Calcium malabsorption in elderly women with vertebral fractures: evidence for resistance to the action of vitamin D metabolites on the bowel

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Summary

1. Radio-calcium absorption, plasma 25-hydroxyvitamin D [25-(OH)D] and 1,25-dihydroxyvitamin D [1,25-(OH)2D] concentrations were measured in 19 elderly women with, and 21 without, vertebral fractures, before and after treatment with 25-hydroxyvitamin D3 [25-(OH)D3], to establish whether malabsorption of calcium in elderly women with vertebral fractures has a cause different from that in elderly women without vertebral fractures.

2. Malabsorption of calcium and low plasma 25-(OH)D and 1,25(OH)2D concentrations were common in both groups of women but there was no significant difference in these variables between the two groups.

3. After treatment with 40 μg of 25(OH)D3 daily for 7 days, there was a significant increase in plasma 25(OH)D and 1,25(OH)2D in both groups of women, but radio-calcium absorption increased significantly only in the group without vertebral fractures.

4. Elderly women with vertebral fractures have malabsorption of calcium which is resistant to the action of vitamin D metabolites at concentrations which correct calcium malabsorption in elderly women without vertebral fractures.

Key words: calcium absorption, osteoporosis, vitamin D metabolites.

Abbreviations: (OH)D, hydroxyvitamin D; PTH, parathyroid hormone.

Introduction

Calcium absorption is lower in women with vertebral crush fractures than in age-matched normal subjects [1-3] and may contribute to the development of osteoporosis in such patients. The cause of the reduced calcium absorption in women with vertebral fractures has never been unequivocally established, though several mechanisms have been suggested. Calcium malabsorption may result from an intrinsic defect in the absorption mechanism [4]; alternatively it may be caused by low plasma 1,25-dihydroxyvitamin D [1,25-(OH)2D] concentrations due either to a failure of the renal 1α-hydroxylase enzyme to respond to parathyroid hormone (PTH) [5] or to suppression of PTH production by the calcium released from increased bone resorption [6]. We, however, have not found any difference in plasma 25-hydroxyvitamin D [25-(OH)D] and 1,25(OH)2D concentrations in women with and without vertebral fractures [7], although others have demonstrated lower plasma 1,25-(OH)2D concentrations in vertebral fracture cases [2].

The difference in calcium absorption between women with and without vertebral fractures is less marked in the elderly because of the decline in calcium absorption in normal subjects with age [8]. We have previously shown that malabsorption of calcium in the elderly is largely due to low plasma 25-(OH)D concentrations and can be corrected by increasing these into the normal range by treatment with oral 25-hydroxyvitamin D3 [25-(OH)D3] [9]. Impaired renal function, which is common in the elderly, is also a significant but relatively less frequent cause of calcium malabsorption [9].

To investigate whether malabsorption of calcium in elderly patients with vertebral fractures differs
in its pathogenesis from calcium malabsorption of the elderly, we have examined the relationship between calcium absorption and the concentration of plasma vitamin D metabolites in elderly women, with and without vertebral fractures, before and after treatment with oral 25-(OH)D₃.

Methods

Subjects

Two groups of women were investigated. The first comprised 19 women (age range 65-86 years) with radiological evidence of two or more vertebral crush fractures and the second 21 women (age range 61-94 years) without vertebral fractures. Each subject was investigated as an in-patient on a constant dietary calcium intake of 25 mmol/day. The osteoporotic patients had not received treatment for their bone disease. The subjects without vertebral fractures had all been in hospital for less than 2 weeks and were convalescing from a minor acute medical illness. All subjects were unselected, other than that they had a plasma creatinine in the hospital normal range (50-140 µmol/l) and were not taking corticosteroids, barbiturates, vitamin D or calcium supplements. Each individual gave informed consent for the study and approval was obtained from the local ethical committee.

Experimental procedure

After an overnight fast, blood and urine were collected for measurement of plasma calcium, phosphate, creatinine, alkaline phosphatase, 25-(OH)D, 1,25-(OH)₂D and parathyroid hormone (PTH), and urine calcium, hydroxyproline and creatinine. Calcium absorption was then estimated with radio-calcium (⁴⁰Ca). Each subject was treated for 7 days with 40 µg of 25-(OH)D₃ daily, which we have previously shown increases plasma 25-(OH)D and 1,25-(OH)₂D through the normal range in adults and elderly women [10]. All investigations were repeated on the day after the last dose. In 13 subjects from each group, renal function was assessed by calculating the mean of two consecutive 24 h creatinine clearances and correcting to a surface area of 1.73 m².

