Relationship of Intestinal Calcium Absorption to 1,25-Dihydroxyvitamin D \([1,25(OH)_2D]\) Levels in Young Versus Elderly Women: Evidence for Age-Related Intestinal Resistance to 1,25(OH)_2D Action*

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ABSTRACT

Intestinal calcium absorption decreases with aging, but it is unclear whether this is attributable to an age-related intestinal resistance to 1,25-dihydroxyvitamin D \([1,25(OH)_2D]\) action. Thus, we assessed the in vivo dose response of active intestinal calcium absorption to a broad range of circulating \(1,25(OH)_2D\) levels in elderly [age (mean ± SD), 72.5 ± 3.0 yr] vs. young women (age, 28.7 ± 5.3 yr; \(n = 20\) per group), who were stratified into 5 subgroups: group 1 was given a high calcium intake of 75 mmol/day, suppressing \(1,25(OH)_2D\) levels; group 2 was given a normal calcium diet of 15–30 mmol/day, representing basal \(1,25(OH)_2D\) levels; group 3 was given a low-calcium diet of 5 mmol/day to stimulate endogenous \(1,25(OH)_2D\) production; group 4 was given the low-calcium diet plus 1 \(\mu g/\text{day} \(1,25(OH)_2D\); and group 5 was given a low-calcium diet plus 2 \(\mu g/\text{day} \(1,25(OH)_2D.\) After 7 days of diet and/or \(1,25(OH)_2D\) treatment, fasting fractional calcium absorption (FCA) was assessed by a double-tracer method using stable calcium isotopes. Serum \(1,25(OH)_2D\) and vitamin D-binding protein levels were measured concurrently, and the free \(1,25(OH)_2D\) index [molar ratio of \(1,25(OH)_2D\) to DBP] was calculated.

FCA was significantly correlated with the free \(1,25(OH)_2D\) index in the young \((R = 0.63, P = 0.003)\) but not in the elderly women \((R = 0.27, P = 0.25)\). Moreover, the slope of the relationship between FCA and free \(1,25(OH)_2D\) index (representing intestinal sensitivity to \(1,25(OH)_2D\)) was significantly greater in the young (compared with the elderly) women \([\text{mean} ± \text{SD}, 0.15 ± 0.04 \text{ (young)} vs. 0.03 ± 0.02, \text{ elder}, P = 0.03]\). Thus, using an experimental design that allowed us to assess FCA over a wide range of \(1,25(OH)_2D\) levels, we demonstrate that elderly women have a resistance to \(1,25(OH)_2D\) action that may contribute to their negative calcium balance, secondary hyperparathyroidism, and bone loss. (J Clin Endocrinol Metab 85: 4023–4027, 2000)

SERUM PTH levels increase with age (1–8), and this increase correlates with the age-related increase in bone turnover (9–12). Moreover, suppression of PTH secretion in elderly vs. young women results in a disproportionately greater decrease in bone resorption in the elderly women than in premenopausal women (13). This indicates that bone resorption is more dependent on circulating PTH levels in elderly (compared with young) women and is consistent with a causal role for PTH in mediating the age-related increase in bone resorption (13). An important cause of the secondary hyperparathyroidism in elderly individuals is an age-related impairment in intestinal calcium absorption (14–16). However, the mechanism(s) for this decrease in calcium absorption in aging women is not well understood.

Several indirect lines of evidence indicate that elderly women may have intestinal resistance to 1,25-dihydroxyvitamin D \([1,25(OH)_2D]\) action. For example, we previously found that, whereas serum \(1,25(OH)_2D\) levels increased in women up to age 65 yr, intestinal calcium absorption remained unchanged (8), consistent with an impaired intestinal responsiveness to \(1,25(OH)_2D\) action. Moreover, studies in humans (17) and in rats (18) have found an age-related decrease in intestinal vitamin D receptor (VDR) concentrations, although this remains controversial (19, 20).

To date, human studies assessing intestinal calcium absorption with aging have been made primarily under basal conditions, over a relatively narrow range of circulating \(1,25(OH)_2D\) levels (8, 17, 21–24), although Ireland et al. (25) did find that the intestinal adaptation to a low-calcium diet was impaired in elderly (compared with young) subjects. However, serum \(1,25(OH)_2D\) levels were not measured in the latter study, so it was unclear whether the impaired intestinal adaptation to a calcium-deficient diet was attributable to smaller increases in circulating \(1,25(OH)_2D\) levels or to reduced intestinal responsiveness to \(1,25(OH)_2D\) action in the elderly (compared with the young) subjects. Thus, in the present study, we used a study design that greatly expanded circulating \(1,25(OH)_2D\) levels, thereby providing the necessary dynamic range in circulating \(1,25(OH)_2D\) levels to allow us to test for a possible reduction in intestinal sensitivity to \(1,25(OH)_2D\) action in elderly (compared with young) women.
**Subjects and Methods**

