Effects of the SERM raloxifene on calcium and phosphate metabolism in healthy middle-aged men

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Summary

Background. **Sex hormones are important regulators of calcium and phosphate homeostasis. Estradiol appears to be a major determinant of bone health in the male gender. However, physiological effects of estrogens on calcium and phosphate homeostatic fluxes in men are still poorly understood.**
Objective. **We investigated the influence of 6 weeks of the SERM raloxifene, an estrogen agonist in bone, but devoid of feminizing actions, on calcium and phosphate metabolism in healthy middle-aged men.**
Design. **In a double-blind, randomized, placebo-controled, cross-over study, we evaluated the influence of 120 mg/day of raloxifene on calciotropic hormones levels, renal tubular reabsorption of calcium and phosphate, and intestinal calcium absorption, as assessed by the calciuric response to an oral calcium load.**
Results. **As compared to the placebo period, raloxifene treatment decreased the response to an oral calcium load, together with a decrease in $1,25(OH)_2D_3$ and in IGF-I serum levels. Maximal renal tubular phosphate reabsorption was decreased in raloxifene-treated men following the calcium load. The renal handling of calcium was not changed.**
Conclusion. **These results are compatible with the hypothesis that raloxifene is associated with lower IGF-I and $1,25(OH)_2D_3$ levels, with consequently reduced intestinal calcium absorption capacity.**

KEY WORDS: renal handling, intestinal calcium absorption, calciotropic hormones, males, bone.

Introduction

Both calcium and phosphate homeostases are maintained through an equilibrium between fluxes of calcium and phosphate taking place in the gut, kidney and bone (1). In addition to calciotropic hormones, sex hormones are major regulators of bone growth and mineral homeostasis (2). Based on association studies, on pathological situations with estrogen deprivation or on estrogen administration trials, estradiol appears to be of major importance for bone health in the male gender (3). However, the effects of estrogens on calcium and phosphate fluxes at various organs level in men are still poorly understood. Indeed, studies of the effects of estradiol in men are limited by its feminizing action. We recently showed that the selective estrogen receptor modulator (SERM); raloxifene decreased bone remodeling in middle-aged healthy men with a spontaneous low endogenous estradiol level (4). This effect was associated with an increase in serum estradiol concentrations (4). This agent, with both selective agonistic and antagonistic effects on estrogen receptor, has been shown to decrease urine NTX excretion in men with low estradiol levels (5), and to increase hip BMD in men with prostate cancer receiving a GnRH agonist (6).
Extending our previous observation (4), we now report on the effects of raloxifene on intestinal calcium absorption, renal tubular transport of calcium and phosphate, and on calciotropic hormones regulating these fluxes in middle-aged healthy men.

Subjects and methods

Subjects

The protocol was approved by the Ethics Committee of the Department of Internal Medicine of the Geneva University Hospitals. The study was carried out in full accordance with the recommendations of the Helsinki declaration 1964, amended Edinburgh 2000. We recruited 43 men aged 55.7 ± 5.2 years (range 50-70) among the hospital employees, their friends or relatives, who all signed an informed consent. At the onset of the study, the subjects underwent a health questionnaire, physical examination including digital rectal examination, blood and urine tests, and bone mineral density measurements at the lumbar and hip levels, as well as whole body bone mineral, fat and lean masses with dual X-ray absorptiometry using a Hologic-4500 device (Waltham, MA, USA). None of the subjects was osteoporotic, as defined by WHO criteria for women as a T-score at spine or hip lower than -2.5 standard deviations (SD) of the normal healthy male peak bone mass. All were healthy, without any serious acute or chronic medical conditions. Exclusion criteria were: known bone disorders, history of cancer within the previous 5 years, endocrine disorders, liver disease, impaired renal function, malabsorption symptoms, intestinal resection, renal stones, excess of alcohol, smoking (i.e. smoking of more than 10 cigarettes per day), history of deep venous thrombosis or thrombo-embolic disorders, or medications known to affect bone, gut and/or kidney metabolisms, including statins and thiazides. The subjects were advised not to father a child while participating in the study and until 3 months afterwards.

