Bouillon *et al.*, 1981).

The ECV was calculated from the plasma Br⁻ concentration, after an intravenous injection of the sodium bromide solution (38.8 mg Br⁻/kg), according to the following formula (Miller *et al.*, 1989).

$$\text{ECV(L)} = \frac{\text{Br}^- \text{ dose (mmol)}}{[\text{Br}^-]_{t=240 \text{ min}} - [\text{Br}^-]_{t=0 \text{ min}} (\text{mmol/l})} \times 0.90 \times 0.95$$

in which 0.90 is the correction factor for the distribution of Br⁻

in the non-extracellular sites (principally red blood cells) and 0.95 is the correction factor for the Donnan equilibrium. Intestinal strontium absorption was calculated according to the following formula (Sips *et al.*, 1996):

$$\text{Fc}_{240}(\%) = \frac{[C_t - C_0] \times V}{D} \times 100$$

in which C_t = plasma concentration at 240 min, C₀ = plasma concentration at 0 min, V is the central volume of distribution represented by the ECV and measured by means of sodium bromide, D = dose of strontium = 5.0 mmol. The short (1 month)- and long-term (6 months) precisions (standard error of reproducibility, SE_R) assessed in our laboratory in 8 healthy male volunteers and 12 healthy postmenopausal women were 1.85% and 1.63%, with intra-assay coefficients of variation (CV) of 19.6% and 28.6%, respectively.

Statistical analysis

Values are expressed as mean (SD). The difference between two months of therapy and basal values was calculated for all biochemical parameters and expressed as delta (Δ). The difference between the placebo and oestrogen treated group was tested for a significant difference between the delta values by a Student *t*-test for independent samples. Statistical difference was defined as *P* < 0.05 (two tailed). All analyses were performed using the Statistical Package for the Social Sciences (SPSS 7.0 for windows).

Results

Baseline characteristics of the participating women are presented in Table 1. There were no significant differences in the basal values between the placebo and the treatment groups. No adverse effects of the intervention were reported.

Table 2 Effect of oestrogen treatment on metabolic parameters. Significant differences from placebo values by Student's *t*-test for independent samples (**P* < 0.005, ***P* < 0.001). Values are expressed as mean $\pm$ SD.

	Basal		Change after treatment (Δ)	
	Placebo (<i>n</i> = 13)	Oestrogen (<i>n</i> = 12)	Placebo (<i>n</i> = 13)	Oestrogen (<i>n</i> = 12)
ECV (l)	16.7 $\pm$ 3.5	16.0 $\pm$ 1.9	+ 0.47 $\pm$ 1.4	+0.88 $\pm$ 0.55
Fc ₂₄₀ (%)	9.2 $\pm$ 3.0	10.1 $\pm$ 5.0	+ 0.98 $\pm$ 2.8	+0.1 $\pm$ 2.1
Calcium (mmol/l)	2.32 $\pm$ 0.06	2.30 $\pm$ 0.07	+0.0009 $\pm$ 0.06	+0.003 $\pm$ 0.03
Phosphate (mmol/l)	0.94 $\pm$ 0.14	0.97 $\pm$ 0.17	-0.002 $\pm$ 0.4	-0.03 $\pm$ 0.40
25(OH)D ₃ (nmol/l)	71 $\pm$ 19	61 $\pm$ 21	-8 $\pm$ 12	+0.1 $\pm$ 9
1,25(OH) ₂ D (pmol/l)	88 $\pm$ 22	88 $\pm$ 30.0	-9 $\pm$ 19	+32 $\pm$ 33*
DBP (mg/l)	325 $\pm$ 54	310 $\pm$ 45	-15 $\pm$ 44	+55 $\pm$ 37**
Free 1,25(OH) ₂ D index	1.6 $\pm$ 0.5	1.6 $\pm$ 0.5	-0.1 $\pm$ 0.4	+0.3 $\pm$ 0.5

Fig. 1 The effect of 2 months of oestrogen supplementation on intestinal strontium absorption (Fc₂₄₀), ECV and biochemical parameters in healthy postmenopausal women. ▽, (▼): mean of the group. *P < 0.005, **P < 0.001.

Oestradiol therapy did not change serum calcium, phosphate, and $25(\text{OH})\text{D}_3$ (Table 2). Furthermore, oestrogen did not show a significant change in extracellular fluid volume (Fig. 1). A 2-month treatment with oestrogen increased serum levels of total $1,25(\text{OH})_2\text{D}$ and vitamin D-binding protein significantly, expressed as Δ values, compared to the placebo-treated group ($P < 0.005$ and $P < 0.001$, respectively). However, the calculated free $1,25(\text{OH})_2\text{D}$ index did not show a significant change. Intestinal calcium absorption, as measured by the strontium absorption test (Fc_{240}), did not change after treatment with oestrogen compared to the placebo-treated group (Fig. 1).

