Calcium Absorption on High and Low Calcium Intakes in Relation to Vitamin D Receptor Genotype*

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ABSTRACT

The finding that the link between polymorphism at the vitamin D receptor (VDR) gene and rates of bone loss from the femoral neck in postmenopausal women is enhanced at low calcium intakes suggests that intestinal calcium absorption is a site of differential action of the VDR alleles. 1,25-Dihydroxyvitamin D [1,25-(OH)\(_2\)D] and its receptor mediate active calcium transport, the major mechanism of calcium absorption at low calcium intakes. We compared fractional calcium absorption in healthy late postmenopausal women with (bb) and without (BB) the BSM-1 restriction site. In 60 women (26 BB and 34 bb), we measured calcium absorption and plasma 1,25-(OH)\(_2\)D after 2 weeks on a high (1500 mg/day) and 2 weeks on a low (<300 mg/day) calcium intake. The mean 45Ca absorption indexes were similar in the two groups on the high calcium intake [19.01 ± 1.12% (±SEM)/L in BB and 20.45 ± 0.97%/L in bb; P = 0.346] and differed significantly on the low calcium intake (20.57 ± 1.10%/L vs. 23.66 ± 0.95%/L; P = 0.044). Calcium restriction induced similar percent increases in plasma 1,25-(OH)\(_2\)D, but the BB group had a smaller increase in the fractional 45Ca absorption index [7.8 ± 3.8% (±SEM) vs. 20.7 ± 3.3% in bb; P = 0.016; increments adjusted for initial absorption value].

In conclusion, compared to women with the bb variants, women with BB allelic variants of the VDR have reduced calcium absorption efficiency on low calcium intake, consistent with a functional defect in the intestinal VDR. The impact of this heritable difference is reduced at higher calcium intakes. (J Clin Endocrinol Metab 80: 3657–3661, 1995)

Subjects and Methods

**Subjects**

Healthy postmenopausal women were enrolled and completed the 4-week study. Of 51 women previously determined to have the BB genotype, 26 were eligible and willing to participate. Of 106 women with the bb genotype, 34 were randomly selected to bring the total sample size to 60. Many of these women had previously participated in calcium and vitamin D supplement trials (22, 23) and in a retrospective study of VDR genotype and rates of bone loss (17).

Exclusion criteria for the study were hepatic or renal disease, malnutrition, diabetes mellitus, or a history of cancer (except nonmelanoma skin cancer). Subjects were also excluded if they had a chronic gastrointestinal or respiratory disease, were taking medications with the potential to interfere with calcium metabolism, were pregnant or nursing, or were using any form of hormone replacement therapy. The study was approved by the institutional review board at Tufts University. Each subject gave written informed consent. The study was supported by the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University (Contract 53–3K06–5-10) and Sequana Therapeutics (La Jolla, CA). The contents of this publication do not necessarily reflect the views or policies of the USDA, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.
absorption, hyperparathyroidism, malignancy in the past 5 yr, and use in the past 3 months of estrogen, glucocorticoids, or other drugs known to influence calcium metabolism. The women agreed to take no calcium or vitamin D supplements except those provided during the study. The protocol was approved by the radiation safety and human investigation review committee at Tufts University, and written informed consent was obtained from each subject.

Study design

Volunteers were counseled to avoid all dairy products and calcium-fortified foods throughout the 4-week study. Counselling took place before and at the midpoint of the study. For the first 2 weeks of the study, the women took 1200 mg/day calcium as the citrate (400 mg at breakfast, dinner, and bedtime). To reduce variation in vitamin D intake, each woman took a multivitamin containing 400 IU vitamin D daily throughout the study. The volunteers, staff who had contact with them, and staff who made calcium absorption and other measurements were blinded to genotype group throughout the study.

Measurements were made after 2 weeks (the high calcium intake period) and after 4 weeks (the low calcium intake period). On both occasions, subjects came to the Metabolic Research Unit at 0800 h after an 8-h fast. Each brought a 24-h urine collection. A blood sample was drawn by venipuncture at 0815 h. At 0830 h, subjects ingested the 45Ca tracer (for method, see below). Blood was analyzed for calcium (total), creatinine, phosphorus, PTH, 25-hydroxyvitamin D, and 1,25-(OH)2D, and urine was analyzed for calcium and creatinine.