Study design

This was a single-center, randomized, placebo-controled, double-blind, two-sequence, cross-over study that consisted of a

screening period, a first therapy period, a washout period, a second therapy period, and a follow-up period (4). Forty-three healthy middle-aged men were randomly assigned to one of the two treatment sequences: raloxifene 120 mg/day for 6 weeks followed by placebo for 6 weeks, or placebo for 6 weeks followed by raloxifene 120 mg/day for 6 weeks. The two treatment phases were separated by a washout period of 8 weeks.

Biochemical determinations

Blood and 2-hour fasting morning urine samples were collected at the end of the 6-week treatment period. All blood and urine samples were collected between 8 and 9 am after 12 hours of fast. 24-hour urine collections were also performed concurrently. Calcium, phosphate and creatinine were measured in blood and in the 2-hour fasting morning urine samples in order to calculate an index of renal tubular reabsorption of calcium (TRCal) (7), and maximal tubular reabsorption of phosphate (TmPi/GFR) (7, 8). The tubular reabsorption of calcium index was calculated from a nomogram based on the relationship between urine calcium excretion per unit of glomerular filtration rate and serum calcium (7). Serum calcium was corrected for albumin concentration (corrected calcium = measured calcium – [(albumin – 40) x 0.02]). The intestinal calcium absorption capacity was evaluated by comparing the urinary calcium-to-creatinine ratio before and after an acute oral calcium load (9). Briefly, after a blood sampling, a 2-hour urine sample was collected. Then, 1000 mg calcium carbonate with a standardized breakfast was given. The subsequent two-hour urine sample was discarded. The next 2-hour urine sample was collected. Blood was drawn in the middle of the last 2 urine collection periods. Serum parathyroid hormone (PTH) was measured with an IRMA (Immulite, Diagnostic Products, Los Angeles, CA, USA) and 25-hydroxy-vitamin D using a RIA (Incstar, Stillwater, Minnesota, USA). An extractive chromatography followed by a radioreceptor assay was used to measure serum 1,25-dihydroxy-vitamin D (Incstar, Stillwater, Minnesota, USA). Serum IGF-I and IGF-BP3 were measured using an RIA (Diagnostic Systems Laboratories Inc., Webster, Texas, USA) and an IRMA (DSL 6600, Diagnostic Systems Laboratories Inc., Webster, Texas, USA), respectively. Total testosterone and 17β estradiol, as well as sex hormone binding protein (SHBG) were measured by radio immuno assay. All the determinations were performed batchwise.

Dietary survey

Dietary calcium and protein intakes were evaluated using a food recall of the last 24 hours. The subjects were asked to maintain their regular diet, except during the last 4 days preceding the end of the treatment periods, where they were asked to abstain from dairy products to obtain a consistent, homogeneous and steady calcium intake of around 400 mg/day of calcium, before undergoing the calcium loading test (9).

Statistical analysis

Results are presented as means ± SD. Analysis of variance (ANOVA) with a repeated measure design was applied to take into account the two-period crossover design. This is an exploratory analysis with multiple endpoints. There was no evidence of a carryover effect for any variable. Thus, to increase the statistical power, the values obtained at the end of each therapy period (visits 3 and 5) were pooled together as a placebo phase and a raloxifene phase, respectively. Paired T-tests and Wilcoxon matched-pairs signed-ranks tests, when dealing with non-normally distributed variables, were used to compare the two phases. The need for Bonferroni's correction, applied to adjust for multiple comparisons, is disputed (10). Thus, by providing an exact p value, we allow the reader to choose whether he/she wants to apply the Bonferroni's adjustment, which involves dividing the usual p threshold set at 0.05 by the number of groups to be compared (here two). Statistical analyses and 95% confidence intervals computation were performed using the STATA program, Release 8.2.

Results

Baseline characteristics

All 43 subjects were healthy middle-aged men with a mean age of 55.7±5.2 yrs (range 50-70) (Table I). Their values of BMD at lumbar spine, proximal femur and whole body were within normal range for young, healthy subjects. Baseline bone remodeling biochemical markers and sex hormones, together with SHBG, were also within the normal range as reported previously (data not shown) (4). In all subjects, baseline 25-OH vitamin D_3 was 67.7±30.0 nmol/l, with 70.2±32.6 in the placebo and 65.4±27.8 in the raloxifene group.

Table I - Baseline characteristics.

