Effects of vitamin D metabolites on intestinal calcium absorption and bone turnover in elderly women[1–3]

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ABSTRACT

Background: The relative importance of vitamin D metabolites in the regulation of gut calcium absorption has not been well studied in elderly women living in an environment with abundant sunlight.

Objective: The objective was to examine the determinants of active gut calcium absorption ($\bar{x} \pm$ SD: 42 $\pm$ 11%) after an overnight fast with the use of a low (10 mg) calcium load.

Design: One hundred twenty elderly women aged 74.7 $\pm$ 2.6 y underwent an active calcium absorption test with a radioactive calcium tracer, dietary analysis, and measurement of markers of bone turnover and calcium metabolism.

Results: The mean serum 25-hydroxyvitamin D [25(OH)D] concentration at the time of the calcium absorption test was 68 $\pm$ 29 nmol/L. Gut calcium absorption was correlated with 25(OH)D but not 1,25-dihydroxyvitamin D (calcitriol), the free calcitriol index, or dietary calcium intake. After adjustment for age, calcitriol concentration, and dietary calcium intake, the significant determinant of fractional calcium absorption was the 25(OH)D concentration ($r = 0.34$, $P = 0.001$). When body weight was included in the regression, both 25(OH)D ($\beta = 1.20 \times 10^{-3}$) and calcitriol ($\beta = 1.00 \times 10^{-3}$) were significantly correlated with calcium absorption. Despite the strong relation between 25(OH)D and gut calcium absorption, there was no relation with other aspects of bone turnover or calcium metabolism.

Conclusion: These data suggest that at low calcium loads, 25(OH)D is a more important determinant of gut calcium absorption than is calcitriol in elderly women exposed to abundant sunlight, but that this relation has little effect on overall calcium metabolism. *Am J Clin Nutr* 2002;75:283–8.

KEY WORDS Elderly women, vitamin D, calcitriol, calcium absorption, bone turnover, calcium intake, 25-hydroxyvitamin D, sunlight

INTRODUCTION

The effect of vitamin D deficiency in impairing intestinal calcium absorption was recognized in the 1920s. In the 1950s it was recognized that vitamin D acted directly on the intestine to stimulate calcium absorption. Once 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D (calcitriol) were characterized (1), the results of animal experiments showed that the affinity of the vitamin D receptor for calcitriol was 1000- to 2000-fold higher than the affinity of the receptor for 25(OH)D and that calcitriol was the hormone principally responsible for the regulation of intestinal calcium absorption. This result is despite the fact that circulating total concentrations of 25(OH)D are $\approx$1000-fold higher than those of calcitriol. Over the years, data have been presented to support the importance of both metabolites of vitamin D in the regulation of intestinal calcium absorption in humans (2–7).

Calcium deficiency plays a central role in the development of age-related bone loss. The major components of this role include increased renal calcium loss and reduced intestinal absorption, which can be alleviated by increasing calcium intake (8–11). Intestinal calcium intake and absorption have important effects on clinical outcomes such as fracture and bone density (12, 13). Thus, studies of the determinants of absorption may play an important role in optimizing regimens for the prevention of fracture. One of the determinants of absorption is the cutaneous synthesis of vitamin D, which is strongly related to altitude and season (14, 15). The photoproduction of vitamin D_3 throughout the year in Perth, Western Australia (latitude 32° south), is likely to be considerable. These observations are based on previous work by Ladizesky et al (15), who examined the significant photoconversion of previtamin D and vitamin D present in winter in Buenos Aries (latitude 34° south) with that in another city, Ushuaia (latitude 55° south), situated further south (15). The present cross-sectional study was undertaken in elderly, ambulatory women residing in an environment with abundant sunlight to examine the relative importance of 25(OH)D and calcitriol on active calcium absorption and their role in bone and calcium homeostasis.

SUBJECTS AND METHODS

Subjects

One hundred twenty women aged ≥70 y were recruited at random from the community by using the electoral roll. The subjects selected were likely to survive a 5-y study, were not receiving bone-active agents, and were not currently using vitamin D supplements or products rich in ergocalciferol. Informed consent was obtained from all subjects, and the study was approved by the Human Rights Committee of the University of Western Australia.

Biochemical assessment

A blood and a urine sample were collected at baseline from each subject after she had fasted overnight. The urine samples were analyzed for creatinine, calcium, and phosphorus by use of routine methods (BM/Hitachi 747 Analyser; Boehringer Mannheim GmbH, Mannheim, Germany). Urine deoxypyridinoline was measured by HPLC (16) and the measurement corrected for creatinine excretion. The blood samples were analyzed for alkaline phosphatase, creatinine, calcium, and phosphorus by routine methods (BM/Hitachi 747 Analyser). Serum osteocalcin was measured by radioimmunoassay techniques, as previously described (17, 18). Serum intact parathyroid hormone (PTH) was measured by an immunochemiluminometric method (19), with intra- and interassay CVs of 3.6% and 6.2%, respectively. This method does not have the nonlinearity problems of other assays at high PTH concentrations.

