Osteoporos Int (2000) 11:43–51

Osteoporosis International

Original Article

Calcium Absorption in Postmenopausal Osteoporosis: Benefit of HRT Plus Calcitriol, but not HRT Alone, in both Malabsorbers and Normal Absorbers

M. L. Holzherr[1], R. W. Retallack[1], D. H. Gutteridge[1,5], R. I. Price[2], D. L. Faulkner[1], S. G. Wilson[1], R. K. Will[3], G. O. Stewart[4], B. G. A. Stuckey[1], R. L. Prince[1,5], R. A. Criddle[6], G. N. Kent[7], C. I. Bhagat[7], S. S. Dhaliwal[1] and K. Jamrozik[8]

Departments of [1]Endocrinology and Diabetes, [2]Medical Technology and Physics, and [6]Geriatric Services, Sir Charles Gairdner Hospital; [3]Department of Rheumatology, Royal Perth Hospital; [4]Department of Endocrinology and Diabetes, Fremantle Hospital; [5]University Department of Medicine, The University of Western Australia; [7]The Western Australian Centre for Pathology and Medical Research; and [8]Department of Public Health, The University of Western Australia, Perth, Western Australia

Abstract. In a randomized trial involving 71 postmenopausal osteoporotic women with vertebral compression fractures, radiocalcium absorption studies using the ^{45}Ca single isotope method (α) were performed at baseline and after 8 months of treatment with either continuous combined hormone replacement therapy (HRT, as piperazine estrone sulfate 0.625–0.937mg daily $\pm$ medroxyprogesterone acetate 2.5 mg daily depending on uterine status) or HRT plus calcitriol 0.25 μg twice daily. A calcium supplement of 600 mg nocte was given to only those women who had a daily calcium intake of less than 1 g per day at baseline, as assessed by recalled dietary intake. There was a significant *decrease* [0.74 ($\pm$ 0.35 SD) to 0.58 ($\pm$ 0.22), $\Delta\alpha = -0.17$ ($\pm$ 0.26), $p<0.0005$] in α at 8 months compared with baseline in the HRT-treated group, but a significant *increase* [0.68 ($\pm$ 0.31) to 0.84 ($\pm$ 0.27), $\Delta\alpha = +0.16$ ($\pm$ 0.30), $p<0.003$] in the HRT-plus-calcitriol treated patients, resulting in α being significantly higher after 8 months in the latter group than in the HRT-only group. Although 72% of the patients had been supplemented with calcium between the first and second studies, separate analyses revealed that the change in calcium intake had not affected the result. Further breakdown of the groups into baseline 'normal' absorbers ($\alpha \geqslant 0.55$) and 'malabsorbers' ($\alpha < 0.55$) revealed that α decreased with HRT treatment only in the normal absorbers, and remained stable in the malabsorbers. Conversely, following HRT plus calcitriol treatment, α increased only in the malabsorbers, the normal absorbers in this group remaining unchanged. In conclusion, our data show that HRT, of the type and dose used in this study, did not produce an increase in absorption efficiency; it was in fact associated with a fall. Increased absorption efficiency cannot be achieved unless calcitriol is used concurrently, and then only in patients with malabsorption. Calcitriol also had a significant effect in normal absorbers in that it prevented the decline in α seen with HRT alone, and thus should be considered in all patients with postmenopausal osteoporosis treated with HRT.

Keywords: Calcitriol therapy; Calcium absorption; Hormone replacement therapy; Osteoporosis

Correspondence and offprint requests to: Ms Marianne L. Holzherr, Department of Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Verdun Street, Nedlands WA 6009, Australia.

Introduction

Malabsorption of calcium has been frequently described in osteoporosis. It has been attributed to calcitriol deficiency [1], resistance to the action of calcitriol in the gastrointestinal tract [2–5], or a lack of estrogen in postmenopausal or oophorectomized women [6,7]. Calcium malabsorption and increased urinary calcium

excretion are the major determinants of calcium balance [8], and therefore could be predictors of low bone mass in women after the menopause.

In the past, a number of studies [9–11] have been able to demonstrate an increase in intestinal calcium absorption in postmenopausal osteoporotic patients with vertebral fractures using estrogen therapy, both with and without the concurrent use of a progestogen. In addition, estrogen therapy has been found to protect oophorectomized women against the fall in calcium absorption seen postoperatively in similar women on placebo [6]. These studies used conjugated equine estrogen or ethinyl estradiol in moderate to large doses.

Calcitriol (1,25-$(OH)_2$ vitamin D), the physiologically active metabolite of vitamin D, is the main regulator of intestinal calcium absorption [12]. Calcitriol, in oral doses of 0.25–0.50 μg per day, has also been shown to increase intestinal calcium absorption in postmenopausal osteoporotic women [10,13].

One way to measure calcium transport across the gut epithelium is to use the fractional rate of calcium absorption, which is calculated from the amount of radiocalcium circulating in the plasma 1 h after an oral bolus dose of radiolabelled calcium [14]. The mechanism of calcium transport depends on the size of the oral calcium load. With calcium loads under 5 mmol, absorption is mainly active, but with loads above 5 mmol the active transport carrier is saturated and any increment in absorption at higher gut luminal calcium concentrations is the result of passive diffusion [15]. The proportion of the active and passive mechanisms will therefore depend on the size of the calcium load [16]. Furthermore, the active transport mechanism has been shown to be vitamin-D-dependent and calcium absorption via this pathway has been shown to be directly proportional to serum calcitriol levels [17].

To assess the importance of malabsorption of calcium in osteoporosis, and to elucidate the possible mechanisms of the actions of estrogen and calcitriol on intestinal calcium absorption, we studied ^{45}Ca absorption in 71 postmenopausal women. The women were enrolled in a randomized trial in which we compared the effect of 8 months of treatment with hormone replacement therapy (HRT) alone or HRT plus calcitriol on the fractional rate of intestinal calcium absorption.