Discussion

In this short-term randomised double-blind placebo controlled clinical study we have shown that treatment with 17β -oestradiol in healthy postmenopausal women did not affect intestinal calcium absorption as measured by the strontium absorption test (Fc_{240} of strontium). The existing data on the effect of oestrogen on intestinal calcium absorption are conflicting. Gallagher *et al.* (1980) and Civitelli *et al.* (1988) described a significant positive effect of oestrogen treatment on intestinal calcium absorption in postmenopausal osteoporotic women, using dual tracer and single tracer absorption tests, respectively. Other reports (Balhorn *et al.*, 1997; Heaney *et al.*, 1978; Selby *et al.*, 1985) demonstrated that oestrogen supplementation did not significantly increase intestinal calcium absorption. Balhorn *et al.* (1997) and Selby *et al.* (1985) used a single tracer absorption test, whereas Heaney *et al.* (1978) measured intestinal calcium absorption by a dual tracer test under balance conditions. Gallagher *et al.* (1980) and Civitelli *et al.* (1988) showed that the efficiency of intestinal calcium absorption in postmenopausal osteoporotic women improved in parallel with an oestrogen-induced rise in serum $1,25(\text{OH})_2\text{D}$ level, suggesting that biologically active $1,25(\text{OH})_2\text{D}$ had indeed increased. They concluded therefore, that oestrogen treatment increases intestinal calcium absorption in postmenopausal osteoporosis by increasing $1,25(\text{OH})_2\text{D}$. However, oestrogen-induced rise in $1,25(\text{OH})_2\text{D}$ serum levels has been found to be due to an increase of serum DBP, resulting in unchanged free $1,25(\text{OH})_2\text{D}$ levels after treatment with estrogens (Bouillon *et al.*, 1981; Dick *et al.*, 1995). In a later study, Balhorn *et al.* (1997) did not find again a significant positive effect on intestinal calcium absorption by oestrogen treatment. However, it should be noted that this study (Balhorn *et al.*, 1997) concerned normal elderly women whereas their first study concerned osteoporotic postmenopausal patients (Gallagher *et al.*, 1980).

In our present study we found that oestrogen significantly increased serum levels of total $1,25(\text{OH})_2\text{D}$ and DBP but did not change the calculated free $1,25(\text{OH})_2\text{D}$ fraction, indicating

that oestrogen treatment has an effect on vitamin D metabolism within two months of treatment. The data support previous studies which have found no effect of oral oestrogen on the free calcitriol level (Bouillon *et al.*, 1981; Dick *et al.*, 1995). Nevertheless, some previous studies have shown that oral oestrogen treatment results in increased levels of free calcitriol (Bikle *et al.*, 1992; Cheema *et al.*, 1989).

The lack of a change in intestinal strontium absorption is in agreement with the unchanged concentration of free serum $1,25(\text{OH})_2\text{D}$. However, it is generally held that the absorption efficiency for calcium is inversely related to the size of the ingested load (Heaney *et al.*, 1989, 1990). At lower carrier loads principally active calcium absorption is measured. At high carrier loads, passive as well as active absorption is measured (Prince, 1994). Therefore, the relatively high oral load used in this test, i.e. 5 mmol strontium and 9 mmol calcium (corresponding with 560 mg calcium) may have reduced the sensitivity of the test. However, Balhorn *et al.* (1997) also failed to demonstrate a significant positive effect of long-term oestrogen treatment on intestinal calcium absorption in normal elderly women using ^{45}Ca with a calcium load of 100 mg.

The contradictory results regarding the effect of oestrogen on intestinal calcium absorption could not be explained by differences in the absorption tests used, i.e. single tracer- or dual tracer absorption tests. Although the most accurate method for assessment of intestinal calcium absorption is the dual tracer procedure, single tracer tests seem to have good options as a calcium absorption test for clinical application when comparing different subjects. Nevertheless, when examining the effect of interventions, for instance oestrogen, on intestinal calcium absorption within an individual subject, (single point) single tracer tests may have some drawbacks. First of all, measuring intestinal absorption using a single point single tracer absorption test can result in an imaginary change in intestinal absorption because of the possibility that oestrogen supplementation may alter the absorption profile of calcium/strontium. Secondly, possible changes in the rate of elimination (kidney, bone, intestine) of calcium can also change the plasma tracer concentration, which might result in an under- or over-estimation of intestinal absorption. Therefore, the findings with a single tracer test have to be confirmed by limited numbers of patients in which the true bioavailability of calcium/strontium is exclusively measured at the basal level and after intervention.

A possible effect of oestrogen on gastric emptying could also not explain the conflicting results of the effect of oestrogen on intestinal calcium absorption since oestrogen does not significantly change gastric emptying in postmenopausal women with or without hormone replacement therapy (Hutson *et al.*, 1989).

In conclusion, the present study demonstrated that 2 months of oestrogen therapy does not change intestinal calcium

absorption. Total 1,25(OH)₂D and vitamin D-binding protein increased, but the free 1,25(OH)₂D fraction remained unchanged.

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