Serum calcitriol was measured by using a column extraction technique followed by an assay with cytosol binding protein from calf thymus (20); the intra- and interassay CVs were 14% and 20%, respectively. Vitamin D binding protein was measured by radioimmunoassay with an antibody donated by R Bouillon; the intra- and interassay CVs were 4% and 11%, respectively (21). The free calcitriol index was calculated as the ratio of calcitriol to vitamin D binding protein. Serum 25(OH)D was measured by using an extraction technique followed by a competitive-binding assay with diluted human serum (22). Intra- and interassay CVs were 8% and 16%, respectively. This technique measures both 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 with equal affinity. Intra- and interassay CVs were 8% and 16%, respectively. Baseline measurements were undertaken during a 4-wk period in September 1999 at the end of the Australian winter.

Dietary calcium intake and absorption

Before the calcium absorption test, the subjects consumed their normal self-selected diet. At the baseline visit, the Anti Cancer Council of Victoria food-frequency questionnaire (23) was used to assess calcium intake. The questionnaire was recently validated against a 7-d weighed-food record in 63 women (24). With use of the weighed-food record as the gold standard, the correlation coefficient for energy-adjusted deattenuated calcium intake was 0.59. The food-frequency questionnaire used a computer-read answer sheet that was analyzed with the NUTTAB95 database (Nuttab 95, Canberra, Australia).

Active calcium transport was measured by using a 10-mg calcium chloride carrier and a 5-uCi (^{45}Ca) tracer. Two blood samples were collected, 1 at baseline before the calcium tracer was administered and 1 precisely 1 h after tracer administration. Subjects were instructed to consume only specific brands of

deionized water during the 12 h before testing. The formula for calculating the fraction absorbed was as follows (25):

$$\text{Fraction absorbed (\%)} = [(\text{cpm } ^{45}\text{Ca/L blood}) \times \text{body weight (kg)} \times 0.15]/\text{total counts administered} \quad (1)$$

The CV for active gut calcium absorption, conducted in 12 women 2 wk apart, was 8.5%.

Statistical analysis

All statistical analyses were performed with SPSSPC for WINDOWS (version 10.0; SPSS Inc, Chicago). Interrelations between gut calcium absorption and biochemical and bone mineral density variables were calculated by using Spearman's correlation coefficient. The homogeneity of variance and normality assumptions were checked by visual inspection of the residuals from analysis of variance models and by using the Kolmogorov-Smirnov goodness-of-fit test, respectively. Multiple regression techniques were used to determine the significant predictors of the dependent variables after adjustment for the independent variables.

RESULTS

The women's mean baseline age, weight, biochemistry indexes, and dietary calcium intake are detailed in **Table 1**. The median 25(OH)D concentration at baseline (end of winter) was 66 nmol/L (interquartile range: 44–86 nmol/L). The lower 5% and 20% cutoffs for 25(OH)D concentrations in this population were 28 and 41 nmol/L, respectively.

Gut calcium absorption

Circulating 25(OH)D concentrations were positively correlated with gut calcium absorption (Table 1, **Figure 1**). 25(OH)D was positively correlated with calcitriol and the free calcitriol index but not with PTH (Table 1). 25(OH)D was negatively correlated with weight. Calcitriol was negatively correlated with weight but not correlated with gut calcium absorption or PTH (Table 1).

Multiple regression analysis was used to determine whether baseline 25(OH)D was a predictor of active gut calcium absorption. After adjustment for age, calcium intake, and calcitriol, the major determinant of gut calcium absorption was 25(OH)D. The regression equation was gut calcium absorption = 0.130 [25(OH)D] + 33.5 ($P < 0.001$). After the model was adjusted for variation in weight, calcitriol and weight were also significant predictors of active gut calcium absorption. The regression equation was gut calcium absorption = 0.12 [25(OH)D] + 0.28 (weight) + 0.10 (calcitriol) + 5.3 ($P < 0.001$).

Bone biochemistry

Potential mechanisms whereby calcitropic hormones may regulate calcium physiology were explored by using regression approaches. With use of both simple and multiple regression techniques, 25(OH)D was not found to be related to bone turnover markers, even after adjustment in the regression model for age and weight. Calcitriol was significantly correlated with osteocalcin (Table 1).

