Effect of age on calcium absorption in postmenopausal women

Christopher Nordin, Allan G Need, Howard A Morris, Peter D O’Loughlin, and Michael Horowitz

ABSTRACT
Background: It is assumed that calcium absorption decreases with age, but this is not well documented. We report a study that addresses this issue.
Objective: The aim was to establish the extent and timing of any age-related change in calcium absorption in postmenopausal women.
Design: We measured radiocalcium absorption (α) in 262 healthy postmenopausal women aged 40–87 y. We also measured the serum vitamin D metabolites, parathyroid hormone (PTH), and other biochemical variables.
Results: Radiocalcium absorption decreased with age (P = 0.018); it was 28% lower in the 25 women aged >75 y than in the rest (P < 0.001). It was significantly related to serum 1,25-dihydroxyvitamin D [1,25(OH)₂D] in the whole set and in both the younger and older subsets, but it was not related to either 25-dihydroxyvitamin D [25(OH)D] or PTH or to any other measured variable. No decrease in 1,25(OH)₂D was seen with age to account for the decrease in calcium absorption, so radiocalcium absorption corrected for serum 1,25(OH)₂D decreased significantly after age 75 y. On multivariate analysis, the serum 1,25(OH)₂D concentration was a positive function of 25(OH)D (P < 0.001), albumin (P = 0.010), and PTH (P = 0.012) and a negative function of serum creatinine (P = 0.003). PTH was a negative function of calculated ionized calcium (P = 0.004) and 25(OH)D (P = 0.009) and a positive function of weight (P = 0.011) and age (P = 0.028).
Conclusions: A late age-related decrease in calcium absorption is seen in postmenopausal women to the decline that occurs at menopause. This decrease could be due to a decline in either the active calcium transport or diffusion component of the calcium absorption system.

KEY WORDS Calcium absorption, age, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone

INTRODUCTION
It has been more than 30 y since an age-related decrease in calcium absorption was first reported in humans (1–3), but there have been few studies since. One of those studies reported a decrease in calcium absorption at menopause and a further decrease with age in women (4), and another report significantly lower radiocalcium absorption in 41 women aged 65–83 y than in 59 women aged 25–35 y (5), but other studies (6–10) essentially describe a decrease in calcium absorption at menopause that is reversible with hormone therapy and probably a result of the loss of a direct effect of estrogen on calcium transport in the gastrointestinal tract (11). It is known that calcium absorption is lower in women with fractures, particularly those of the vertebrae and hip, than in age-matched control subjects (12–14), but it is not certain whether there is an age-related decrease in calcium absorption over and above the effect of menopause, and still less what might be its pathogenesis. We now report radiocalcium absorption, serum vitamin D metabolites, parathyroid hormone (PTH), and other relevant variables in a cohort of 262 essentially healthy postmenopausal women.

SUBJECTS AND METHODS
The subjects of this study were 262 community-dwelling, white, postmenopausal women aged 40–87 y, all of whom had undergone spinal radiography to exclude significant osteoporotic vertebral compression. We included 72 women with one wedged vertebra in whom mean radiocalcium absorption was the same as that in women with no wedged or crushed vertebrae (0.67 compared with 0.68/h), as well as height (157 cm) and all other measured variables. Menopause was defined as cessation of menstruation for ≥1 y or, if the subject had undergone simple hysterectomy, a follicle-stimulating hormone concentration >20 U/L. None of the subjects had suffered a hip fracture or had a serum creatinine concentration >0.10 mmol/L, and none was taking estrogen, corticosteroids, or any other therapy or suffered from parathyroid dysfunction, Paget disease, or any other disorder likely to affect calcium metabolism.
All subjects attended our laboratory for venesection and measurement of height and weight at 0900 after an overnight fast. They were then given a standard dose of radiocalcium [0.2 mBq (5 μCi) ⁴⁵Ca] in 250 mL water with 20 mg calcium carrier (as the chloride), and another blood sample was taken exactly 1 h later. Radioactivity in the serum at 1 h was determined by liquid scintillation counting by using the first serum sample as the blank. The fractional rate of calcium absorption (α) was calculated as previously described (15). Serum calcium, proteins, and electrolytes were measured on Olympus AU-5400 (Olympus Optical

