Caffeine intake increases the rate of bone loss in elderly women and interacts with vitamin D receptor genotypes

Prema B Rapuri, J Christopher Gallagher, H Karimi Kinyamu, and Kay L Ryschon

ABSTRACT

Background: The role of caffeine as a risk factor for bone loss is controversial.

Objective: Our goals were 1) to compare in both a cross-sectional study and a 3-y longitudinal study the bone mineral density (BMD) of postmenopausal women consuming high or low amounts of caffeine and 2) to study the interaction between caffeine intake, vitamin D receptor (VDR) polymorphism, and BMD in the longitudinal study.

Design: The results are derived from cross-sectional measurements of BMD in 489 elderly women (aged 65–77 y) and from longitudinal measurements made in 96 of these women who were treated with a placebo for 3 y. Changes in BMD were adjusted for confounding factors and were compared between groups with either low (≤300 mg/d) or high (>300 mg/d) caffeine intakes and between the VDR genotype subgroups of the low- and high-caffeine groups.

Results: Women with high caffeine intakes had significantly higher rates of bone loss at the spine than did those with low intakes (−1.90 ± 0.97% compared with 1.19 ± 1.08%; P = 0.038). When the data were analyzed according to VDR genotype and caffeine intake, women with the tt genotype had significantly (P = 0.054) higher rates of bone loss at the spine (−8.14 ± 2.62%) than did women with the TT genotype (−0.34 ± 1.42%) when their caffeine intake was >300 mg/d.

Conclusions: Intakes of caffeine in amounts >300 mg/d (=514 g, or 18 oz, brewed coffee) accelerate bone loss at the spine in elderly postmenopausal women. Furthermore, women with the tt genetic variant of VDR appear to be at a greater risk for this deleterious effect of caffeine on bone. 

Key Words Caffeine, bone loss, vitamin D receptor genotype, bone mineral density, elderly women

INTRODUCTION

Osteoporosis is a multifactorial disease with a major socioeconomic impact. Nutrition, lifestyle, and genetics contribute to the pathogenesis of osteoporosis. Caffeine is consumed regularly by most of the US population either in beverages, the diet, or medications. According to the US Department of Agriculture (1) and the National Coffee Association, each cup (172 g, or 6 oz) of brewed coffee contains ≈103 mg caffeine. Studies of caffeine as a probable risk factor for osteoporosis have yielded conflicting results. Caffeine consumption has been reported to decrease bone mineral density (BMD) (2–4), increase the risk of hip fracture (5–8), and negatively influence calcium retention (9–11). However, most of the studies reported no overall association between caffeine intake and BMD, fracture rate, or calcium metabolism (12–21). In 1994, Morrison et al (22) first reported an association between vitamin D receptor gene (VDR) polymorphism and BMD of the spine and hip in adults. After this initial report, the relation between VDR polymorphism and BMD, bone turnover, and bone loss has been extensively evaluated. The results of some studies support an association between VDR polymorphism and BMD (23–25), whereas other studies showed no evidence for this association (26, 27). To some extent, the conflicting data may reflect the interaction of the VDR alleles with environmental factors. One potential environmental factor in relation to bone and calcium homeostasis is calcium intake. Rubin et al (28) reported an interaction between calcium intake and VDR genotypes in determining BMD in young white women. In older subjects with low calcium intakes, significant differences in fractional calcium absorption and changes in BMD were associated with VDR genotype (29–32). Ferrari et al (33) also reported in a prospective study that the BMD response to calcium supplementation varied according to VDR genotype. The aims of the present study were 1) to study the association between caffeine intake and BMD in postmenopausal elderly women both cross-sectionally and longitudinally, with control for confounding factors, and 2) to assess the influence of a less-investigated dietary factor, caffeine intake, on the association between VDR genotype and the rate of bone loss in the same population.

1 From the Bone Metabolism Unit, Creighton University, School of Medicine, Omaha; the National Institute of Environmental Health Sciences, Laboratory of Reproductive and Developmental Toxicology, Research Triangle Park, NC; and Ryschon Health and Technology Services, Valentine, NE.