**Experimental subjects**

We studied 20 young premenopausal women (age range, 20–35 yr; mean ± SD, 28.7 ± 5.3 yr) and 20 elderly postmenopausal women (age range, 65–80 yr; mean ± SD, 72.5 ± 3.0 yr). None of the study subjects had any diseases such as renal failure (creatinine > 133 μmol/L), malabsorption, or congestive heart failure, or was taking any drugs known to affect calcium metabolism (calcium supplements > 12.5 mmol/day, vitamin D supplements > 1,000 IU/day, sodium fluoride, oral bisphosphonates, glucocorticoids, diuretics, calcium channel blockers, or anticonvulsants). All participants had a bone mineral density, of the lumbar spine and hip, within the age-adjusted 95% confidence levels for normal women. None of the elderly participants had vertebral fractures on thoracic and lumbar spine radiographs. The studies were approved by the Mayo Institutional Review Board. Signed informed consent forms were obtained from all subjects. All studies were carried out in the Mayo General Clinical Research Center (GCRC).

**Study protocol**

To expand the circulating range of 1,25(OH)2D levels, the young and elderly subjects were divided into 5 groups of 4 subjects each: group 1 was given a high calcium intake of 75 mmol (3000 mg)/day [15 mmol of calcium in the diet and 60 mmol of oral calcium supplement as calcium citrate (Citracal) in 3 divided doses]; group 2 was given a normal calcium intake [15–30 mmol (600–1200 mg)/day]; group 3 was given a low calcium intake [5 mmol (200 mg)/day]; group 4 was given a low calcium intake (5 mmol/day) and received oral 1,25(OH)2D (Rocaltrol), 1 μg/day; group 5 was given a low calcium intake (5 mmol/day) and received 1,25(OH)2D (Rocaltrol), 2 μg/day. Subjects were started on all dietary and 1,25(OH)2D interventions 7 days before the study. All subjects were ambulatory, and consumed diets were prepared by the dietitians in the GCRC during the 8-day study period. Each subject was admitted into the GCRC on the evening of day 7 of the study and stayed overnight for 2 nights. The intestinal calcium absorption study was performed on the morning on day 8, after an overnight fast.

**Assessment of fractional calcium absorption (FCA)**

FCA was assessed as previously described (26), except that the oral tracer dose was given only once (in the morning after an overnight fast) with a fixed calcium carrier rather than being mixed with each of the meals. Stable isotopes of calcium (44Ca for iv administration and 42Ca for oral administration) were purchased from Oak Ridge National Laboratories (Oak Ridge, TN) and prepared as sterile solutions of calcium chloride (26). At 0800 h on day 8, a blood sample was drawn through an indwelling catheter for measurement of serum calcium, phosphate, PTH, 25-hydroxyvitamin D [25(OH)D], 1,25(OH)2D, and vitamin D binding protein (DBP). The serum was separated and stored at −70 C until analysis. Subjects in groups 4 and 5 took their assigned doses of 1,25(OH)2D, and then all subjects received an iv dose of 0.075 mmol (3 mg) 44Ca and an oral dose of 0.045 mmol (18 mg) 42Ca in a carrier of 2.05 mmol (82 mg) of calcium, as CaCl2 [total oral dose of calcium, 2.5 mmol (100 mg)]. Previous studies (27) have shown that, at this oral calcium load, calcium adsorption is mainly active [1, 25(OH)2D-mediated]. Each subject remained fasting for 4 h after the oral dose of 42Ca, and then a second blood sample was drawn for measurement of 1,25(OH)2D. The mean of the two 1,25(OH)2D values in each subject was used to relate to intestinal FCA. A 24-h urine was collected, beginning at 0730 h on day 8, for measurement of the 44Ca/42Ca ratio. Subjects were dismissed from the GCRC on the following morning after completion of the 24-h urine collection.

An aliquot of the 24-h urine specimen was prepared for mass spectrometric analysis by an oxalate precipitation procedure, as previously described (28). Stable isotopic ratios were measured by thermal ionization mass spectrometry with a Finnigan MAT Thermoquad (THQ) Quadrupole mass spectrometer (Bremen, Germany). All ratios were measured relative to 48Ca. The relative precision of all determinants was 0.3–0.5%. Each sample was assayed in duplicate. FCA was calculated from the urine ratio of 44Ca/42Ca as previously described (26).