Intake assessment

Self-selected intake of calcium and other nutrients was estimated at enrollment with use of the Fred Hutchinson Cancer Research Center Food Frequency Questionnaire (version 06.10.88, 1988, Fred Hutchinson Cancer Research Center, Seattle, WA). On the final visit, the questionnaire was readministered to evaluate calcium intake during the study.

Laboratory assays

Plasma 25-hydroxyvitamin D was measured by the method of Preece et al. (24), with intra- and interassay coefficients of variation of 5.0% and 7.3%, respectively. Plasma 1,25-(OH)2D was measured by the competitive protein binding method of Reinhardt et al. (25), with intra- and interassay coefficients of variation of 4.9% and 7.7%, respectively. Serum intact PTH was measured with Allegro immunoradiometric assay kits from Nichols Institute (San Juan Capistrano, CA), with intra- and interassay coefficients of variation of 5.6% and 6.6%, respectively. Serum calcium was measured with a Nova 7 analyzer (Nova Biomedical, Waltham, MA). Serum phosphorus and serum and urinary creatinine levels were assayed by colorimetry with a Cobas Fara centrifugal analyzer (Roche Instruments, Belleville, NJ). Urinary calcium was measured by direct current plasma emission spectroscopy with a Spectroscan 6 (Beckman Instruments, Palo Alto, CA). All samples for individual subjects were measured in a single assay.

Densitometry

Lumbar spine (L2-L4) and femoral neck BMD were measured with a model DPX dual energy x-ray absorptiometer (Lunar Radiation Corp., Madison, WI), with coefficients of variation in our laboratory of 1% and 2%, respectively.

Calcium absorption

We estimated fractional 45Ca absorption from the appearance of 45Ca in blood after the ingestion of 100 mL of an aqueous solution containing 3 μCi 45Ca and 100 mg cold calcium as the chloride (26). This was followed by ingestion of 300-mL deionized water rinses. Before ingestion, duplicate 60-μL standards were removed from each test dose for counting. Exactly 3 h after ingestion of the tracer, 15 mL blood were drawn. Two 2-mL aliquots of serum and the standards were each added to 18 mL scintillation fluid, and β-emissions were counted in a scintillation counter (model LS3801, Beckman Instruments, Fullerton, CA).

Counts were corrected for quenching. In 25 women, blood was drawn immediately before the second tracing and counted as described above to evaluate residual counts from the first tracer dose. Residual background counts represented a mean of 8.9% of the absorbed counts on the second measurement. Regression analysis revealed a positive linear association between background counts and the count rate in blood on the first measurement. All of the second absorption measurements, therefore, were corrected for residual background counts with use of the linear regression equation. Based on evidence compiled by Manery (27) and used by others (28), the fraction of 45Ca counts per L serum was corrected for appropriate pool size (fraction of dose in extracellular fluid) by multiplying by 15% of the body weight (in kilograms). The coefficient of variation of the method in our laboratory in 12 subjects measured twice each, 5 days apart, is 9.1%. Thus, the outcome variable in this study, the fractional 45Ca absorption index, is the fraction of the 45Ca counts ingested and multiplied by 15% of the body weight.

45Ca was purchased from Amersham Corp. (Chicago, IL), and a spectral analysis was carried out on each batch before its use to ensure purity. Total radiation exposure from the study was 150 mrem to bone, the critical organ, and 15 mrem to the whole body.

VDR genotyping

DNA was extracted from peripheral leukocytes and amplified using the polymerase chain reaction method (17). The enzyme BsmI endonuclease was used to define the VDR-gene allelic polymorphisms, with BB representing the absence and bb the presence of the restriction site on both alleles.

