Relations between calcium intake, calcitriol, polymorphisms of the vitamin D receptor gene, and calcium absorption in premenopausal women\textsuperscript{1,2}

Judith M Wishart, Michael Horowitz, Allan G Need, Franca Scopacasa, Howard A Morris, Peter M Clifton, and BE Christopher Nordin

ABSTRACT  The relations between calcium absorption, dietary calcium intake, 1,25-dihydroxyvitamin D\textsubscript{3} (calcitriol), and vitamin D receptor (VDR) gene polymorphisms were evaluated in 99 healthy women who were approaching menopause (mean age: 47 y, range: 43–53 y). Dietary calcium was assessed by food-frequency questionnaire and calcium absorption was measured by a single-isotope radiocalcium test. VDR alleles were classified according to the presence (b, t, a) or absence (B, T, A) of the BsmI, TaqI, and Apal restriction enzyme cutting sites. Radiocalcium absorption was positively related to serum calcitriol (r = 0.23, \(P < 0.05\)) and inversely related to dietary calcium intake (r = −0.26, \(P < 0.01\)). There was, however, no significant relation (r = 0.10) between serum calcitriol concentrations and dietary calcium. Radiocalcium absorption was higher in the \(bb\) genotype (P < 0.05) and the \(au\) genotype (P < 0.05), polymorphisms said to be associated with a higher bone density. We conclude that serum calcitriol and dietary calcium are independent determinants of calcium absorption in premenopausal women and that VDR gene polymorphisms influence calcium absorption. \textit{Am J Clin Nutr} 1997:65:798–802.

KEY WORDS  Calcium absorption, vitamin D receptor alleles, calcium intake, calcitriol, 1,25-dihydroxyvitamin D, women, menopause

INTRODUCTION

Calcium balance is critically important to the maintenance of bone mass and prevention of bone loss. A major determinant of calcium balance is intestinal calcium absorption and \(\approx 50\%\) of postmenopausal women with osteoporosis have malabsorption of calcium (1). There is also a strong genetic component to bone density (2–7). Recent studies have suggested that polymorphisms of the vitamin D receptor (VDR) gene may account for a substantial proportion of the heritable component of bone density (8–12), although this issue remains highly controversial (13–16).

Calcium absorption can be measured in different ways (17–21). Balance studies are probably the gold standard but are of limited practical usefulness because they are expensive, cumbersome, and can be imprecise (20). Calcium isotopes are now used. We originally described a six-blood-sample, single-isotope procedure (21), but subsequently showed that results obtained from a single blood sample taken 60 min after ingestion of radiocalcium correlated closely with those obtained from balance studies and were almost as accurate (17). Calcium is absorbed in the small intestine by both an active, vitamin D–dependent transport mechanism, particularly in the duodenum, and a passive diffusion component (22, 23). At dietary calcium intakes < 1000 mg/d, the majority of calcium absorption occurs by active transport (> 80\% at calcium intakes < 400 mg/d) (24, 25) and, accordingly, an inverse relation between dietary calcium and calcium absorption is to be expected (26–29). The rate of intestinal calcium absorption is also influenced by serum concentrations of 1,25-dihydroxyvitamin D\textsubscript{3} (calcitriol), as shown in healthy postmenopausal women (30).

Calcium absorption efficiency falls with age (26, 31), and this may be a result of a fall in calcitriol concentration due to renal dysfunction (32). In osteoporotic postmenopausal women malabsorption of calcium may reflect reduced gastrointestinal sensitivity to calcitriol (30, 33) and, possibly, a fall in serum calcitriol itself (31). It has been suggested that calcium absorption falls at menopause, but there is little information about the determinants of calcium absorption in women who are approaching menopause (26). A study of a small group of premenopausal women failed to show any relation between calcitriol or dietary calcium and calcium absorption (34). This may, however, be because the test used to measure calcium absorption was not particularly sensitive to the active transport component because a relatively high calcium load (200 mg) was used (24).

Previous studies of the relation between bone density and polymorphisms of the VDR gene reported an association with the VDR genotypes \(BsmI\) (B, b), \(ApaI\) (A, a), and \(TaqI\) (T, t) (12). Bone density has been reported in some (12), but not all (16) studies to be higher in subjects with the \(bb\) haplotype than in those with the \(BB\) haplotype. The mechanism by

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which polymorphisms in the VDR gene exert their effect on bone is unclear, but theoretically this could be via an effect on calcium absorption. There is a lack of consensus as to whether there is any relationship between VDR genotype and calcium absorption. Dawson-Hughes et al. (34) reported that calcium absorption was less with a low calcium intake in postmenopausal women with the BB genotype than in those with the bb genotype. Hayes et al. (35) found that radiocalcium absorption was lower in a group of nonhysterectomized postmenopausal women homozygous for the TaqI endonuclease site (TT) than in those without (TT). However, it has been reported that there is no association between calcium absorption status and VDR genotype (15).