No. of subjects	43
Weight (kg)	82.6 ± 12.9
Height (cm)	176.1 ± 4.8
BMI (kg/m^2)	26.7 ± 4.0
Lumbar spine BMD (g/cm^2)	1.09 ± 0.15 (T-score: 0.18 ± 1.14)
Femoral neck BMD (g/cm^2)	0.88 ± 0.12 (T-score: 0.70 ± 0.82)
Usual dietary calcium intake (g/d)	932 ± 393

Results are means ± SD.

Effects of raloxifene on calcium and phosphate fluxes

The administration of 120 mg/day for 6 weeks was well tolerated, without any difference in adverse events incidence between the raloxifene and placebo periods (data not shown). No subject dropped out.

Raloxifene treatment caused a small but significant decrease in plasma phosphate (Table II). TmPi/GFR was significantly lower in the raloxifene group as compared to placebo after, but not before the acute oral calcium load (1.25 ± 0.19 vs. 1.33 ± 0.23 mmol/l GFR, $p < 0.01$) at the end of the raloxifene period (Table III). Albumin-corrected plasma calcium, renal tubular reabsorption of calcium index, fasting calcium-to-creatinine ratio, taken as a reflection of net bone resorption, were not affected. The 20% decrease in 24-hour urinary calcium excretion in raloxifene-treated men was not statistically significant.

To evaluate calcium intestinal absorption capacity, urinary calcium-to-creatinine ratio was measured before and after an acute oral calcium load of 1000 mg together with a standardized breakfast (9). This oral calcium load increased plasma calcium and phosphate, and TmPi/GFR, although no discernable difference between the raloxifene and placebo periods was observed (Table III). This was associated with a similar decrease of serum PTH in both groups. As expected, oral calcium load increased the urinary calcium-to-creatinine ratio. This increase was significantly lower ($p = 0.018$) in raloxifene-treated men.

Table II - Effects of raloxifene on calcium-phosphate fluxes in healthy middle-aged men.

	Placebo	Raloxifene	p
Albumin corrected serum calcium, mmol/L	2.13 ± 0.10	2.13 ± 0.07	0.965
Serum phosphate, mmol/L	1.10 ± 0.13	1.06 ± 0.13	0.017
Serum creatinine, µmol/L	82.7 ± 11.4	83.5 ± 12.4	0.490
Net bone resorption index (fasting urinary calcium/creatinine), mmol/mmol	0.17 ± 0.15	0.17 ± 0.13	0.940
Tubular reabsorption of calcium index (TRCaI/GFR), mmol/GFR	2.48 ± 0.13	2.49 ± 0.14	0.527
Maximal renal tubular Pi reabsorption (TmPi/GFR), mmol/GFR	1.10 ± 0.21	1.06 ± 0.22	0.259
Daily urinary calcium excretion, mmol/day	4.66 ± 1.86	3.53 ± 2.29	0.620

Results are means ± SD.
After 6 weeks of 120 mg/day raloxifene or placebo, 43 healthy middle-aged men had blood and urine sampled after an overnight fast.

Table III - Effects of raloxifene on the response to an oral acute calcium load in healthy middle-aged men.

Difference after/before acute oral calcium load (Δ)	Placebo	Raloxifene	Raloxifene-Placebo	p (Raloxifene-Placebo)
Δ Serum albumin corrected calcium	0.17 ± 0.12***	0.15 ± 0.09***	-0.017 ± 0.130	0.411
Δ Serum phosphate	0.10 ± 0.16***	0.08 ± 0.11***	-0.018 ± 0.135	0.390
Δ Serum intact PTH	-1.94 ± 1.11***	-1.85 ± 1.14***	+0.93 ± 1.17	0.604
Δ Urinary calcium/creatinine	0.36 ± 0.27***	0.31 ± 0.18***	-0.092 ± 0.172	0.018
Δ TmPi/GFR	0.23 ± 0.21***	0.20 ± 0.15***	-0.042 ± 0.233	0.272
Δ TRCaI/GFR	0.10 ± 0.12	0.02 ± 0.09	0.010 ± 0.150	0.677

Results are means±SD. After 6 weeks of 120 mg/day raloxifene or placebo, an oral calcium loading test was performed as described in Subjects and Methods. The results are shown as the difference after/before the oral calcium load.
*** $p < 0.001$ as compared with values before the oral calcium load.