Materials and Methods

Patients

Eighty-one ambulant Caucasian postmenopausal women aged 53–79 years were recruited into the study over a period of 2 years. Seventy-one of these women completed 8 months of follow-up. All the women had osteoporosis as defined by the presence of at least one grade 2 or two grade 1 vertebral fracture(s) [18]. In addition, at least one lumbar vertebra had a bone density of 2 standard deviations or more below the mean of peak premenopausal bone density (i.e., *T*-score below -2) on either anteroposterior (AP) or lateral study (Hologic QDR 2000 densitometer). Only 7 of the women had received HRT during the past 5 years, and none had received any of the following during the previous 6 months: HRT, vitamin D (ergocalciferol or calcitriol), antiepileptic medication or anabolic steroids. None of the women had ever received bisphosphonate or sodium fluoride therapy. Corticosteroids were limited to the equivalent of 10 mg prednisolone daily for less than 1 month in the previous 2 years, and inhaled steroids were limited to 1000 μg/day over the same period. (Two patients, one from each treatment group, commenced oral prednisolone for asthma between the first and second studies. Removing these patients from the analysis did not change the results of the study.) No patient had received more than 150 μg thyroxine/day in the past 2 years. Patients with a history of liver or kidney disease, small bowel resection, current malignancy, or any disease other than osteoporosis known to affect calcium metabolism were excluded.

Study Protocol

Patients were studied at baseline and after 8 months of treatment. On entry into the trial, any patients who were taking any form of calcium supplements stopped them for at least 7 days prior to the baseline tests, and all the women fasted from 2200 hours the previous night, drinking only distilled water. The following blood tests were performed at each time: ionized calcium, intact parathyroid hormone (PTH), osteocalcin, 25-(OH) vitamin D and 1,25-$(OH)_2$ vitamin D plus plasma total calcium, creatinine, phosphate, albumin and alkaline phosphatase; the third morning urine specimen was tested for calcium/creatinine ratio, fasting calcium excretion, hydroxyproline/creatinine ratio, hydroxyproline excretion and renal phosphate threshold (Tmp_i/GFR), using analytical methods published by Henderson et al. [19]. Serum total 1,25-$(OH)_2$ vitamin D, vitamin D binding protein (DBP) and the free 1,25-$(OH)_2$ vitamin D index were measured according to the method described by Wilson et al. [20]. Two consecutive 24 h urine collections were taken at baseline and after 3 months for analysis of calcium and creatinine; baseline AP and lateral thoracic and lumbar spine radiographs were performed; bone densitometry was measured at the proximal femur (femoral neck, trochanter, intertrochanter and total hip) and AP lumbar spine using dual-energy X-ray absorptiometry (DXA; Hologic QDR 2000, Hologic, Waltham, MA). Dietary calcium intake was assessed at baseline and 8 months using recalled dietary intake with the food frequency questionnaire of Angus et al. [21]. Baseline Ca^{45} absorption tests were performed on all women, according to the 1993 version of the method of Marshall and Nordin [14]. This technique used a low dose (0.5 mmol) calcium carrier as calcium chloride together with 5 μCi ^{45}Ca in 250 ml distilled water.

Following the baseline tests the women were randomly assigned to two treatment groups using a method which balances the treatment assignment for certain prognostic factors [22]. These were calcium absorption status, uterine status, years since menopause and number of vertebral fractures: group 1 received HRT while group 2 received HRT plus calcitriol (Rocaltrol, Roche) 0.25 μg twice daily. The HRT was in the form of piperazine estrone sulfate (Ogen, Upjohn) in the dosage described below. In addition, medroxyprogesterone acetate (MPA) (Provera, Upjohn) 2.5 mg per day was given to women with an intact uterus. Calcium carbonate (Caltrate, Whitehall) 0.6 g nocte was given to patients who had a recalled dietary calcium intake of less than 1 g per day. The treatment was given according to the following protocol: calcium and/or MPA were taken for 1 week before adding estrone sulfate 0.312 mg/day. After 3 weeks of this combined therapy, the estrone sulfate was increased to 0.625 mg/day. Ten weeks after commencing treatment, and provided no adverse effects had been experienced by the patient, the estrone sulfate was further increased up to 0.937 mg/day, the average dose at the second test being 0.830 mg/day. Patients assigned to group 2 commenced calcitriol after 6 weeks of treatment with the other agents. The drugs were started sequentially rather than concomitantly to help the patients interpret side effects. MPA preceded estrone sulfate by 1 week to more effectively block endometrial estrogen receptors, with the aim of reducing vaginal bleeding. The estrogen dose was increased slowly to limit the development of breast discomfort. Calcitriol was deferred until the patients had been on a moderate dose of estrogen (0.625 mg/day) for 2 weeks.

^{45}Ca absorption tests were repeated 8 months after commencing HRT (i.e., 6½ months after commencing calcitriol). The women again fasted overnight, drinking only distilled water. Calcium supplements were omitted the night before the test to ensure that the intestine was free of calcium. Patients took their regular tablets including estrone sulfate, MPA and calcitriol, as applicable, the night before the test. No tablets were taken on the morning of the test. Dietary history was taken as before, and the patients were weighed. The biochemical measurements on fasting blood and urine specimens were also repeated at 8 months. The 24 h urinary calcium study was repeated after 3 months of treatment.

Calculations and Statistics

Radiocalcium absorption was calculated from the plasma radioactivity 1 h after ingestion of the ^{45}Ca. The fraction of the dose per liter of plasma at 1 h was multiplied by 15% of body weight and expressed as the hourly fractional absorption rate (α) by reference to a calibration curve [23]. Dietary calcium intake was calculated as milligrams per day [21]. Changes within and between groups over time were assessed using analysis of variance (ANOVA) with repeated measures, with and without the covariates: age, years since menopause, dietary calcium intake, progestogen dose and plasma creatinine. Unpaired *t*-tests were used for comparisons between the groups where appropriate. Malabsorption of calcium was defined as α <0.55 [24]. In the comparisons of 'normal absorber' and 'malabsorber' groups, the change in α with treatment as a function of baseline α was tested for regression to the mean effects using the methods of Blomqvist [25]. Forward stepwise multiple linear regression was performed on baseline data with α as the dependent variable.

Results

There was no significant difference between the two groups in any of the pretreatment baseline measurements (Table 1).