We evaluated the relation between calcium absorption, dietary calcium intake, serum calcitriol, and VDR genotypes (BsmI, Apal, and TaqI polymorphisms) in a group of normal older premenopausal women.

**SUBJECTS AND METHODS**

The series comprised 99 normal premenopausal women aged (x ± SE) 47 ± 0.2 y (range: 43–53 y), weighing 69 ± 1.3 kg, and having a body mass index (in kg/m²) of 25.6 ± 0.56, who were recruited by advertisement. None had any illness or was taking medication known to affect calcium metabolism. All had regular menses and serum follicle stimulating hormone concentrations were in the premenopausal range (≤ 20 U/L). The study was approved by the Research Ethics Committees of the Royal Adelaide Hospital and the Commonwealth Scientific and Industrial Research Organisation (CSIRO), South Australian Division of Human Nutrition.

All subjects attended our laboratory for venesecation at 0900 after an overnight fast from 2100. 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 (21). 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 was calculated according to the method of Marshall and Nordin (17).

Dietary calcium and phosphorus intakes were evaluated with a quantitative food-frequency questionnaire developed by the CSIRO, South Australian Division of Human Nutrition (36). Serum calcitriol was measured by HPLC and radioimmunoassay (37). The restriction fragment length polymorphisms for the VDR gene at the BsmI, TaqI, and Apal sites were measured with the polymerase chain reaction through use of the method and primers described by Morrison et al. (9).

**Statistical analysis**

Data were evaluated by using single- and multiple-linear regression and analysis of variance and covariance, and are shown as means ± SEMs. A P value < 0.05 was considered significant. All analyses were performed with MINITAB (release 9 for Windows; State College, PA).

**RESULTS**

The mean fractional radiocalcium absorption rate (α) was 0.79 ± 0.03 h⁻¹, (the interindividual CV was 38%), the serum calcitriol concentration was 122 ± 3.8 pmol/L, dietary calcium intake was 1.153 ± 0.039 g/d, and dietary phosphorus intake was 1.514 ± 0.042 g/d. There was a positive relation between α and serum calcitriol (Figure 1) with a correlation coefficient of 0.23 (P < 0.05). Serum calcitriol was not significantly related to calcium intake (r = 0.10) but was inversely related to body weight (r = −0.25, P < 0.05). There was no significant relation between radiocalcium absorption and phosphorus intake (r = −0.17). Multiple regression analysis showed a close correlation between dietary calcium and phosphorus (r = 0.84, P < 0.001). As would be predicted from the calculation, α was related to body weight (r = 0.22, P < 0.05) (21). When α was regressed on serum calcitriol, calcium intake, and body weight simultaneously, it was significantly related to all variables as follows:

\[
\alpha = (0.002 \times \text{calcitriol}) - (0.0002 \times \text{dietary calcium}) + (0.007 \times \text{body weight}) + 0.281
\]

![Figure 1](https://academic.oup.com/ajcn/article-abstract/65/3/798/4655301)  
**FIGURE 1.** Relation between radiocalcium absorption and serum calcitriol (r = 0.23, P < 0.05).
where the $P$ values for the first three terms in the equation are 0.0006, 0.001, and 0.001; and $R^2 = 21.9\%$. The relation between the radiocalcium absorption and dietary calcium was $r = -0.26$ ($P < 0.01$) (Figure 2).

Genotype frequencies for $BSMI$, $TAQI$, and $APAI$ polymorphisms are shown in Table 1. There was a high degree of association between the genotypes, i.e., all $tt$ cases were found with $BBAA$; 72% of $TT$ cases were $bb$ and $aa$ homozygotes. One-way analysis of variance did not show any significant differences between the mean values of calcitriol, dietary calcium, or $\alpha$ in the VDR genotypes individually, or the haplotypes $BBAA\text{tt}$ and $bbaaTT$. After radiocalcium absorption was corrected for dietary calcium intake and serum calcitriol and body weight by covariance analysis, the genotype $aa$ (Table 1) and the haplotype $bbaaTT$ (Table 2) were associated with higher calcium absorption ($P < 0.05$ for both), i.e., the genotype generally associated with the highest bone mass had the highest calcium absorption.