Statistical methods

Baseline characteristics and laboratory values of the genotype groups were compared with standard two-sample t tests and χ2 tests. Within each genotype, paired t tests were used to compare laboratory values during the high and low calcium periods. Despite uniform intervals between each individual’s first and second absorption tests (2 weeks) and the short duration of the overall absorption measurement period (17 weeks), there was a small time-dependent decline in changes in calcium absorption fraction over the study period. This decline was linear and similar in the two genotype groups. As a χ2 test of measurement month by VDR group was borderline significant (P = 0.089), we adjusted for time of measurement in all analysis of covariance models in which fractional absorption indexes and their changes were compared across VDR groups. All statistical tests were conducted at the 0.05 level, and except for the χ2 test, all were two-tailed. Analyses were performed in SPSS (29) and SAS (30).

Results

Women in the BB and bb groups were similar in age and other clinical characteristics (Table 1). The femoral neck BMD was 5% lower in the BB than in the bb group, a difference that was not statistically significant. During the study, mean dietary calcium intake was 248.3 ± 57.0 (±sd) mg/day in the BB and 283.4 ± 137.9 mg in the bb group (P = 0.187). Plasma 25-hydroxyvitamin D levels were similar in the two groups during the study, i.e. at the midpoint [76.4 ± 18.4 (±sd) nmol/L vs. 75.2 ± 23.3 nmol/L]. On the high calcium intake, the fractional 45Ca absorption index was similar in the two groups [19.01 ± 1.12% (±sem)/L in BB and 20.45 ± 0.97%/L in bb]. After calcium restriction (i.e. discontinuation of calcium supplements), both groups increased their fractional calcium absorption, but the increase in the BB group was less than that in the bb group. The mean unit changes were 1.37 ± 0.69%/L in BB and 3.35 ± 0.60%/L in bb (P = 0.041). The mean percent changes were also lower in the BB group (7.8
TABLE 1. Clinical characteristics at enrollment

<table>
<thead>
<tr>
<th>Measure</th>
<th>Genotype</th>
<th>Significance of difference ($P$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>26</td>
<td>34</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>67.1 ± 4.7</td>
<td>68.2 ± 6.0</td>
</tr>
<tr>
<td>Time since menopause (yr)*</td>
<td>19.6 ± 6.8</td>
<td>19.6 ± 7.4</td>
</tr>
<tr>
<td>Ht (cm)</td>
<td>160.6 ± 6.1</td>
<td>160.8 ± 7.2</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>68.5 ± 11.2</td>
<td>70.9 ± 14.3</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>709.7 ± 545.5</td>
<td>594.7 ± 253.4</td>
</tr>
<tr>
<td>Vitamin D intake (IU/day)</td>
<td>215.3 ± 166.0</td>
<td>175.0 ± 85.0</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>11.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Baseline BMD (g/cm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral neck</td>
<td>0.80 ± 0.11</td>
<td>0.84 ± 0.11</td>
</tr>
<tr>
<td>Spine (L2-L4)</td>
<td>1.02 ± 0.15</td>
<td>1.05 ± 0.16</td>
</tr>
</tbody>
</table>

Values are the mean ± SD.
* Age of menopause was assigned as 50 yr in the three BB and three bb women for whom exact age at menopause was unknown.

On the low calcium intake, the $^{45}$Ca absorption index was significantly lower in the BB than in the bb group (20.57 ± 1.0%/L vs. 23.66 ± 0.95%/L; $P = 0.044$). Intakes of phosphorus, magnesium, protein, and energy were similar in the two groups. Adjustment for a group difference in fiber intake [14.4 ± 4.5 (±sd) g/day in BB and 11.8 ± 4.0 g in bb; $P = 0.02$] did not alter any of these results.

On high and low calcium intakes, circulating levels of 1,25-(OH)$_2$D, calcium, and PTH and 24-h urine calcium excretion (as the calcium/creatinine ratio) did not differ significantly in the two groups (Table 2). With calcium restriction, plasma 1,25-(OH)$_2$D increases, adjusted for initial values, were similar in the two groups (Fig. 1). The diet change also induced similar increases in PTH and decreases in serum calcium and in the urinary calcium/creatinine ratio in the BB and bb groups (Table 2).