DISCUSSION

These results suggest that at low calcium loads, 25(OH)D is a more important determinant of gut calcium absorption than is

TABLE 1
Baseline characteristics of the elderly women and the linear correlation of each characteristic with 25-hydroxyvitamin D [25(OH)D] and calcitriol[1]

Characteristic	Value	25(OH)D regression coefficient	Calcitriol regression coefficient
Age (y)	74.7 ± 2.6[2]	NS	NS
Weight (kg)	70.4 ± 12.7	−0.19[3]	−0.45[4]
Biochemistry indexes			
Plasma calcium (mmol/L)	2.34 ± 0.10	NS	NS
Plasma phosphate (mmol/L)	1.14 ± 0.13	NS	NS
Plasma creatinine (μmol/L)	72.2 ± 12.4	NS	NS
Serum PTH (pmol/L)	3.94 ± 1.83	NS	NS
Serum calcitriol (pmol/L)	91.3 ± 25.7	0.36[4]	—
Free calcitriol index	13.5 ± 4.6	0.27[4]	0.76[4]
Serum vitamin D binding protein (μmol/L)	7.1 ± 1.7	NS	NS
Serum 25(OH)D (nmol/L)	68.0 ± 28.7	—	0.36[4]
Bone turnover markers			
Serum ALP (U/L)	82.6 ± 19.1	NS	NS
Serum osteocalcin (μg/L)	4.07 ± 2.44 [118]	NS	0.30[4]
Urine deoxypyridinoline/creatinine (μmol/L)	29.1 ± 11.7 [117]	NS	NS
Calcium intake and absorption			
Dietary calcium intake (mg)	906 ± 319	NS	NS
Active gut calcium absorption (%)	42.3 ± 10.9	0.34[4]	NS

[1] n = 120 unless otherwise stated in brackets. PTH, parathyroid hormone; ALP, alkaline phosphatase.
[2] $\bar{x}$ ± SD.
[3] $P < 0.05$.
[4] $P < 0.001$.

calcitriol in healthy elderly women. Despite differences in the populations studied and the radioactive calcium doses used, similar fractional calcium absorption rates and associations with 25(OH)D were reported by others (26, 27). In our population and in a younger cohort (28), a strong negative correlation was observed between weight and calcitriol. In both studies, calcitriol became a positive determinant of gut calcium absorption only when the variance due to weight was controlled for in the multiple regression models. This indicates that at any given weight, calcitriol concentrations were positively related to gut calcium absorption in both the present study and the study by Wishart et al (28). The negative relation between weight and calcitriol remains unexplained but may be related to the reductions shown in the synthesis and processing of vitamin D in obese subjects (29).

After adjustment for the wide range of calcium absorption values measured (16–70%), calcitriol or 25(OH)D accounted for equal portions of net active absorption. Thus, the equivalence of potency of 25(OH)D and calcitriol may be due to the lower receptor affinity of 25(OH)D being offset by its higher circulating concentration, as was found in previous cross-sectional studies (30). In addition, supplemental vitamin D_3 (6, 7, 26) and 25(OH)D (6) were shown to increase the net absorptive capacity of calcium with (26) and without (6) a detectable rise in calcitriol concentrations. In both cases, the change in absorption efficiency was related to 25(OH)D concentrations and not to circulating calcitriol concentrations. Moreover, calcitriol concentrations accounted for only 20–30% of the differences in calcium absorption between healthy and elderly women (31) and between healthy and osteoporotic, postmenopausal women (5). Whereas 5% of the variance in calcium absorption was accounted for by calcitriol (28) in a study of young women (28), in a cohort of men (17–91 y), 56% of the variance in calcium absorption was accounted for by declining age-related calcitriol concentrations (32). In neither study, however, was 25(OH)D measured; thus, neither study was able to examine the relation between calcitriol

and its precursor. The question remains as to the relative absorptive capacity due to 25(OH)D compared with its metabolite calcitriol in osteoporotic, elderly, and young groups.

Dosing studies using bowel washout techniques in young subjects clearly show a dependence of gut calcium absorption on calcitriol concentrations and calcium intakes across the physiologic range (33). Sheikh et al (33) calculated that in their subjects, the vitamin D–independent fraction of calcium absorption was ≈30% across the range of calcium intakes. Calcitriol regulated calcium absorption across the physiologic range and could stimulate fractional calcium absorption from close to 0% at low calcitriol concentrations to >60% at very high concentrations. Thus, in a healthy individual with a calcitriol concentration of 120 pmol/L who consumed a meal containing 300 mg Ca, vitamin D–dependent processes would absorb ≈75 mg Ca. Con-
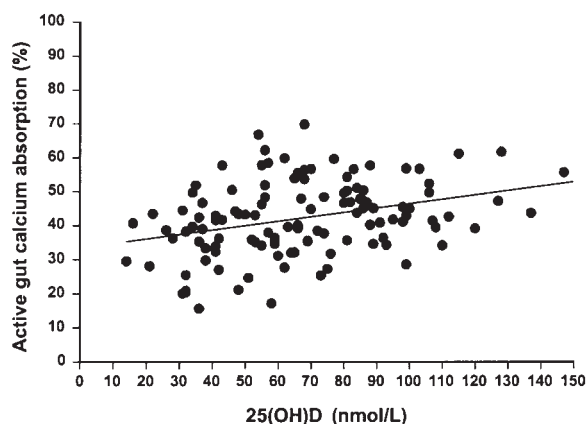