3 Supported by research grants UO1-AG10373 and RO1-AG10358 from the National Institutes of Health.

4 Address reprint requests to PB Rapuri, Bone Metabolism Unit, Creighton University, School of Medicine, 601 North 30th Street, Room 6718, Omaha, NE 68131. E-mail: thiyyari@creighton.edu.

Received December 21, 2000.
Accepted for publication March 28, 2001.
SUBJECTS AND METHODS

Subjects

A total of 489 elderly women aged 66–77 y participated in a double-blind randomized clinical trial (STOP IT: Sites Testing Osteoporosis Prevention or Intervention) that was intended to test the efficacy of 3 therapies in reversing bone loss in the proximal femur and spine compared with placebo: an estrogen-progestin combination [0.625 mg Premarin (Wyeth Ayerst Laboratories, Philadelphia) + 2.5 mg Provera (Pharmacia Corporation, Peapack, NJ)], a vitamin D analogue [1,25-dihydroxyvitamin D3, (Rocaltril; Hoffmann-La Roche Inc, Nutley, NJ)], and a combination of both the vitamin D analogue and estrogen-progestin. The subjects were recruited through advertisements in local newspapers or through mass mailing of letters inviting them to participate in a 3-y study. All subjects signed an informed consent form before participating in the study and were free-living, in good health, and ambulatory. Additional inclusion criteria were normal liver and kidney function. Of the 489 women, 470 were white, 13 were black, 4 were Hispanic, 1 was Asian, and 1 was of mixed race. Women taking medications or those who had diseases known to influence calcium or phosphorus metabolism were not included in the study. The study was approved by the Creighton University Institutional Review Board.

Study design

For the cross-sectional analysis, the baseline data of the 489 women enrolled in the STOP IT study were used. Women excluded from the analysis were those taking thiazide diuretics (n = 43), one subject with suspected Paget disease, and one subject with a doubtful smoking status. Additionally, 7-d food diary data were not available for one woman. Thus, the present analysis was performed with data from the remaining 443 women. The biochemical indexes and BMD measures were compared between groups with low (≤300 mg/d) and high (>300 mg/d) caffeine intakes. The cutoff of 300 mg was chosen on the basis of previously published work (12).

For the longitudinal analysis, the data of 96 women who received the placebo treatment and completed the 3-y study were used to compare the low- and high-caffeine groups. For this analysis, average caffeine intake at baseline and at 3 y was used to divide the women into groups according to caffeine intake, and the percentage change over 3 y (corrected for baseline values) in the biochemical indexes and BMD measures was compared between the low- and high-caffeine groups.

The interaction between VDR genotype and caffeine intake was analyzed by using the longitudinal data. Of the 96 women in the placebo group who completed the study, 4 women belonged to ethnic groups other than white and genotype data were not available for 5 women. Thus, the number of subjects available for the analysis was 87. The percentage of change over 3 y in the biochemical indexes and BMD measures were compared between genotype groups defined by use of the TaqI restriction enzyme in both the low- and high-caffeine groups.

Dietary caffeine intake, smoking, and alcohol history

Dietary intake at baseline and at the end of the study (3 y) was assessed by using 7-d food diaries. A dietitian asked the subjects to complete a 7-d food dairy and nutrient supplement record. Plastic food models (NASCO, Fort Atkinson, WI) were used to help participants better estimate the quantities consumed. Average daily caffeine and calcium intakes were calculated by using the FOOD PROCESSOR II PLUS nutrition and diet analysis system (version 5.1; Esha Research, Salem, OR).

At baseline and at 3 y, participants were provided with a questionnaire to report their smoking and alcohol history, reproductive history, and present use of medications, vitamins, and mineral supplements. Current smokers were classified as smokers, whereas past smokers and women who had never smoked were classified as nonsmokers. Alcohol use was stratified as drinkers and nondrinkers.