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TABLE 1
Measured variables in 262 postmenopausal women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young reference range (16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>63.2 ± 8.52</td>
</tr>
<tr>
<td>Time since menopause (y)</td>
<td>—</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157 ± 6.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.3 ± 12.6</td>
</tr>
<tr>
<td>Radiocalcium absorption (fx/h)</td>
<td>0.67 ± 0.27</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>60.0 ± 23.0</td>
</tr>
<tr>
<td>Serum 1,25(OH)2D (pmol/L)</td>
<td>116 ± 37.8</td>
</tr>
<tr>
<td>Serum PTH (pmol/L)</td>
<td>4.65 ± 2.26</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>2.34 ± 0.10</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>41.8 ± 3.1</td>
</tr>
<tr>
<td>Ionized calcium (mmol/L)</td>
<td>1.17 ± 0.04</td>
</tr>
<tr>
<td>Serum creatinine (mmol/L)</td>
<td>0.068 ± 0.012</td>
</tr>
</tbody>
</table>

1 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)2D, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone.
2 x ± SD (all such values).

RESULTS

The mean (± SD) of the measured variables is shown in Table 1. The mean serum 25(OH)D was near the lower end of the young reference range (16) (with 45 subjects below it), and the mean serum PTH was near the upper end of the young reference range (16) (with 60 subjects above it).

On univariate analysis, α was a significant positive function of serum 1,25(OH)2D (r = 0.40, P < 0.001) (Figure 1) and a negative function of age (r = −0.15, P < 0.05) but not of years since menopause. Simple inspection showed that the decrease in α with age was essentially due to the low calcium absorption in the 25 subjects >75 y old; up to age 75 y, there was no significant correlation between age and α (Figure 2). The analysis of variance (ANOVA) and Tukey test showed that the only significant differences between groups were between each of the groups up to age 75 y on the one hand and the group >75 y old on the other hand. Despite the decrease in α with age, there was no significant change with age in serum 1,25(OH)2D (Figure 2) or in serum 25(OH)D, although there was a downward trend in the latter. However, there was a significant increase in PTH with age (r = 0.14, P = 0.020), which was essentially due to an increased value in the subjects >75 y old, which was significantly positive on ANOVA with Tukey test. The only other biochemical variables that changed with age were albumin that decreased (r = −0.16, P = 0.011) and creatinine that increased (r = 0.17, P = 0.003), but the change with age in both these variables was essentially continuous.

On multivariate analysis, the only variables to which α was significantly related were age (negative) and serum 1,25(OH)2D (positive) (Table 2). When age was represented by dummy variables for subjects up to age 75 y and for subjects >75 y old, the percentage of the variance accounted for (19.1) was not different from that when age was used as a continuous variable (18.3). To test for parallelism between the slopes of α on 1,25(OH)2D in the groups up to age 75 y (n = 237) and >75 y old (n = 25), we used classical analysis of covariance. The linear dependence of absorption on 1,25(OH)2D was highly significant (P < 0.001). The model was significantly improved by fitting separate parallel lines for the 2 age groups (P = 0.0013). However, there was no further improvement (P = 0.71) when separate slopes were fitted to the 2 age groups.

The consequence of the age-related decrease in α without change in serum 1,25(OH)2D was that the mean residual deviations of α from the values predicted by the serum 1,25(OH)2D was significantly negative (P < 0.001) in the oldest age group.
TABLE 2
Regression of radiocalcium absorption (α) on age and serum 1,25-dihydroxyvitamin D [1,25(OH)₂D] concentrations in 262 postmenopausal women

<table>
<thead>
<tr>
<th>Equation</th>
<th>SE</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using actual ages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \alpha = -0.0050 \times \text{age} + 0.0029 \times 1,25\text{(OH)}_2\text{D} + 0.66/\text{h} )</td>
<td>0.0018</td>
<td>0.006</td>
</tr>
<tr>
<td>( R^2 = 18.3% )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using dummy variables for 237 women up to age 75 y and 25 women aged &gt; 75 y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \alpha = -0.17 \times \text{age} + 0.0028 \times 1,25\text{(OH)}_2\text{D} + 0.54/\text{h} )</td>
<td>0.052</td>
<td>0.001</td>
</tr>
<tr>
<td>( R^2 = 19.1% )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( /t\) test.