**Biochemical methods**

Serum calcium was measured by atomic spectroscopy, and serum phosphate and creatinine were measured by kinetic centrifugal analyzer methods. Serum PTH was determined by two-site immunochemiluminesometric assay [Diagnostics Systems Laboratories, Inc., Webster, TX; intraassay coefficient of variation (CV), 6.3%]. Serum 25(OH)D and 1,25(OH)2D were measured by competitive protein binding assays [Nichols Institute Diagnostics, San Juan Capistrano, CA; intraassay CVs, 9.2% for 25(OH)D and 7.3% for 1,25(OH)2D]. DBP was measured by immunonephelometric assay (29) (intraassay CV, 2.9%).

**Statistical methods**

Statistical analyses were carried out by using the Statistical Analysis System software program (30). Paired t tests were used to determine significance, between the young and elderly subjects in each group, for each of the measured variables. Results were considered significant for P < 0.05. Correlation among variables was assessed through the calculation of Pearson’s product moment correlation coefficient. Linear regression was used to assess the relationship between 1,25(OH)2D and the free 1,25(OH)2D index [molar ratio of 1,25(OH)2D to DBP] and FCA in the young and elderly women. Bivariate linear regression models were used to simultaneously assess the impact of 1,25(OH)2D or the free 1,25(OH)2D index and 25(OH)D on FCA.

**Results**

Table 1 shows the baseline characteristics of the study subjects. Body mass index was significantly higher, and creatinine clearance was significant lower, in the elderly (compared with the young) women.

Figure 1 shows serum 1,25(OH)2D levels and the molar ratios of 1,25(OH)2D to DBP [free 1,25(OH)2D indices] in the young and elderly women in the different groups. As evident, our study design did expand both values over a wide range in the young and elderly women. Both 1,25(OH)2D and the free 1,25(OH)2D indices tended to be higher in the elderly women in groups 4 and 5, compared with the younger women, although these differences did not reach statistical significance.

| Table 1. Baseline characteristics of the study subjects |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Young women     | Elderly women   | P-value         |
|                                | (n = 20)        | (n = 20)        |                 |
| Age, years                     | 29.2 ± 1.2      | 72.6 ± 0.7      |                 |
| Body mass index, kg/m²         | 24.2 ± 0.9      | 26.9 ± 0.8      | 0.04            |
| Serum                          |                 |                 |                 |
| Calcium, mmol/L                | 2.20 ± 0.01     | 2.23 ± 0.02     | NS              |
| Phosphorus, mmol/L             | 1.19 ± 0.03     | 1.19 ± 0.03     | NS              |
| Creatinine, μmol/L             | 79.6 ± 1.8      | 79.6 ± 3.5      | NS              |
| 25-Hydroxyvitamin D, nmol/L    | 79.9 ± 8.5      | 67.4 ± 4.7      | NS              |
| Creatinine clearance, mL/min   | 98.7 ± 5.3      | 78.0 ± 3.2      | 0.002           |

NS, Not significant.
Figure 2 shows the serum PTH levels in the different groups of the young and elderly women. Serum PTH levels were higher in the elderly women (compared with the young women) in group 2, which represents basal conditions (although this did not achieve statistical significance because of the small number of subjects per group). The major difference between the young and elderly women, however, was in group 3, where the low-calcium diet induced significantly higher PTH levels in the elderly.

To better assess the relationship between intestinal calcium absorption and 1,25(OH)₂D levels, we took advantage of the expanded range for serum total 1,25(OH)₂D levels and free 1,25(OH)₂D indices achieved with our interventions, and we plotted these against FCA (Figs. 3 and 4). As shown in Fig. 3, FCA was significantly correlated with 1,25(OH)₂D levels in the young (but not in the elderly) women. FCA was even more strongly related to the free 1,25(OH)₂D index than to total 1,25(OH)₂D levels in the young women but again was not significant in the elderly women (Fig. 4). In addition, the slope of the relationship between FCA and free 1,25(OH)₂D index was significantly greater in the young women (0.15 ± 0.04 (young) vs. 0.03 ± 0.02 (elderly), P = 0.03). To test for any impact of circulating 25(OH)D levels on the relationship between FCA and 1,25(OH)₂D and/or the free 1,25(OH)₂D index, we also entered 25(OH)D levels as a covariate in the analyses in Figs. 3 and 4, but it was not significant.