Laboratory methods

The plasma for measurement of calcium, phosphate and creatinine concentration and alkaline phosphatase activity was stored at 4°C until the estimations were performed by standard techniques. Urine was also stored at 4°C until the calcium and creatinine concentrations were measured by autoanalyser and the hydroxyproline was estimated by using a resin-catalysed hydrolysis and an automated colorimetric procedure [11]. Plasma for estimation of 25-(OH)D, 1,25-(OH)₂D and PTH concentrations was quick-frozen and stored at −30°C until assayed as paired untreated and treated samples. After preliminary extraction with ether and separation by high pressure liquid chromatography [12], plasma 25-(OH)D was measured by a radiocompetitive protein binding assay [13] and plasma 1,25-(OH)₂D by radioimmunoassay [14]. Plasma PTH was measured by C-terminal radioimmunoassay [15]. Calcium absorption was estimated from plasma radioactivity [16] 1 h after an oral dose of ⁴⁰Ca by using the formula

\[
\text{radiocalcium absorption (fraction of the dose/h)} = 1.17f + 2.54f^2
\]

where \( f \) = % of administered dose/l at 1 h x body weight (kg) x 0.0015.

Statistical analysis

The statistical significance of the differences in the basal data from the two groups was calculated by using Student's t-test; the significance of changes on treatment was calculated by using Student's t-test for paired observations, the values for radio-calcium absorption, plasma 25-(OH)D, 1,25(OH)₂D and PTH undergoing a preliminary logarithmic transformation to convert them to a normal distribution. The correlation of two variables was assessed by Pearson's correlation test.

Results

The mean (±SEM) age for the group with vertebral fractures was 76.8 ± 1.3 years, which was not significantly different from the group without vertebral fractures (80.4 ± 1.6 years). The mean basal radio-calcium absorption, plasma 25-(OH)D and 1,25-(OH)₂D concentrations were at the lower end of the normal range in both groups (Table 1). Radio-calcium absorption, plasma 25-(OH)D, 1,25-(OH)₂D, calcium, phosphate and creatinine concentrations, alkaline phosphatase activity, and urine calcium/creatinine and hydroxyproline/creatinine ratios, were not significantly different between the two groups but the plasma PTH was higher in the group without vertebral fractures (\( P < 0.05 \)). Mean (±SEM) creatinine clearance was not significantly different in the two groups (75.6 ± 8.6 ml min⁻¹ 1.73 m⁻² in the group with vertebral fractures compared with 57.9 ± 6.4 in the group without vertebral fractures).
Calcium absorption in osteoporosis

TABLE 1. Biochemical findings in 21 subjects without and 19 patients with vertebral fractures before and after 7 days' treatment with 40 μg of 25-(OH)D$_3$/day

Means ± SEM are shown. Significance between variables before and after treatment: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The normal range for radio-calcium absorption was calculated from 152 normal subjects between the ages of 20 and 60 years. The normal range for plasma 25-(OH)D and PTH was from 66 healthy laboratory staff with an age range 18–55 years, and the range for plasma 1,25-(OH)$_2$D from 41 of the same group. The normal ranges for plasma calcium and creatinine were obtained from a large number of healthy blood donors between the ages of 18 and 65 years.