Calcitropic hormones and bone markers

Fasting blood samples and 24-h urine collections were obtained from the subjects at baseline, at 6-mo intervals, and at the end of the study (at 3 y). Serum was separated from the blood samples after the blood was allowed to clot and was then centrifuged at 2056 × g for 15 min at 4 °C. The serum samples were stored at −70 °C until analyzed. Serum intact parathyroid hormone was measured with the Allegro immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA). The interassay CV was 5% and the limit of detection was 1 ng/L (1 pg/mL). Serum 25-hydroxyvitamin D was assayed with a competitive protein binding assay (34) after prepurification of serum on Sep-Pak cartridges (Waters Associates, Milford, MA) (35). The limit of detection for this assay was 12.5 nmol/L (5 μg/L) and the interassay CV was 5%. Serum concentrations of osteocalcin were measured by radioimmunoassay (Incstar Corporation, Stillwater, MN). The limit of detection was 0.78 μg/L and the interassay CV was 5%. Urinary collagen cross-links were measured by enzyme-linked immunosorbent assay (Osteomark International, Seattle) as N-telopeptides, a marker for bone type I collagen. The lower limit of detection was 20 nmol bone collagen equivalents, and the interassay CV was 6%. These data are expressed as nmol bone collagen equivalents/mmol creatinine.

Bone mineral density

BMD (in g/cm²) was measured by dual-energy X-ray absorptiometry (model Lunar DPX-L; Lunar Radiation Corp, Madison, WI). BMD at the lumbar spine (L1–L4), the total hip, 2 sites in the proximal femur (femoral neck and trochanter), and the whole-body was calculated by using standardized protocols for uniform subject positioning, scan mode, and scan analysis. The hip and spine scans were performed in duplicate and the mean was used for the analysis. The percentage of change in BMD was calculated as the difference between baseline and follow-up BMD (3-y value), divided by baseline BMD, and multiplied by 100.

VDR restriction fragment length polymorphism

VDR genotypes were identified as TaqI restriction fragment length polymorphisms by polymerase chain reaction (PCR) amplification as described earlier (36). DNA was extracted from white blood cells by phenol-chloroform extraction and stored at −20 °C as described earlier (36). PCR amplification of the DNA sequence flanking the TaqI restriction sites of VDR was performed with the forward oligonucleotide primer 5'-CCAAGACTACAAGTGACACC-3' and the reverse oligonucleotide primer 5'-TGAGGAGGGCTGCTGAGTAC-3'. The PCR product (∼2035 base pairs) was digested with TaqI (New England Biolabs, Beverly, MA) according to the manufacturer's instructions. The digested products were fractionated by gel electrophoresis.
on 2%-agarose gels, and the gels were stained with ethidium bromide, visualized under ultraviolet light, and photographed. The presence of a restriction site for TaqI was genotyped as the \( t \) allele and the absence of the restriction site was genotyped as the \( T \) allele. The absence of the \( TaqI \) site resulted in a single band. The absence of the \( B_{sm}I \) site resulted in a single band.

The presence of a restriction site for \( N_{p}t \) was seen as the \( t \) allele. The absence of the \( N_{p}t \) site resulted in a single band.

The presence of the \( B_{sm}I \) site resulted in a single band.

and high-caffeine groups were compared by using ANCOVA. Fixed effects in this model were baseline smoking, alcohol intake, caffeine intake, and \( VDR \) genotype. Covariates were average calcium intake, respective baseline BMD, and other significant covariates identified by the correlation analysis. The percentage of change in biochemical indexes and BMD over 3 y between the \( VDR \) genotypes within low- and high-caffeine groups was analyzed by using the ANCOVAs determined above for each caffeine group. For each ANCOVA analysis, a full factorial model was first examined. Only significant interactions were included in the final analysis. The residuals of the final models were tested by graphic methods for deviation from normality.

The effects of caffeine intake and \( VDR \) genotypes on BMD and biochemical indexes are summarized by estimated marginal means and their respective SEs. Bonferroni’s post hoc multiple-comparison test was used to determine post hoc significance between the \( VDR \) genotypes.