In response to calcium restriction, increments in 1,25-(OH)$_2$D and the $^{45}$Ca absorption index were significantly correlated in the bb group ($r = 0.41; P = 0.016$), but not in the BB group ($r = 0.23; P = 0.269$). Correlations between 1,25-(OH)$_2$D and the $^{45}$Ca absorption index on high and low calcium diets were not significant in either genotype group.

![Graph](image)

**Fig. 1.** Changes in fractional $^{45}$Ca absorption and circulating 1,25-(OH)$_2$D after calcium restriction, adjusted for differences in initial (high calcium intake) values and expressed as a percentage of the initial values. The star indicates a significant difference between the genotypes ($P = 0.016$).

**Discussion**

This study links calcium absorption to heredity with the finding that women with the BB alleles absorb calcium less efficiently than those with the bb alleles under selected conditions. The BB homozygotes appear to have less efficient active calcium transport because their defect is exposed at a low calcium intake, the setting in which active transport is enhanced by higher circulating levels of 1,25-(OH)$_2$D, but not at high calcium intakes, at which absorption by 1,25-(OH)$_2$D-independent passive diffusion plays an increasing role (21). The observed genotype difference in absorption performance despite similar levels of 1,25-(OH)$_2$D points to the VDR as the element that is functionally different between the bb and BB alleles. Other studies of VDR genotype and calcium absorption are not yet available for comparison. Our findings may help explain the earlier observation that intersubject variability in calcium absorption is greater at low than at high calcium intakes (31). Although the differences were not statistically significant, the BB group had somewhat higher PTH and lower urinary calcium excretion levels than the bb group on both diets.

The calcium intake-dependent relationship between VDR genotype and calcium absorption that we observed in this study is consistent with our earlier finding that genotype differences in femoral neck bone loss depend on calcium intake level (17). Accelerated bone loss occurred in the BB women who had a low mean calcium intake (394 mg/day) but not in those who were supplemented and had an intake of 900 mg/day. In a longitudinal study in elderly men and women, Ferrari et al. (16) found that spinal loss was inversely correlated with calcium intake in the heterozygotes ($n = 37$), but not in the BB homozygotes ($n = 9$), perhaps because of limitations related to sample size. Estimates of calcium intake have not been reported in the cross-sectional studies of genotype and BMD (9–15). Population differences in past and current calcium intakes may account for some of the different results reported. In our study, femoral neck BMD was 5% lower in the BB than in the bb group, a difference that was not statistically significant, perhaps because of the sample size.
TABLE 2. Laboratory values in 26 BB and 34 bb women after 2 weeks on a high calcium intake and after 2 weeks on a low calcium intake

<table>
<thead>
<tr>
<th>Measure</th>
<th>BB genotype (pmol/L)</th>
<th>bb genotype (pmol/L)</th>
<th>Difference (CI95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (\text{1,25(OH)}_2\text{D})</td>
<td>78.92 ± 3.16</td>
<td>88.80 ± 3.04</td>
<td>-9.88 ± 1.85^a</td>
</tr>
<tr>
<td>Serum PTH (ng/L)</td>
<td>36.62 ± 2.55</td>
<td>46.38 ± 3.49</td>
<td>-9.77 ± 2.05^a</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>2.28 ± 0.02</td>
<td>2.23 ± 0.01</td>
<td>-0.05 ± 0.09</td>
</tr>
<tr>
<td>Serum phosphorus (mmol/L)</td>
<td>1.13 ± 0.03</td>
<td>1.10 ± 0.02</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>24-h urinary calcium (mmol/day)</td>
<td>4.15 ± 0.42</td>
<td>2.56 ± 0.26</td>
<td>1.59 ± 0.27^a</td>
</tr>
<tr>
<td>24 h urinary calcium/creatinine (mmol/mol)</td>
<td>533.8 ± 61.3</td>
<td>323.6 ± 36.0</td>
<td>210.2 ± 35.1^a</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. CI95, Ninety-five percent confidence interval.

References


Acknowledgments

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increased prevalence of the BB vitamin D receptor genotype in severe osteoporosis [Abstract]. J Bone Miner Res. 9(Suppl 1):111.