Discussion

Although calcium absorption is impaired in elderly women (8, 17), it is unclear whether this is caused by an intestinal resistance to 1,25(OH)₂D action or to some other mechanism. Using dietary and pharmacologic interventions to expand the dynamic range of circulating 1,25(OH)₂D levels, we demonstrate here that the relationship between FCA and the free 1,25(OH)₂D index is clearly different between young vs. elderly women. Thus, whereas we found a strong correlation between FCA and the free 1,25(OH)₂D index in the young subjects, this correlation was not significant in the elderly subjects. Moreover, the slope of this relationship, a measure of intestinal responsiveness to 1,25(OH)₂D action, was markedly reduced in the elderly women. Taken to-
together, these data indicate a fundamental breakdown in the relationship between FCA and free 1,25(OH)2D levels in elderly women. This is further reflected by the greater dispersion of data points in the elderly subjects (Fig. 4B) vs. the young subjects (Fig. 4A). Of note, several elderly subjects had relatively high FCA values, even at low 1,25(OH)2D and free 1,25(OH)2D index values (Figs. 3 and 4). The reasons for this are unclear, but this could be attributable simply to biologic variability, because FCA tends to be fairly variable, even in inbred strains of rat (19). Alternatively, because calcium absorption has both a passive (1,25(OH)2D-independent) and an active (1,25(OH)2D-dependent) component (27), our findings in Fig. 4 may be attributable to a greater contribution of passive vs. active calcium absorption in some of the elderly subjects. Thus, while our 100-mg calcium load should have led mainly to active [1,25(OH)2D-mediated] calcium transport (27), this may not have been true in some of the elderly subjects. A third possibility for these findings is that some elderly subjects were unable to modulate (up or down) FCA in response to changes in circulating 1,25(OH)2D levels. Indeed, these possibilities are not mutually exclusive, and all could, in part, explain the data in Fig. 4. We should stress, however, that when the entire data set is analyzed as a whole, the fundamental observations are a breakdown of the relationship between FCA and 1,25(OH)2D levels in elderly subjects, with a reduced slope of this relationship.

In a recent study, Wood et al. (19) performed a similar regression analysis between intestinal calcium absorption and circulating 1,25(OH)2D levels in young vs. aged rats, with results that were virtually identical to our findings in humans. Although the reason(s) for the breakdown in the relationship between FCA and 1,25(OH)2D levels in elderly humans and animals is unclear, we have previously reported that intestinal VDR levels were reduced in elderly compared with young women (17). However, other studies in humans (20) failed to demonstrate a reduction, and the data in animals is also conflicting (18, 19). Thus, a postreceptor defect in 1,25(OH)2D action may also be present.

Because the elderly women were also estrogen deficient, our study cannot dissociate the effects of aging from those of estrogen deficiency. However, several lines of evidence suggest that estrogen may enhance intestinal calcium absorption. Thus, in perimenopausal women, before and 6 months after oophorectomy, Gennari et al. (31) found that the increase in calcium absorption, in response to treatment with 1,25(OH)2D, was blunted in the presence of E deficiency, suggesting a direct enhancing effect of estrogen on the intestinal response to 1,25(OH)2D. More recently, Liel et al. (32) demonstrated that estrogen increased VDR levels and the response of 1,25(OH)2D target genes to 1,25(OH)2D treatment in ovariecotomized rats. In contrast, Bolscher et al. (33) found that estrogen directly stimulated intestinal calcium absorption in the rat, independent of 1,25(OH)2D action, and the presence of estrogen receptors has been demonstrated in rat intestine (34). Clearly, further studies, using the present study design in estrogen-treated vs. untreated elderly women, will be needed to resolve the issue of whether the impaired intestinal sensitivity to 1,25(OH)2D that we observed in elderly women is, at least in part, caused by estrogen deficiency.

Though our data indicate that intestinal sensitivity to 1,25(OH)2D is reduced in elderly women, clearly there are other factors that may contribute to the age-related decline in calcium absorption. These include vitamin D deficiency (35), although baseline 25(OH)D levels were similar in the young and elderly women in our study, and 25(OH)D was not a significant covariate in the analyses in Figs. 3 and 4. In addition, age-related reductions in renal mass may result in a defect in 1-α-hydroxylation of 25(OH)D (36), resulting in low 1,25(OH)2D levels in some elderly individuals. Our studies suggest, however, that (on average) there is intestinal resistance to 1,25(OH)2D action in elderly women, and these other abnormalities may be superimposed on this defect.

We also found, as expected, that baseline serum PTH levels tended to be higher in the elderly (compared with the young) women. With the induction of calcium deficiency in Group 3, however, serum PTH levels were markedly increased in the elderly women, consistent with our previous observations that elderly women have a functional defect in parathyroid function consistent with parathyroid hyperplasia (37).

In summary, we conclude that elderly women have an impaired intestinal response to 1,25(OH)2D. This defect may contribute to the negative calcium balance, secondary hyperparathyroidism, and bone loss in aging women. Further studies are needed to define whether this abnormality is caused by a primary impairment in calcium absorption associated with aging or is secondary to estrogen deficiency.

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