**DISCUSSION**

However calcium absorption is measured, it is clear that a wide range of values is encountered in normal subjects and this is equally true in our group of premenopausal subjects. Although the absorption rate was significantly related to the serum calcitriol concentration, the latter only accounted for $\approx 5\%$ of the variance. A part of the residual variance was accounted for by dietary calcium intake and inclusion of both of these individual variables accounted for $\approx 13\%$ of the variance of radiocalcium absorption.

Assessment of dietary calcium intake is relatively imprecise (38) but the finding of an inverse correlation between dietary calcium intake and $\alpha$ in premenopausal women is not unexpected (25, 39). It has, however, generally been assumed that this relation was mediated through the serum concentration of calcitriol. In our data set this is not the case; we did not find the expected inverse correlation between dietary calcium and serum calcitriol—the trend was the reverse though not significant. It is therefore probable that the inverse relation between dietary calcium and $\alpha$ is operating at the receptor level. Perhaps high calcium intake reduces either small intestinal receptor density or the sensitivity to calcitriol. The association between serum calcitriol concentrations and body weight is an unexpected finding and is not accounted for by dietary calcium intake. In some studies, calcium absorption has been shown to relate to plasma concentrations of 25-hydroxyvitamin D (39). However, this almost certainly reflects subclinical 25-hydroxyvitamin D deficiency due to nutritional inadequacies and lack of sunlight exposure (39). We showed that 25-hydroxyvitamin D deficiency is nonexistent in the population we studied (40).

We sought to account for some of the residual variance of calcium absorption by the VDR genotype, anticipating that the genotypes reportedly associated with the highest bone densities ($bb$, $aa$, $TT$) would be associated with higher rates of calcium absorption. Mean radiocalcium absorption was marginally, but significantly, higher in subjects with genotypes generally associated with high bone density. This observation suggests that

<table>
<thead>
<tr>
<th>VDR genotype</th>
<th>Radiocalcium absorption fraction/h</th>
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<tbody>
<tr>
<td>$BB$ ($n = 18$)</td>
<td>0.83 ± 0.06</td>
</tr>
<tr>
<td>$Bb$ ($n = 41$)</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td>$bb$ ($n = 40$)</td>
<td>0.83 ± 0.04</td>
</tr>
<tr>
<td>$AA$ ($n = 20$)</td>
<td>0.79 ± 0.06</td>
</tr>
<tr>
<td>$Aa$ ($n = 50$)</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td>$aa$ ($n = 29$)</td>
<td>0.89 ± 0.05</td>
</tr>
<tr>
<td>$tt$ ($n = 15$)</td>
<td>0.80 ± 0.07</td>
</tr>
<tr>
<td>$Tt$ ($n = 44$)</td>
<td>0.75 ± 0.04</td>
</tr>
<tr>
<td>$TT$ ($n = 40$)</td>
<td>0.83 ± 0.04</td>
</tr>
</tbody>
</table>

1 Least square means ± SE. $B$, $b$: $BSMI$; $A$, $a$: $APAI$; $T$, $t$: $TAQI$.
2 Significantly different from other genotypes. $P < 0.05$ (one-way ANOVA).

**FIGURE 2.** Relation between radiocalcium absorption and dietary calcium intake ($r = -0.26$, $P < 0.01$).
TABLE 2
Mean radiocalcium absorption by vitamin D receptor (VDR) haplotype after correction for dietary calcium and serum calcitriol and body weight by covariance analysis.

<table>
<thead>
<tr>
<th>VDR haplotype</th>
<th>Radiocalcium absorption</th>
</tr>
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<tbody>
<tr>
<td>BBAAtt (n = 15)</td>
<td>fraction/h 0.79 ± 0.07</td>
</tr>
<tr>
<td>bbattT (n = 29)</td>
<td>0.89 ± 0.05</td>
</tr>
<tr>
<td>Other haplotypes (n = 55)</td>
<td>0.74 ± 0.03</td>
</tr>
</tbody>
</table>

1 Least square mean ± SE. B: BSMI; A: a; APAI; T: r; TAQI.
2 Significantly different from all other haplotypes. P < 0.05 (one-way ANOVA).

VDR polymorphisms are likely to influence calcium absorption. In more recent data (published as yet only in abstract form) some workers have failed to find any association with genotype and calcium absorption (15, 41). However, another study found that the magnitude of the increase in calcium absorption in response to calcium restriction was less in subjects with the BB genotype than in those with bb genotype, although the increase in serum calcitriol was similar in both groups (34). It has also been reported, in a cohort of postmenopausal women, that there is a significant relation between α and VDR genotypes at the TaqI endonuclease site in those women whose age at menopause was known, but not in the group as a whole (35). Our findings, therefore, together with the inconsistent observations of others, suggest that VDR genotypes have a minor, albeit significant, role in calcium absorption.

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REFERENCES