(Figure 3). ANOVA and Tukey test showed that the only significant differences between the age groups were between each of the 3 groups up to age 75 y on the one hand and the group >75 y old on the other hand.

The significant predictors of serum 1,25(OH)₂D are shown in Table 3. Serum 1,25(OH)₂D was a positive function of serum 25(OH)D, albumin, and PTH and an inverse function of serum creatinine and height.

The significant predictors of serum PTH are shown in Table 4. It was a negative function of body weight (\( P = 0.011 \)) and age (\( P = 0.028 \)). Marginally less of the variance was accounted for when total calcium was used (9.7%) than when calculated ionized calcium was used (10.7%).

DISCUSSION

Three main findings are reported here. First, calcium absorption remained essentially constant in a group of apparently healthy postmenopausal women until they reached the age of 75 y, when it decreased by nearly 30%. Second, this late decrease could not be accounted for by a decrease in the serum concentrations of either 25(OH)D or 1,25(OH)₂D. Third (and consequently), there was an apparent decrease in gastrointestinal responsiveness to 1,25(OH)₂D in the oldest women that was analogous to, but in addition to, the decrease that occurs at menopause.

The validity of our data and the conclusions we draw from them depend, of course, on the selection of subjects and the validity of our method. As far as the subjects are concerned, the 262 untreated postmenopausal women in this study make up a valid sample of the postmenopausal population. As far as the subjects are concerned, the 262 untreated postmenopausal women in this study make up a valid sample of the postmenopausal population.

The radiocalcium absorption test was used, with modifications by our group, for nearly 40 y (17) and by others (10, 18, 19). We validated it against the serum calcitriol concentration in several cohorts that is as normal as it is possible to find without actually enlisting volunteers. All of the women had gone through routine history taking and screening for suspected disorders of calcium metabolism, and all had undergone spinal radiography. We included patients with one wedged vertebra because we found elsewhere (14) and again in this study that calcium absorption in such subjects does not differ from that in women with no vertebral deformity. Nor did the women with one vertebral wedge differ from the rest with respect to any other measured variable.

We are, therefore, satisfied that our 262 postmenopausal women can be regarded as normal with regard to bone. We excluded women with a history of hip fracture because of good evidence of calcium malabsorption in this group (13, 14), but we did not exclude women with other peripheral fractures, because their calcium absorption is much closer to normality (14) and again in this study that calcium absorption in such subjects does not differ from that in women with no vertebral deformity. Nor did the women with one vertebral wedge differ from the rest with respect to any other measured variable.

We validated it against the serum calcitriol concentration in several

TABLE 4
Significant predictors of serum parathyroid hormone (PTH) concentrations by multiple linear regression in 212 postmenopausal women

<table>
<thead>
<tr>
<th>PTH</th>
<th>SE</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-8.57 \times \text{ionized calcium calculated} )</td>
<td>2.96</td>
<td>0.004</td>
</tr>
<tr>
<td>(-0.015 \times 25\text{(OH)}_2\text{D} )</td>
<td>0.0059</td>
<td>0.009</td>
</tr>
<tr>
<td>(0.027 \times \text{weight} )</td>
<td>0.011</td>
<td>0.011</td>
</tr>
<tr>
<td>(+0.035 \times \text{age} )</td>
<td>0.016</td>
<td>0.028</td>
</tr>
<tr>
<td>(+11.6 \text{pmol/L} )</td>
<td>3.75</td>
<td>0.002</td>
</tr>
</tbody>
</table>

\( R^2 = 10.7\% \)

\( /t\) test.
series of patients (7, 12, 20–24) and showed it to correlate with calcium absorption (adjusted for intake) measured by the balance technique (15). We are satisfied that it is a valid measure of intestinal calcium transport and can be used with confidence to detect disorders of calcium absorption and to monitor treatment. It gives rather higher readings than a test that uses more calcium carrier (13), but the clinical conclusions are much the same.