FIGURE 1. Relation between active gut calcium absorption and 25-hydroxyvitamin D [25(OH)D] concentration in postmenopausal women. Active gut calcium absorption = 0.13 × 25(OH)D + 33.5 (r = 0.35, $P < 0.001$).

versely, passive processes would absorb ≈25 mg. Our findings show that calcitriol concentrations in elderly women are not as important as shown previously in younger women.

The test used in this study examined active calcium absorption at a very low dose of calcium (10 mg), unlike other studies in which both active and passive calcium absorption were measured (6, 27). Extrapolation to a high calcium intake of 600 mg Ca would suggest that 180 mg Ca would be absorbed by passive means with no increment in active absorption. These fractional absorption data are supported by other studies conducted with high calcium doses (34, 35). Thus, assuming that calcium absorption does deteriorate with age, if high-calcium meals are ingested, the vitamin D status of the individual would have little effect on calcium absorption. If, however, low-calcium meals are consumed, as is common in the elderly, vitamin D status becomes critical. Under these circumstances, and if there is a deficiency of calcitriol, there may well be net calcium loss from the bowel (33).

There was no association of 25(OH)D or calcitriol concentrations with age in the present study, even though we (36) and others (2, 5, 32, 37, 38) showed such an association previously in a larger age range. Elderly individuals with a suboptimal concentration of 25(OH)D are reported to have secondary hyperparathyroidism as a result of negative calcium balance (39, 40). This finding, however, was not supported in the present study, especially because only a small percentage of subjects had suboptimal circulating 25(OH)D concentrations. A rise in PTH is thought to be predictive of increased bone turnover and bone loss so that calcium balance is maintained. In this study of bone turnover markers, we were unable to show a relation between 25(OH)D and these direct markers of bone function. This, however, does not preclude a possible relation between calcium intake and 25(OH)D concentrations and small differences in bone turnover that could be measured with use of bone histomorphometric techniques but that are not reflected in bone turnover markers. Calcitriol was not related to PTH, suggesting that these subjects were in calcium balance and did not have secondary hyperparathyroidism. This was reflected in their mean calcium intake of 906 ± 319 mg/d, which may have been sufficient to suppress the stimulatory mechanisms by which PTH increases calcitriol production. Indeed, the mean intake of this older population was higher than previously reported by us (827 ± 316 mg/d) (41) and nationally (686 mg/d) (42).

Many factors are known to influence individual 25(OH)D concentrations and consequently gut calcium absorption (31, 37, 38, 40, 43–47). More recently, genetic and environmental factors have been investigated that may directly influence calcium absorption. Vitamin D receptor genotype was reported to be associated with differences in absorptive capabilities in young and older women by some investigators (28, 48–50), a finding that has been refuted by others (51–53). The interaction between genotype and environment and its effect on gut calcium absorption has also been investigated (12, 13, 44, 54). On the basis of these analyses, some have reported associations between gut calcium absorption and bone mineral density (12) and increased rates of fracture (13). Other dietary components and lifestyle factors have been shown to play an important role in calcium absorption via other mechanisms. Recent findings suggest that low-fat, high-fiber diets reduce calcium absorption as a result of increased gut transit time (53). This evidence parallels previous suggestions by Barger-Lux et al (27) that the

variation in gut motility may have as much effect on calcium absorption as "systemic control factors." A negative effect of alcohol intake was also found to be associated with calcium absorption, even when the intake is modest (53). Yet, others have shown that phosphorus and protein intake are not related to calcium absorption (55). In a study of young men, levels of physical activity were related to calcium absorption (56). Consequently, many factors need to be considered when examining the determinants of calcium absorption.

Although it is generally assumed that calcitriol is the principal regulator of calcium absorption, only a small fraction of the variance in calcium absorption has been explained by calcitriol in many of the studies reported to date (5, 28, 31). Because of the significant relation between 25(OH)D and calcium absorption found by us and others (26, 27) with use of both active and total calcium absorption tests, it is evident that 25(OH)D is more biologically active in gut calcium absorption than is calcitriol. Despite the strong relation between 25(OH)D and gut calcium absorption at low calcium loads, this relation had little effect on calcium balance in the present study because there was no relation with other aspects of bone turnover or calcium metabolism.

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