The effect of treatment with either HRT or HRT plus calcitriol on biochemical parameters is shown in Table 2.

Figure 1 demonstrates the effect of HRT alone, compared with HRT plus calcitriol, on the fractional rate of ^{45}Ca absorption in postmenopausal women with vertebral fractures. The fractional rate of intestinal calcium absorption decreased significantly over 8 months in the patients treated with HRT (p<0.0005), and increased significantly over the same time period in the patients on HRT plus calcitriol (p<0.003). There was also a significant difference between $\Delta\alpha$ in the two groups (p<0.001). Thus the addition of calcitriol had an overall significant effect on α. Age, years since menopause, dietary calcium intake, progestogen dose and plasma creatinine were not significant covariates.

Separate analyses were then performed within each treatment group to examine the possibility of a confounding effect of calcium supplementation on the results. Seventy-two per cent of all the patients (81% in the HRT-only group and 63% in the HRT-plus-calcitriol group, NS by χ^2) were given a daily supplement of 600 mg calcium after the baseline measurements had been completed. Each treatment group was thus divided into patients who were supplemented with calcium, and those

Table 1. Baseline physical measurements on 71 postmenopausal women, divided by adaptive assignment into two treatment groups

Measurement	HRT (n = 36)	HRT + calcitriol (n = 35)
Age (years)	69.9 (6.7)	69.9 (7.4)
Height (cm)	155.2 (7.8)	156.2 (7.0)
Weight (kg)	64.6 (13.7)	61.9 (9.6)
Years since menopause	22.2 (8.1)	23.5 (9.3)
Uterine status (% intact)	71	64
Ca absorption status (α) (Fx/h)[a]	0.74 (0.35)	0.68 (0.31)
L1–4 bone density (g/cm^2)	0.760 (0.156)	0.748 (0.115)
Total hip density (g/cm^2)	0.721 (0.138)	0.689 (0.118)
Vertebral fractures (no.)	2.6 (2.1)	2.7 (2.5)

Values are mean (SD). None of the differences between groups is significant.

[a] Fx/h is the hourly fractional radiocalcium absorption rate.

Table 2. Comparison of baseline and 8-month biochemistry and absorption measurements for the two treatment groups

Measurement	HRT (n = 36)		HRT + calcitriol (n = 35)		
	0 months	8 months	0 months	8 months	
Dietary calcium[a] (mg/day)	712 (389)	1191 (289)*	879 (429)	1224 (329)*	
α (Fx/h)	0.74 (0.35)	0.58 (0.22)*	0.68 (0.31)	0.84 (0.27)*	δ
Fasting plasma biochemistry					
25-OH vitamin D (nmol/l)	73 (39)	–	68 (31)	–	
1,25-$(OH)_2$ vitamin D (pmol/l)	104 (37)	119 (41)	92 (35)	–	
Vitamin D binding protein (μmol/l)	6.24 (2.13)†	6.81 (1.35)	5.16 (1.30)†	6.51 (1.28)*	
Free 1,25-D index (mol/mol $\times$ 10^6)	18.15 (9.69)	18.48 (7.58)	17.88 (6.37)	–	
Ionized calcium[b] (mmol/l)	1.22 (0.04)	1.21 (0.03)*	1.23 (0.04)	1.23 (0.05)	
Total calcium[c] (mmol/l)	2.32 (0.07)	2.26 (0.07)*	2.34 (0.08)	2.32 (0.11)	
Creatinine (μmol/l)	77 (12.1)	77 (14.6)	77 (12.0)	80 (14.4)*	
Phosphate (mmol/l)	1.09 (0.14)	0.95 (0.11)*	1.11 (0.13)	1.01 (0.13)*	
Albumin (g/l)	43.5 (2.7)	42.6 (2.3)*	42.7 (2.6)	42.9 (2.4)	δ
Alkaline phosphatase (U/l)	97 (24)	79 (27)*	109 (38)	72 (16)*	δ
Osteocalcin (μg/l)	8.67 (3.13)	6.43 (2.91)*	10.32 (5.66)	5.87 (4.14)*	δ
Intact PTH (pmol/l)	2.45 (1.17)	3.30 (1.72)*	2.23 (0.89)	1.89 (1.34)	δ
Fasting urine biochemistry					
Calcium/creatinine (mol/mol)	0.37 (0.32)	0.18 (0.12)*	0.31 (0.17)	0.34 (0.23)	δ
Calcium excretion (μmol/l GF)	28.2 (27.5)	13.7 (9.3)*	23.8 (13.7)	28.1 (20.0)	δ
Hydroxyproline/creatinine (mmol/mol)	22.2 (8.3)	15.7 (5.5)*	23.0 (8.0)	14.1 (4.1)*	
Hydroxyproline excretion (μmol/l GF)	1.68 (0.55)	1.17 (0.34)*	1.73 (0.56)	1.13 (0.38)*	
Tmp_i/GFR (mmol/l GF)	0.99 (0.18)	0.86 (0.16)*	0.98 (0.21)	0.87 (0.17)*	
24 h Urinary Calcium[d] (mmol/day)	3.12 (1.34)	2.99 (1.42)	3.31 (1.70)	5.34 (2.48)*	δ

Values are mean (SD).
* Significant difference from baseline (p<0.05).
[a] Eight month dietary calcium inclusive of supplements.
[b] Ionized calcium corrected to pH 7.40.
[c] Total calcium corrected to an albumin of 44.
† Significant difference at baseline.
[d] The 24 h urine calcium was repeated at 3 months.
δ Significant difference between the change from baseline to 8 months in the two treatment groups (p<0.05).