In this cohort, as in earlier ones (7, 12, 14, 21), the main determinant of calcium absorption was the serum 1,25(OH)₂D₃ concentration, which was, in turn, positively related to its substate serum 25(OH)D (25) and inversely related to serum creatinine as a marker of renal function, despite the exclusion of all women with creatinine concentrations >0.10 mmol/L. We cannot explain the positive correlation of serum 1,25(OH)₂D₃ with height, but its correlation with serum albumin could reflect the binding of this metabolite to albumin (26) and compensatory upward adjustment of total 1,25(OH)₂D concentration in response to homeostatic maintenance of the free fraction. The positive correlation between serum calcitriol and PTH indicates that, in this population, it was PTH that maintained the calcitriol concentration, rather than, as might be thought, low calcitriol that drove PTH. In turn, PTH was inversely related, as expected, to 25(OH)D and calculated ionized calcium, and the decrease in these 2 variables in the subjects >75 y old explains the rise in PTH at that age, which cannot be attributed to the simultaneous decrease in calcium absorption. We cannot explain the correlation between PTH and weight, which was also reported elsewhere (27, 28).

It could be asked why previous studies did not note the decrease in calcium absorption around age 75 y that we now report. The answer is that visual inspection of the data of Avioli et al (1) and Bullamore et al (2) shows both their results to be compatible with an accelerated decrease in calcium absorption after age 70–75 y. The duodenal calcium infusion studies of Ireland and Fordtran (3) contained only 7 young and 6 elderly subjects and so are irrelevant to this issue. In the study by Ebeling et al (6), the comparison was actually between 15 premenopausal women and 15 women with a mean age of 74 y, so it is impossible to disentangle the effect of age from that of menopause in their series, and the same is true of other earlier studies in women (5, 29).

That being said, we must consider why calcium absorption should decrease around age 75 y in apparently healthy women. It might be tempting to attribute this decrease to the more or less simultaneous, although nonsignificant, decrease in serum 25(OH)D, but we have again failed to show any correlation between serum 25(OH)D and radiocalcium absorption after adjustment for serum calcitriol. Nor can the decrease in calcium absorption in the oldest age group be attributed to renal insufficiency; the rise in serum creatinine with age was continuous, and it was only marginally higher in the subjects >75 y old than in the preceding age group. This apparent age-related "resistance" to the action of calcitriol should be expressed in a flattening of the slope or lowering of the intercept of α on 1,25(OH)₂D₃, both of which were reported in old rats (30). In our study, although the trends were in the expected direction, the differences were not significant. However, previous studies failed to show a decrease in intestinal vitamin D receptor content with age in humans (5) or rats (30), which raises the possibility that the age-related decrease in calcium absorption might be due to the diffusion rather than the active transport component of the absorption process. We reported before (12) that the intercept, not the slope, of calcium absorption on serum calcitriol is reduced in women with vertebral compression. This finding might imply that it is diffusion rather than active transport that is reduced in elderly women in general and osteoporotic women in particular.

We conclude that, in our series of apparently healthy postmenopausal women, an age-related decrease in calcium absorption was essentially expressed after the age of 75 y. Because it could not be attributed to a decline in serum 1,25(OH)₂D₃, this decrease suggests a decline in the responsiveness of the small intestine to serum calcitriol, analogous to the corresponding event at menopause but occurring rather later in life than was generally supposed. The ultimate cause of this intestinal resistance to 1,25(OH)₂D₃ in old age remains uncertain, but we speculate that it might reside in the diffusion rather than the active transport component of the calcium absorption mechanism.

We thank Peter Baghurst for statistical advice.

The first draft of the paper was prepared by BECN and progressively revised in the light of comments from the other authors. Revision in the light of reviewers' comments was performed in the same way. BECN, AGN, and MH were responsible for the clinical management of the patients, and HAM and PDO were responsible for the laboratory investigations. None of the authors had a conflict of interest.

REFERENCES