Table IV - Effects of raloxifene on calciotropic hormones in healthy middle-aged men.

	Placebo	Raloxifene	Δ (%) (Raloxifene-Placebo)	95% CI	p
Intact PTH, pmol/L	4.04 ± 1.50	3.92 ± 1.33	-2.2	[-7.17;+11.56]	0.531
1,25 $(OH)_2D_3$, pmol/L	131.16 ± 24.20	119.11 ± 26.30	-6.4*	[-14.80;+2.05]	0.017
IGF-I, nmol/L	23. 98 ± 8.65	20.68 ± 7.15	-11.2*	[-16.84;-5.59]	<0.001
IGF-BP3, µmol/l	2.96 ± 0.52	3.15 ± 0.65	7.0*	[1.88;12.17]	0.018

Results are means ± SD.
After 6 weeks of 120 mg/day raloxifene or placebo, blood was sampled after an overnight fast.
* $p < 0.05$ as compared with zero.

Renal tubular reabsorption of calcium index was not modified (Table III).

Effects of raloxifene on calciotropic hormones

There was no difference in PTH in raloxifene-treated men and placebo group, neither in the mean serum levels nor in the relationship between serum PTH values and ionized calcium (before and after calcium load) (Table IV, Figure 1). This suggests that PTH secretion was not modified by raloxifene administration. Despite a trend toward lower value in raloxifene-treated subjects, the relationship between maximal renal tubular reabsorption of phosphate (TmPi/GFR) and PTH was not statistically different compared to placebo (Figure 2). IGF-I and 1,25 $(OH)_2D_3$ were significantly lower in the raloxifene-treated group (Table IV). In contrast, IGF-BP3 was slightly higher. The median of the IGF-I-to-IGF-BP3 ratio, taken as a reflection of IGF-I bioactivity (the values were not normally distributed) was 7.1 and 6.8 ($p = 0.011$) in placebo and raloxifene groups, respectively. This indicates a likely further decrease in bioactive IGF-I. As previously reported (4), total testosterone was 16.9 ± 5.0 vs. 14.9 ± 4.2 nM ($p < 0.01$), total estradiol 109.9 ± 4.1 vs. 99.2 ± 3.8 pM ($p = 0.002$), and SHBG 35.5 ± 17.2 vs. 32.7 ± 13.7 nM ($p = 0.024$), in raloxifene-treated and control healthy middle-aged men, respectively.

Discussion

In this double-blind randomized, placebo-controled, cross-over trial carried out in healthy middle-aged men, we found that the SERM, raloxifene, given at a dose of 120 mg/day for 6 weeks, was associated with lower serum levels of IGF-I, 1,25 $(OH)_2D_3$, decreased TmPi/GFR (after an oral calcium load), and a lower calciuric response to an acute oral calcium load, taken as a reflection of intestinal calcium absorption. The relationship be-