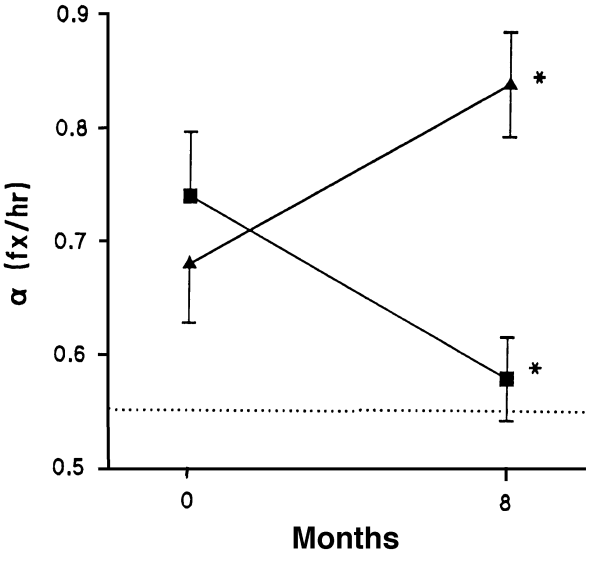

Fig. 1. Effect of HRT and HRT plus calcitriol on the fractional rate of intestinal ^{45}Ca absorption. *Squares*, patients on HRT only (n = 36); *triangles*, patients on HRT plus calcitriol (n = 35). *Significant difference from baseline in each group (p<0.005), assessed using ANOVA with repeated measures. Values for α at 8 months for the two groups are also significantly different from each other (p<0.0001). Error bars are $\pm$ SEM. The *dotted line* at α = 0.55 represents the division between 'normal' gut calcium absorption and 'malabsorption', as defined by Need et al. [24].

who were not. The differential effect of calcium supplementation on intestinal calcium absorption is presented in Fig. 2.

Adaptation of intestinal calcium transport to the increased calcium load was not responsible for the fall in α seen in the HRT-treated patients as the unsupplemented patients demonstrated a significant fall from baseline in α (p<0.05), despite small numbers (n = 7). Conversely, in the patients treated with HRT plus calcitriol, α increased significantly above baseline only in the patients not on calcium. Therefore, calcium supplementation seemed to blunt the response of α to each treatment.

We next analyzed the responses of all the patients in each treatment group, having first divided them into a 'normal absorber' subgroup and a 'malabsorber' subgroup, as determined from the baseline α measurement. An α value of less than 0.55 was taken to indicate malabsorption of calcium [24]. The baseline absorption results were thus divided into those above and those below 0.55, and the respective responses to each treatment regimen were analyzed separately (Fig. 3).

As seen in Fig. 3a, α did not change over the period of treatment in the malabsorbers given HRT alone. In the patients who were normal absorbers at baseline, however, α decreased significantly, although the mean

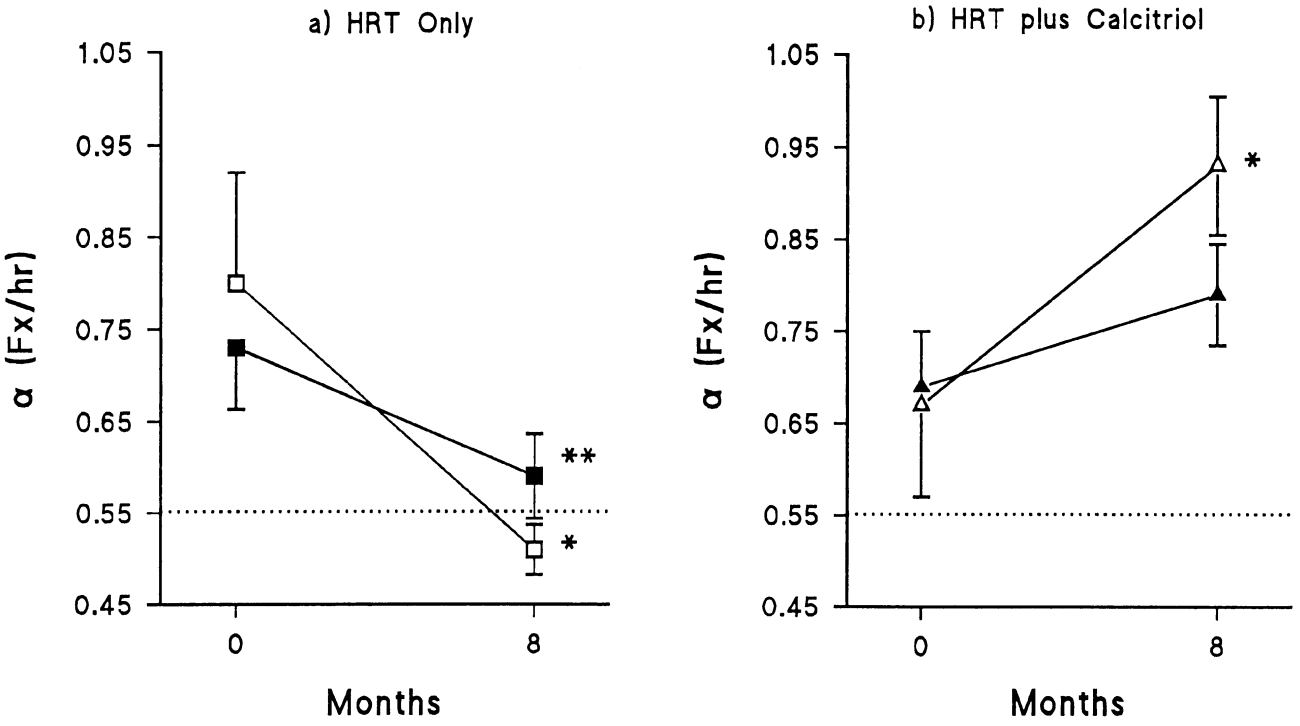

Fig. 2. a Effect of calcium supplementation and HRT on intestinal calcium absorption. *Open squares*, patients not on calcium (n = 7); *filled squares*, patients on calcium (n = 29). *Significant difference from baseline (p<0.05); **p<0.003. Error bars are ± SEM. **b** Effect of calcium supplementation and HRT plus calcitriol treatment on intestinal calcium Absorption. *Open triangles*, patients not on calcium (n = 13); *filled triangles*, patients on calcium (n = 22). *Significant difference from baseline (p<0.01).