<table>
<thead>
<tr>
<th>Without vertebral fractures</th>
<th>Vertebral fractures</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radio-calcium absorption</strong></td>
<td><strong>Before</strong></td>
<td><strong>Vertebral fractures</strong></td>
</tr>
<tr>
<td>(fraction of dose/h)</td>
<td>$0.360 ± 0.027$</td>
<td>$0.412 ± 0.041$</td>
</tr>
<tr>
<td><strong>After</strong></td>
<td>$0.571 ± 0.049$</td>
<td>$0.440 ± 0.069$</td>
</tr>
<tr>
<td>Plasma 25-(OH)D (nmol/l)</td>
<td><strong>Before</strong></td>
<td><strong>Vertebral fractures</strong></td>
</tr>
<tr>
<td></td>
<td>$11.08 ± 1.40$</td>
<td>$20.18 ± 4.44$</td>
</tr>
<tr>
<td></td>
<td><strong>After</strong></td>
<td>$59.46 ± 6.00$</td>
</tr>
<tr>
<td>Plasma 1,25-(OH)$_2$D (pmol/l)</td>
<td><strong>Before</strong></td>
<td><strong>Vertebral fractures</strong></td>
</tr>
<tr>
<td></td>
<td>$79.3 ± 6.6$</td>
<td>$89.7 ± 11.6$</td>
</tr>
<tr>
<td></td>
<td><strong>After</strong></td>
<td>$141.7 ± 15.7$</td>
</tr>
<tr>
<td>Plasma PTH (pg/ml)</td>
<td><strong>Before</strong></td>
<td><strong>Vertebral fractures</strong></td>
</tr>
<tr>
<td></td>
<td>$446.4 ± 36.6$</td>
<td>$339.2 ± 24.0$</td>
</tr>
<tr>
<td></td>
<td><strong>After</strong></td>
<td>$381.1 ± 32.2$</td>
</tr>
<tr>
<td>Plasma calcium (mmol/l)</td>
<td><strong>Before</strong></td>
<td><strong>Vertebral fractures</strong></td>
</tr>
<tr>
<td></td>
<td>$2.384 ± 0.027$</td>
<td>$2.405 ± 0.021$</td>
</tr>
<tr>
<td></td>
<td><strong>After</strong></td>
<td>$2.435 ± 0.035$</td>
</tr>
<tr>
<td>Plasma creatinine (μmol/l)</td>
<td><strong>Before</strong></td>
<td><strong>Vertebral fractures</strong></td>
</tr>
<tr>
<td></td>
<td>$83.8 ± 4.1$</td>
<td>$74.1 ± 4.5$</td>
</tr>
<tr>
<td></td>
<td><strong>After</strong></td>
<td>$88.8 ± 4.6$</td>
</tr>
</tbody>
</table>

TABLE 2. Change in biochemistry in subjects without and patients with vertebral fractures on treatment with 25-(OH)D$_3$

Results are expressed as the mean values ± SEM. The significance of the difference in response in the two groups is indicated. NS, Not significant.

<table>
<thead>
<tr>
<th>Without vertebral fractures</th>
<th>Vertebral fractures</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radio-calcium absorption</strong></td>
<td>(fraction of dose/h)</td>
<td>$+0.210 ± 0.044$</td>
</tr>
<tr>
<td>Plasma 25-(OH)D (nmol/l)</td>
<td>$+48.4 ± 5.5$</td>
<td>$+48.7 ± 9.5$</td>
</tr>
<tr>
<td>Plasma 1,25-(OH)$_2$D (pmol/l)</td>
<td>$+62.4 ± 16.2$</td>
<td>$+43.5 ± 16.1$</td>
</tr>
<tr>
<td>Plasma PTH (pg/ml)</td>
<td>$−65.3 ± 22.3$</td>
<td>$−51.9 ± 22.2$</td>
</tr>
<tr>
<td>Plasma calcium (mmol/l)</td>
<td>$+0.050 ± 0.024$</td>
<td>$−0.005 ± 0.022$</td>
</tr>
<tr>
<td>Plasma creatinine (μmol/l)</td>
<td>$+5.0 ± 3.2$</td>
<td>$+3.9 ± 1.5$</td>
</tr>
</tbody>
</table>

In patients with vertebral fracture before treatment, radio-calcium absorption was related to plasma 1,25-(OH)$_2$D ($r = 0.53$, $P < 0.02$) and plasma PTH correlated with plasma creatinine ($r = 0.52$, $P < 0.02$), but there were no other significant correlations between the variables. In the subjects without vertebral fractures, radio-calcium absorption correlated with plasma 1,25-(OH)$_2$D ($r = 0.55$, $P < 0.01$), but there were no other significant correlations.