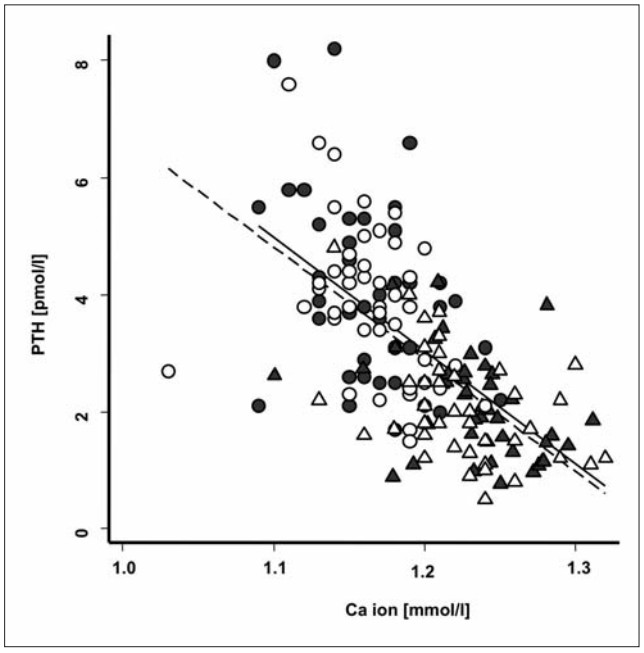

Figure 1 - Relationship between serum PTH and serum ionized calcium in healthy middle-aged men receiving placebo (closed symbols) or 120 mg/day raloxifene (open symbols) for 6 weeks. The circles represent values before and the triangles after an acute oral calcium load. Combining values before and after the oral calcium load, the regressions are PTH = –19.4 x Ca^{++} +26.3, and PTH = –19.2 x Ca^{++} + 25.9 in placebo (solid line) and raloxifene (dashline) groups, respectively (both $p < 0.01$). The two regressions were not statistically different.

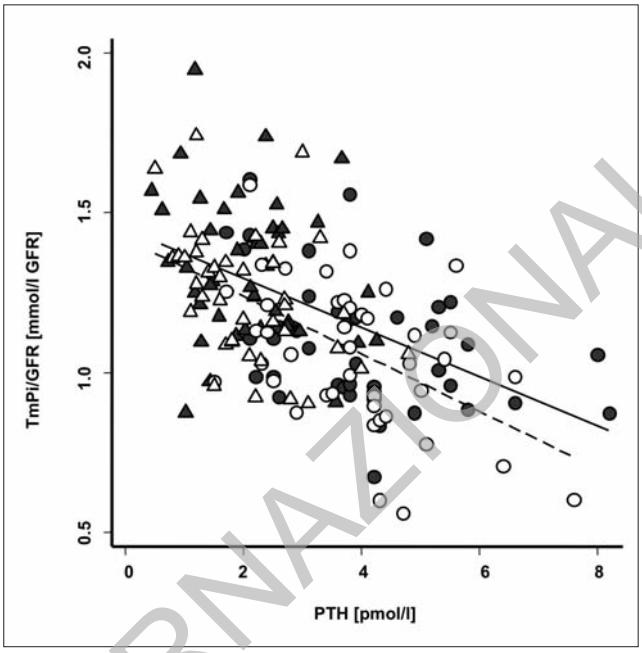

Figure 2 - Relationship between maximal renal tubular reabsorption of phosphate (TmPi/GFR) and serum PTH in healthy middle-aged men receiving placebo (closed symbols) or 120 mg/day raloxifene (open symbols) for 6 weeks. The circles represent values before, and the triangles values after the acute oral calcium load. Combining values before and after the acute oral calcium load, the regressions are TmPi/GFR = (-0.08 x PTH) + 1.45, and TmPi/GFR = (-0.09 x PTH) + 1.42, in placebo (solid line) and raloxifene (dashed line) groups, respectively (both $p < 0.01$). The two regressions were not statistically different.

tween serum PTH and ionized calcium, or between TmPi/GFR and PTH was not significantly altered by raloxifene treatment, suggesting that neither PTH secretion nor its action on Pi transport was modified by this agent. Though statistically significant, the differences detected were modest and within the normal range. The homogenous group of healthy middle-aged men and the cross-over experimental design probably contributed to reach statistical significance.

Calcium and phosphorus homeostases are controled by fluxes occurring at the level of intestine, kidney and bone. Regarding intestinal calcium absorption, it has been shown that estradiol was able to increase intestinal calcium transport in ovariectomized rats through a 1,25 $(OH)_2D_3$-independent mechanism (11). Estradiol administration led to an increased duodenal mRNA expression of TRPV5, TRPV6, CBgl and PMCA1b (12). Similarly, estrogen has been shown to upregulate the calcium influx channel TRPV6, through a specific interaction with estrogen receptor α (13).

In the present study, we found a significant reduction in the calciuric response to an acute oral calcium load in raloxifene-treated men. This was observed despite the fact that we previously reported an increase in estradiol levels in these healthy, middle-aged males (4). These results would be compatible with the hypothesis that raloxifene acts as an estrogen antagonist at the intestinal level. Alternatively, since in the current study raloxifene treatment was associated with a lower serum 1,25 $(OH)_2D_3$ concentration, it is possible that an indirect, vitamin D-dependent mechanism may be involved, hence lower 1,25 $(OH)_2D_3$ leading to a decreased calcium intestinal absorption.