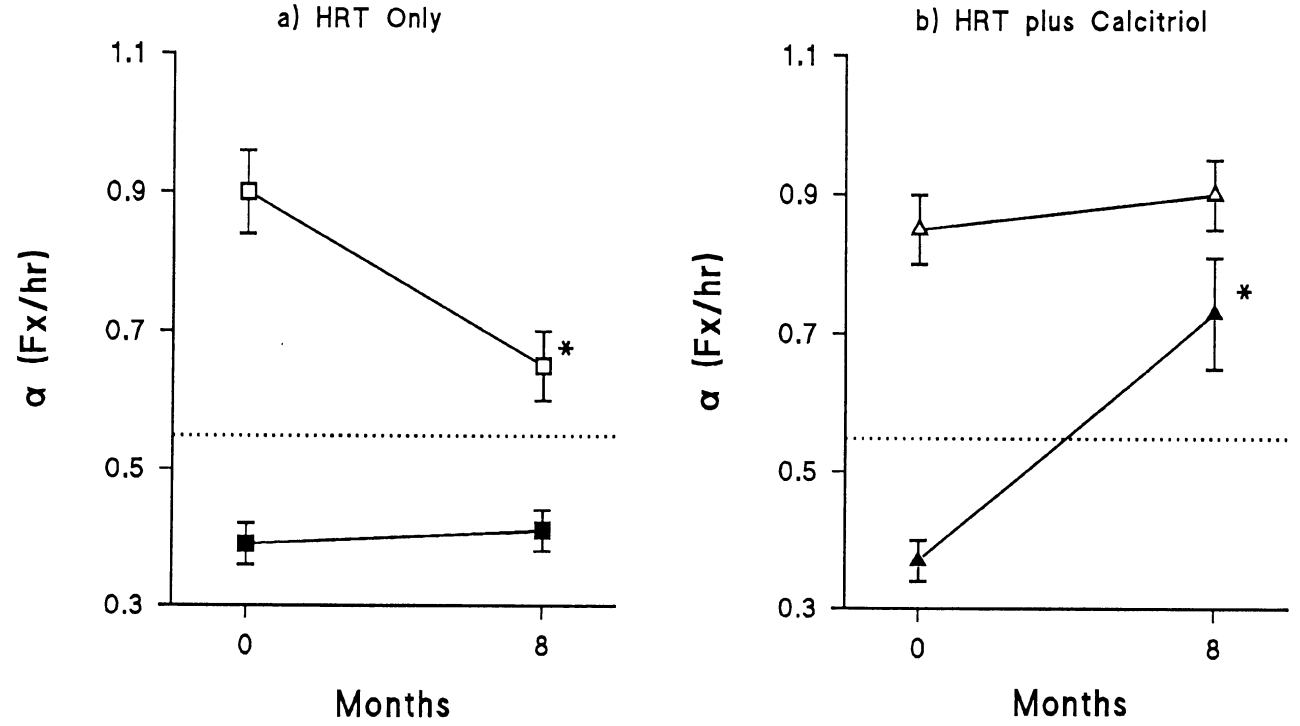

Fig. 3. Effect of HRT with or without calcitriol on α in normal absorbers and malabsorbers. *Significant difference from baseline (p<0.001). The patients were divided into either normal absorbers [*open squares*, HRT (n = 25), *open triangles*, HRT + calcitriol (n = 23)] or malabsorbers [*filled squares*, HRT (n = 11); *filled triangles*, HRT + calcitriol (n = 12)] depending on their baseline test result. The *dotted line* represents the cutoff for malabsorption of calcium. Error bars are ± SEM.

remained in the normal range and did not fall below the malabsorption threshold of 0.55. Conversely, in the patients treated with HRT plus calcitriol (Fig. 3b), the malabsorbers responded with a significant improvement in intestinal calcium absorption to within the normal range, but the normal absorbers showed no change. Thus $\Delta\alpha$ was related to baseline α in each of the two treatment groups. There were also significant differences between the responses to the two treatments ($\Delta\alpha$) within the malabsorbers ($p<0.005$), and between the responses to the two treatments within the normal absorbers ($p<0.0001$).

We then examined whether the differing responses of 'normal absorbers' versus 'malabsorbers' in the two treatment groups could have been influenced by the regression to the mean effect [25], i.e., whether the overall dependence of $\Delta\alpha$ on baseline α real or not? An estimate of within-subject variability (λ) at baseline (0.047 for the HRT-only group, and 0.059 for the HRT-plus-calcitriol group) was determined from data on 27 Adelaide postmenopausal osteoporotic women (A. Need, personal communication, 1997). Repetition of the procedure utilizing assumed values of λ showed that the negative relationship between $\Delta\alpha$ and baseline α persisted for all plausible values of λ (i.e., up to 0.4). The conclusions drawn from Fig. 3 are therefore valid and not influenced by a regression to the mean effect.

Table 3 shows the results of the forward stepwise multiple linear regression performed on 81 patients at baseline with α as the dependent variable. The following variables were also included in the regression but were not found to be significantly related to α: dietary calcium intake, number of vertebral fractures, total L1–4 bone density, serum ionized calcium (corrected to pH 7.40), osteocalcin, intact PTH, 25-(OH) vitamin D, total 1,25-$(OH)_2$ vitamin D, vitamin D binding protein (DBP), free 1,25-$(OH)_2$ vitamin D index, plasma total calcium (corrected to albumin of 44), plasma creatinine and alkaline phosphatase; fasting urinary calcium/creatinine ratio, fasting calcium excretion, hydroxyproline excretion, hydroxyproline/creatinine ratio and Tmp_i/GFR. Weight was excluded from the regression since it is an integral part of the calculation of α, and height was excluded because of its close relationship with weight.

In simple regression, α correlated with total 1,25-$(OH)_2$ vitamin D ($r = 0.26$, $p<0.02$) and the free 1,25-$(OH)_2$ vitamin D index ($r = 0.31$, $p<0.007$). Total 1,25-$(OH)_2$ vitamin D_3 was found to be significantly correlated with albumin ($r = 0.49$), explaining 24% of the variance. Albumin was also correlated with the free 1,25-$(OH)_2$ vitamin D index ($r = 0.35$), but not with DBP. These correlations suggested that albumin may have been acting as a surrogate for 1,25-$(OH)_2$ vitamin D in the multiple linear regression. However, when the regression was repeated without albumin, the significant correlates of α were: 24 h urinary calcium, fasting hydroxyproline/creatinine ratio, plasma phosphate, age, 1,25-OH_2 vitamin D and years since menopause. These variables accounted for a total of 38% of the variance in α, the contribution of 1,25-$(OH)_2$ vitamin D being only 4%. This would indicate that albumin was not acting as a surrogate for 1,25-$(OH)_2$ vitamin D.