After 7 days' treatment with 40 μg of 25-(OH)D$_3$/day, there was a similar and significant increment in plasma 25-(OH)D in both groups (Tables 1 and 2) such that all the previously low plasma 25-(OH)D concentrations increased into the normal range. Plasma 1,25-(OH)$_2$D increased significantly in both groups and although the increment was greater in the subjects without vertebral fractures it was not significantly different between the two groups. Radio-calcium absorption increased significantly in the group without vertebral fractures with treatment whereas in the group with vertebral fractures there was no significant increase in radio-calcium absorption and eight patients still had malabsorption of calcium (Tables 1 and 2). Radio-calcium absorption was related to plasma 1,25-(OH)$_2$D concentrations before and after treatment with 25-(OH)D$_3$ (Fig. 1) in both
FIG. 1. Relationship between radio-calcium absorption and plasma 1,25-(OH)\(_2\)D in subjects without vertebral fracture (○) and patients with vertebral fractures (●) before and after treatment with 25-(OH)D\(_3\). The regression line for the normal subjects is shown (y = 0.0018x + 0.265). Results for the majority of the vertebral fracture patients are below this line.

groups (in patients with vertebral fractures \(r = 0.40, P < 0.01\); in subjects without vertebral fractures \(r = 0.54, P < 0.001\)).

There was a significant increase in plasma calcium in the subjects without vertebral fractures but not in the patients with vertebral fractures and plasma PTH decreased significantly in both groups.

Discussion

In this study we have demonstrated that calcium absorption and plasma 25-(OH)D and 1,25-(OH)\(_2\)D concentrations are commonly reduced in elderly women both with and without vertebral fractures. Treatment with 25-(OH)D\(_3\) increased the plasma 25-(OH)D and 1,25-(OH)\(_2\)D to similar levels in both groups of women, but only the group without vertebral fractures showed a significant increase in radio-calcium absorption and correction of calcium malabsorption. This confirms our earlier study \([9]\), which showed that malabsorption of calcium in elderly women without vertebral fractures is predominantly due to vitamin D deficiency. The elderly women with vertebral fractures also had evidence of vitamin D deficiency, but the failure of treatment with 25-(OH)D\(_3\) to correct malabsorption of calcium, despite increasing plasma 25-(OH)D and 1,25-(OH)\(_2\)D into the normal range, strongly suggests that there is also vitamin D resistance in the bowel. The resistance does not occur in all patients with vertebral fractures (Fig. 1), and it can probably be overcome if the relationship between calcium absorption and plasma 1,25-(OH)\(_2\)D is log-dose related as it is most likely to be.

The similar increment in plasma 25-(OH)D in both groups after treatment with 25-(OH)D\(_3\) indicates that patients with vertebral fractures have normal absorption and metabolism of 25-(OH)D\(_3\). Although plasma 1,25-(OH)\(_2\)D increased significantly in both groups, three patients with vertebral fractures still had low plasma 1,25-(OH)\(_2\)D concentrations after treatment, indicating that there may be a failure of 1,25-(OH)\(_2\)D production in some patients with vertebral fractures, as predicted from PTH infusion \([5]\) and low calcium diet studies \([4]\).

In the elderly, plasma PTH is commonly raised because of vitamin D deficiency, renal impairment or both \([9]\). Plasma PTH concentrations were high in a proportion of women from both groups, but were significantly higher in those without vertebral fractures, suggesting that as a group they were slightly more vitamin D deficient and had more renal impairment. Although the patients without vertebral fractures cannot be considered representative of the elderly population in general, because of the circumstances which necessitated
admission, their plasma 25-(OH)D concentrations were comparable with those of a group of randomly selected elderly women living at home [17].

The maintenance of normal calcium absorption is generally accepted as beneficial in reducing bone loss. In age-related bone loss in subjects without vertebral fractures this can be achieved in the majority by ensuring that plasma 25-(OH)D concentrations do not fall below the normal range. In patients with vertebral fractures, the plasma 25-(OH)D concentration needs to be maintained at a higher level to prevent calcium malabsorption, confirming previous studies which suggest that pharmacological doses of vitamin D are necessary to correct malabsorption [1]. It remains to be established, however, whether the levels of plasma 25-(OH)D necessary for maintaining normal calcium absorption in osteoporosis will have a deleterious effect on the skeleton by increasing bone resorption.

Acknowledgment

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References