As evaluated by the tubular calcium reabsorption index (TRCal) (7), the renal handling of calcium was not influenced by raloxifene. This index calculated from a nomogram and fasting serum and urine values, provides information on the renal tubular capacity to reabsorb calcium (7). Serum PTH was identical in the raloxifene-treated and placebo groups. This suggests that sensitivity to PTH was not altered by raloxifene, although estrogen deficiency has been reported to lower the renal responsiveness to PTH (14).

In women, estrogen replacement therapy stimulates the renal reabsorption of calcium (15), and there is a positive association between free estradiol and the renal handling of calcium (16). Together, these results can account for the renal calcium leak observed under estrogen deficient conditions (17). Moreover, estrogen has been shown to stimulate the renal expression of the epithelial calcium transporter TRPV5 (ECaC1) (18). It is not possible, however, to draw any conclusion as to whether raloxifene has some influence on the renal handling of calcium in healthy middle-aged men.

A series of reports have established a link between estrogens and renal tubular reabsorption of phosphate. Ovariectomy is associated with an increase in TmPi/GFR, a well-established assessment of the renal tubular reabsorption of phosphate (19). An increase in free estradiol is related to a decrease in phosphate reabsorption (16), and hormone replacement therapy in women decreases TmPi/GFR (20). Our results indicate a decrease in plasma Pi in men taking raloxifene. TmPi/GFR was significantly lower after the oral calcium load. This data could be related to the higher estradiol levels previously reported in these subjects (4). It is also compatible with the hypothesis that raloxifene is unable to exert antagonistic or partial agonistic effects at this organ level. It is not possible, however, to make such a conclusion with the current evidence.

Regarding bone-ion fluxes, some estrogen-like effects of raloxifene have been observed with raloxifene-dependent decreases in bone resorption markers, specifically in those patients with low serum estradiol levels (4, 5). We previously reported

that the increase of estradiol may contribute to the anti-resorptive effect of raloxifene (4). Finally, in a 12-month controled trial of men with non-metastatic prostate cancer receiving a GnRH agonist, raloxifene produced a significant increase in hip bone mineral density (6).

Fluxes maintaining calcium and phosphate homeostases are mainly controled by the calciotropic hormones PTH and 1,25 $(OH)_2D_3$. In the present study, neither PTH secretion nor action appeared to be influenced by raloxifene. In contrast, estrogen replacement has been shown to decrease the set point of PTH stimulation by calcium in normal postmenopausal women, without affecting the maximal PTH response to hypocalcemia, the suppressibility of PTH, or the slope of PTH vs. calcium (21).

We found a decrease in serum IGF-I concentration and, possibly, in bioactive IGF-I, as reflected by the ratio IGF-I/IGF binding protein 3. Through a direct effect on the liver, estradiol decreases IGF-I (22-26). In the liver, raloxifene appears to behave as an estrogen receptor agonist (24, 27). Despite lower IGF-I levels, women treated with raloxifene display a sustained decrease in vertebral fracture risk (28, 29). Our results are in total agreement with raloxifene impairing liver IGF-I production, as shown in women (24), possibly via an estrogen-like effect, since IGF-I levels were lower in raloxifene-treated men. On the other hand, IGF-I is known to directly stimulate the renal tubular reabsorption of phosphate and to increase 1,25 $(OH)_2D_3$ synthesis (30). Thus, the results of the present study are compatible with the following mechanism: raloxifene directly inhibits IGF-I liver production. Then, lower IGF-I concentrations are associated with lower TmPi/GFR (the decrease was detectable only after calcium load in the present study), hence lower phosphatemia. Lower IGF-I would also be associated with lower 1,25 $(OH)_2D_3$ levels (30), though hypophosphatemia is a stimulus of 1,25 $(OH)_2D_3$ production (31). The lower 1,25 $(OH)_2D_3$, in turn, is accompanied by decreased intestinal calcium absorption. However, some estrogen-like agonistic or antagonistic effects of raloxifene on the various fluxes cannot be ruled out.

This study has some limitations. It concerns a relatively small number of subjects. The subjects were on their regular diet. The analysis was exploratory with multiple endpoints. The evaluation of calcium intestinal absorption was indirect, based on a calcium response to an oral load (9). The differences detected are of small magnitude.

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