Table 3. Baseline correlates of α

Variable	r^2	B	p value
Albumin	0.17	0.03	0.009
Total hip bone density	0.08	0.59	0.02
24 h urinary calcium	0.06	0.26	0.006
Plasma phosphate	0.03	−0.57	0.02
Age	0.04	−0.02	0.004
Years since menopause	0.03	−0.01	0.05

B is the non-standardized regression coefficient.

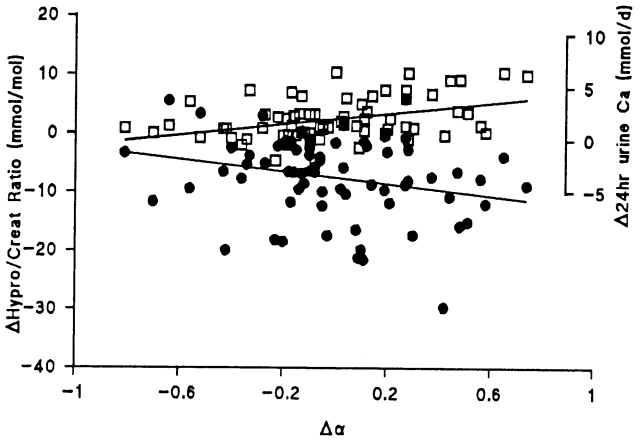

Fig. 4. Correlations between $\Delta\alpha$ and Δ24 h urinary calcium (*filled circles*), plus $\Delta\alpha$ and Δfasting hydroxyproline/creatinine ratio (*open squares*), in all patients.

When the changes in the variables in all patients were regressed on the variable $\Delta\alpha$, the significant correlates were restricted to Δ24 h urinary calcium positively ($r^2 = 0.18$, $p<0.0007$) and Δ fasting hydroxyproline/creatinine ratio negatively ($r^2 = 0.07$ $p<0.01$): see Fig. 4. In the HRT-only group, $\Delta\alpha$ correlated negatively with Δosteocalcin ($r^2 = 0.08$). In the HRT-plus-calcitriol group, $\Delta\alpha$ correlated positively with Δplasma total calcium ($r^2 = 0.06$), Δ24 h urinary calcium ($r^2 = 0.04$) and negatively with Δhydroxyproline/creatinine ratio ($r^2 = 0.06$).

Discussion

Our data show a reduction in gut calcium absorption with HRT which was prevented by concomitant treatment with calcitriol. Our results with HRT alone are therefore in conflict with those of several previous studies [9–11] and this may be due to a number of factors.

Firstly, the previous studies which demonstrated an improvement in calcium absorption with HRT involved smaller numbers (10 or 11 subjects [9–11] vs 36 women in our HRT group) and therefore were more open to random error.

Secondly, the effective estrogen dosage in this study was considerably smaller than those used by any of the previous authors. Furthermore, piperazine estrone sulfate is a less potent estrogen [26] than conjugated equine estrogens [10,11] or ethinyl estradiol [9] used in these earlier studies. Also, one of these previous studies used cyclic estrogen with a progestogen [9], another used cyclic estrogen alone [10], and one used continuous estrogen with no progestogen [11]. Direct comparisons are therefore not possible, and it is conceivable that the different forms of HRT could influence intestinal calcium absorption in different ways.

Thirdly, calcium supplementation in some patients, between the pre- and post-treatment calcium absorption tests, could have caused the gut to adapt to the increased dietary calcium load. The intestine absorbs calcium by vitamin-D-dependent and vitamin-D-independent mechanisms. Vitamin-D-dependent absorption is mediated by an active carrier, whereas vitamin-D-independent absorption is a passive response to chemical gradients [17]. It has been shown that the active component of intestinal calcium absorption can adapt to changes in the calcium load in the diet [7]. In our study, calcium supplementation was given to some patients to ensure that all women had adequate and approximately equal intakes. However, to exclude any potential confounding effect of calcium supplementation on calcium absorption, we analyzed the two groups of patients separately, with and without calcium, and were able to exclude such an effect (Fig. 2a). Direct comparisons with previous studies [9–11] are again difficult as they did not supplement any of their patients with calcium between the first and second tests.

The response of α to the two treatments produced quite divergent results. In the HRT-treated patients the biochemical evidence points to a failure in the renal 1α-hydroxylase response to PTH. Although HRT predictably caused renal calcium conservation (by combined direct and PTH stimulated effects [27]) which alone would lead to a significant rise in plasma calcium, plasma ionized and total calcium levels actually fell, presumably due to direct inhibition of bone resorption and decreased efflux of calcium from bone to the extracellular fluid. Thus the antiresorptive effect on plasma calcium levels was greater than the effect on renal calcium conservation. PTH rose significantly, which should have led to an increase in renal calcitriol production. However, there was no increase in total 1,25-$(OH)_2$ vitamin D, DBP or the free 1,25-$(OH)_2$ vitamin D index in our patients. Renal resistance to the stimulatory effects of PTH has previously been demonstrated in patients with age-related osteoporosis [28]. This could have accounted for the lack of an increase in 1,25-$(OH)_2$ vitamin D seen in the HRT-only group, but it does not explain the fall in α. We are, therefore, unable to fully explain the decrease in α with HRT, although we cannot discount the possibility that in the age-related osteoporosis phenotype, HRT has a direct inhibitory effect on the ability of the intestine to absorb calcium.

In the HRT-plus-calcitriol group, however, the entire mechanism is dominated by calcitriol. Unlike the response with HRT alone, which is essentially driven by PTH, calcitriol has a direct effect on the gut to increase α regardless of the body's need for calcium. Extra calcium is therefore lost in the urine seen as an increased 24 h urinary calcium, while the fasting urinary calcium/creatinine ratio remains unchanged, and the expected rise in PTH seen with HRT alone is suppressed (see Table 2).

To our knowledge this is the first time a study of intestinal calcium absorption has compared the effect of HRT with or without calcitriol in normal absorbers and malabsorbers as separate groups. We found a profound difference in the way these two groups responded to HRT alone. Patients with malabsorption showed no change in absorption status, but α fell significantly in patients with initially normal absorption (see Fig. 3a). The patients on HRT plus calcitriol also differed in their responses, depending on whether they were normal absorbers or malabsorbers at baseline. The malabsorbers showed the best response, with α rising above the malabsorption threshold of 0.55 in this group. The normal absorbers, however, did not change. These data agree with those previously reported using calcitriol alone, where α in patients with postmenopausal osteoporosis on calcitriol treatment improved only if the patient had malabsorption of calcium [24].

Perusal of the data presented by Civitelli et al. [11] suggests that the estrogen-induced increase in intestinal calcium absorption was of greatest magnitude in the malabsorbers within their sample. The patients with higher baseline calcium absorption had relatively small improvements after 12 months of estrogen treatment. However, the responses of our HRT-only patients were in marked contrast to these previous findings. Our data do not back up the assumption that HRT increases intestinal calcium absorption in either malabsorbers or normal absorbers of calcium.

We cannot assume that HRT corrects all the negative features of postmenopausal osteoporosis. Estrogen improves renal calcium conservation [27,29,30] and decreases bone resorption [10,11,31,32] and these actions in turn improve calcium balance. However, our data indicate that HRT, of the type and dose used in this study, decreases intestinal absorption of calcium in normal absorbers and produces no improvement in malabsorbers.

Calcitriol has in the past been shown to improve calcium absorption in postmenopausal women with vertebral fractures [10,33]. A recent study [24] found that calcitriol in a dose of 0.25 μg daily for 6–12 weeks failed to improve α in osteoporotic patients whose initial α was above 0.55, but did improve absorption in patients with an initial α below 0.55. Their patients all received a calcium supplement of 1 g per day during the study period. We did not have a 'calcitriol-only' group to form direct comparisons with these previous data; however, the present study shows that although HRT plus

calcitriol will improve α only in patients who have malabsorption of calcium, the combined therapy also prevented the HRT-related decline in normal absorbers.

The biochemical effects of HRT plus calcitriol on α are supported by the greater effect of added calcitriol on alkaline phosphatase and osteocalcin, two indices of bone formation and turnover (see Table 2). The differences in the decline of bone resorption indices did not reach significance.

Treatment with calcitriol has been shown to improve spinal bone density [34] and to reduce the rate of fractures in some [13,35] but not all [22,36] studies of postmenopausal osteoporotic women. Studies in progress on the patients in our trial will show whether those with initially good calcium absorption go on to develop these other favorable responses to HRT and calcitriol, or whether these bone improvements are limited to those patients in whom malabsorption has been corrected with concomitant calcitriol.

In conclusion, our data suggest that in postmenopausal osteoporotic patients on HRT, calcitriol should be used as part of the treatment even when calcium malabsorption is not present. While the greatest benefit was for the patients with calcium malabsorption, the addition of calcitriol also prevented the significant decrease in α seen in normal calcium absorbers treated with HRT alone. There is, therefore, a potential role for calcitriol in addition to HRT treatment for osteoporotic patients who are normal absorbers of calcium. Calcitriol treatment is well known to carry the potential risks of hypercalcemia and/or hypercalciuria, and occasionally nephrolithiasis or nephrocalcinosis. Thus periodic measurements of plasma and urinary calcium are vital during such treatment, with reduction of the calcitriol and/or calcium dosage as indicated. Radiocalcium absorption tests to distinguish malabsorbers from normal absorbers are not readily available in the community, and knowledge of the beneficial effects of calcitriol in both absorption categories presented in this study should be valuable for doctors prescribing treatment for osteoporosis.

Acknowledgements. This study was supported by the National Health and Medical Research Council (Australia), and by Roche (Australia) P/L. We are grateful to Professor B. E. C. Nordin for helpful advice on establishing the ^{45}Ca absorption test, and for constructive criticism of this paper. We also thank Ms Kristyn Willson of the Department of Public Health, University of Adelaide, for her calculation of the regression to the mean effect, and Professor Allan Need for supplying the data on the reproducibility of the calcium absorption test.

References

1. Gallagher JC, Aaron J, Horsman A, Marshall DH, Wilkinson R, Nordin BEC. The crush fracture syndrome in postmenopausal women. Clin Endocrinol Metab 1973;2:293–315.
2. Francis RM, Peacock M,Taylor GA, Storer JH, Nordin BEC. Calcium malabsorption in elderly women with vertebral fractures: evidence for resistance to the action of vitamin D metabolites on the bowel. Clin Sci 1984;66:103–7.
3. Eastell R, Yergey AL, Vieira NE, Cedel SL, Kumar R, Riggs BL. Interrelationship among vitamin D metabolism, true calcium absorption, parathyroid function, and age in women: evidence of an age-related intestinal resistance to 1,25-dihydroxyvitamin D action. J Bone Miner Res 1991;6:125–32.
4. Morris HA, Need AG, Horowitz M, O'Loughlin PD, Nordin BEC. Calcium absorption in normal and osteoporotic women. Calcif Tissue Int 1991;49:240–3.
5. Ebeling PR, Sandgren ME, DiMagno EP, Lane AW, DeLuca HF, Riggs BL. Evidence of an age-related decrease in intestinal responsiveness to vitamin D: relationship between serum 1.25-dihydroxyvitamin D_3 and intestinal vitamin D receptor concentrations in normal women. J Clin Endocrinol Metab 1992;75:176–82.
6. Gennari C, Agnusdei D, Nardi P, Civitelli R. Estrogen preserves a normal intestinal responsiveness to 1,25-dihydroxyvitamin D_3 in oophorectomised women. J Clin Endocrinol Metab 1990;71:1288–93.
7. Heaney RP, Recker RR Stegman MR, Moy AJ. Calcium absorption in women: relationships to calcium intake, estrogen status, and age. J Bone Miner Res 1989;4:469–75.
8. Nordin BEC. Calcium and osteoporosis. Nutrition 1997;13:664–86.
9. Caniggia A, Gennari C, Borello G, et al. Intestinal absorption of calcium-47 after treatment with oral oestrogen-gestogens in senile osteoporosis. BMJ 1970;iv:30–2.
10. Gallagher JC, Riggs BL, DeLuca HF. Effect of estrogen on calcium absorption and serum vitamin D metabolites in postmenopausal osteoporosis. J Clin Endocrinol Metab 1980;51:1359–64.
11. Civitelli R, Agnudei D, Nardi P, Zacchei F, Avioli LV, Gennari C. Effects of one-year treatment with estrogens on bone mass, intestinal calcium absorption, and 25-hydroxyvitamin D-1α-hydroxylase reserve in postmenopausal osteoporosis. Calcif Tissue Int 1988;42:77–86.
12. Riggs BL. Overview of osteoporosis. West J Med 1991;154:63–77.
13. Caniggia A, Nuti R, Lore F, Martini G, Turchetti V, Righi G. Long-term treatment with calcitriol in postmenopausal osteoporosis. Metabolism 1990;39:43–9.
14. Morris HA, Chatterton BE, Ross PD, et al. Biochemical investigations. In: Nordin, BEC, Need AG, Morris HA, eds. Metabolic bone and stone disease, 3rd ed. Edinburgh: Churchill Livingstone, 1993:339–79.
15. Parker TF, Vergne-Marini P, Hull AR, Pak CYC, Fordtran JS. Jejunal absorption and secretion of calcium in patients with chronic renal disease on hemodialysis. J Clin Invest 1974; 54:358–65.
16. Tellez M, Reeve J, Royston JP, Veall N, Wooton R. The reproducibility of double-isotope deconvolution measurements of intestinal calcium absorption. Clin Sci 1980;59:169–72.
17. Sheikh MS, Ramirez A, Emmett M, Ana SA, Schiller LR, Fordtran JS. Role of vitamin D-dependent and vitamin D-independent mechanisms in absorption of food calcium. J Clin Invest 1988;81:126–32.
18. Black DM, Cummings SR, Stone K, Hudes E, Palermo L, Steiger P. A new approach to defining normal vertebral dimensions. J Bone Miner Res 1991;6:883–92.
19. Henderson NK, Price, RI, Cole, JH, Gutteridge DH, Bhagat CI. Bone density in young women is associated with body weight and muscle strength but not dietary intakes. J Bone Miner Res 1995;10:384–93.
20. Wilson SG, Retallack RW, Kent JC, Worth GK, Gutteridge DH. Serum free 1,25-dihydroxyvitamin D and the free 1,25-dihydroxyvitamin D index during a longitudinal study of human pregnancy and lactation. Clin Endocrinol 1990;32:613–22.
21. Angus RM, Sambrook PN, Pocock NA, Eisman JA. A simple method for assessing calcium intake in caucasian women. J Am Diet Assoc 1989;89:209–14.
22. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 1975;31:103–15.
23. Francis RM, Peacock M, Barkworth SA, Marshall DH. A

comparison of the effect of sorbitol and glucose on calcium absorption in postmenopausal women. Am J Clin Nutr 1986;43:72–6.
24. Need AG, Morris HA, Horowitz M, Nordin BEC. The response to calcitriol therapy in postmenopausal osteoporotic women is a function of initial calcium absorptive status. Calcif Tissue Int 1997;61:6–9.
25. Blomqvist N. On the relation between change and initial value. J Am Stat Assoc 1977;72:746–9.
26. Mashchak CA, Lobo RA, Dozono-Takano R, et al. Comparison of pharmacodynamic properties of various estrogen formulations. Am J Obstet Gynecol 1982;144:511–8.
27. McKane WR, Khosla S, Burritt MF, et al. Mechanism of renal calcium conservation with estrogen replacement therapy in women in early postmenopause: a clinical research center study. J Clin Endocrinol Metab 1995;80:3458–64.
28. Prince RL, Dick IM, Lemmon J, Randell D. The pathogenesis of age-related osteoporotic fracture: effects of dietary calcium deprivation. J Clin Endocrinol Metab 1997;82:260–64.
29. Prince RL, Smith M, Dick IM, et al. Prevention of postmenopausal osteoporosis: a comparative study of exercise, calcium supplementation, and hormone-replacement therapy. N Engl J Med 1991;325:1189–95.
30. Prince RL. Counterpoint: estrogen effects on calcitropic hormones and calcium homeostasis. Endoc Rev 1994;15:301–9.
31. Nordin BEC, Horsman A, Crilly RG, Marshall DH, Simpson M. Treatment of spinal osteoporosis in postmenopausal women. BMJ 1980;280(6212):451–4.
32. Turner RT, Riggs BL, Spelsberg TC. Skeletal effects of estrogen. Endocr Rev 1994;15:275–300.
33. Need AG, Horowitz M, Philcox JC, Nordin BEC. 1,25-Dihydroxycalciferol and calcium therapy in osteoporosis with calcium malabsorption. Miner Electrolyte Metab 1985;11:35–40.
34. Gallagher JC, Goldgar D. Treatment of postmenopausal osteoporosis with high doses of synthetic calcitriol. Ann Intern Med 1990;113:649–55.
35. Tilyard MW, Spears GFS, Thomson J, Dovey S. Treatment of postmenopausal osteoporosis with calcitriol or calcium. N Engl J Med 1992;326:357–62.
35. Ott SM, Chestnut CH. Calcitriol treatment is not effective in postmenopausal osteoporosis. Ann Intern Med 1989;110:267–74.
36. Kanis JA, McKloskey EV, de Takats D, Bernard J, Zhang DM. Treatment of osteoporosis with vitamin D. Osteoporos Int 1997;7(Suppl 3):S140–6.

Received for publication 6 October 1998
Accepted in revised form 3 May 1999