**RESULTS**

**Characteristics of the study population**

In the cross-sectional study, age, height, and weight did not differ significantly between the low- and high-caffeine groups (Table 1). Total calcium intake (including both dietary and supplemental calcium at baseline) and age at menopause, however, were significantly lower in the high-caffeine group than in the low-caffeine group. In the longitudinal study (Table 2), the baseline age, height, weight, and age at menopause of the low- and high-caffeine groups were not significantly different. Additionally, average total

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Baseline characteristics, biochemical indexes, and bone mineral density (BMD) data of the study population (all ethnic groups) in the cross-sectional study</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Low caffeine:</td>
<td>High caffeine:</td>
<td></td>
</tr>
<tr>
<td>( \leq 300 ) mg/d</td>
<td>&gt; 300 mg/d</td>
<td></td>
</tr>
<tr>
<td>(( n = 265 ))</td>
<td>(( n = 178 ))</td>
<td></td>
</tr>
<tr>
<td>Age (y)(^{2} )</td>
<td>71.6 ± 0.22</td>
<td>71.2 ± 0.26</td>
</tr>
<tr>
<td>Height (cm)(^{2} )</td>
<td>159.3 ± 0.40</td>
<td>158.9 ± 0.49</td>
</tr>
<tr>
<td>Weight (kg)(^{2} )</td>
<td>68.9 ± 0.76</td>
<td>67.1 ± 0.94</td>
</tr>
<tr>
<td>Total calcium intake (mg/d)(^{2} )</td>
<td>777.5 ± 21.1</td>
<td>704.6 ± 19.30(^{1} )</td>
</tr>
<tr>
<td>Age at menopause (y)(^{2} )</td>
<td>48.2 ± 0.4</td>
<td>46.9 ± 0.50(^{1} )</td>
</tr>
<tr>
<td>Serum PTH (ng/L)(^{2} )</td>
<td>35.5 ± 1.19</td>
<td>36.12 ± 1.25</td>
</tr>
<tr>
<td>Serum 25(OH)D (pmol/L)(^{2} )</td>
<td>75.80 ± 2.2</td>
<td>72.5 ± 2.25</td>
</tr>
<tr>
<td>Serum osteocalcin (μg/L)(^{2} )</td>
<td>3.65 ± 0.13</td>
<td>4.01 ± 0.13</td>
</tr>
<tr>
<td>Urine NTx:Cr (nmol BCE/mmol Cr)(^{2} )</td>
<td>52.31 ± 2.86</td>
<td>54.72 ± 2.72</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Spine (g/cm(^{2} ))(^{2} )</td>
<td>0.994 ± 0.014</td>
</tr>
<tr>
<td>Femoral neck (g/cm(^{2} ))(^{2} )</td>
<td>0.763 ± 0.007</td>
</tr>
<tr>
<td>Trochanter (g/cm(^{2} ))(^{2} )</td>
<td>0.686 ± 0.010</td>
</tr>
<tr>
<td>Total body (g/cm(^{2} ))(^{2} )</td>
<td>1.003 ± 0.008</td>
</tr>
<tr>
<td>Total femur (g/cm(^{2} ))(^{2} )</td>
<td>0.813 ± 0.010</td>
</tr>
</tbody>
</table>

\(^{1} \)PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; NTx, N-telopeptide; Cr, creatinine; BCE, bone collagen equivalents.

\(^{2} \)\( \bar{x} \) ± SEM.

\(^{1} \)Significantly different from the low-caffeine group, \( P < 0.05 \) (Student’s \( t \) test).

\(^{1} \)Adjusted \( \bar{x} \) ± SEM.

**TABLE 2**

Baseline characteristics, biochemical indexes, and bone mineral density (BMD) data of the study population (all ethnic groups) in the longitudinal study.

<table>
<thead>
<tr>
<th></th>
<th>Low caffeine:</th>
<th>High caffeine:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>( \leq 300 ) mg/d</td>
<td>&gt; 300 mg/d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(( n = 63 ))</td>
<td>(( n = 33 ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)(^{2} )</td>
<td>71.2 ± 0.49</td>
<td>70.2 ± 0.56</td>
<td></td>
</tr>
<tr>
<td>Height (cm)(^{2} )</td>
<td>160.4 ± 0.83</td>
<td>159.3 ± 1.01</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)(^{2} )</td>
<td>69.7 ± 1.7</td>
<td>68.1 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>Average calcium intake (mg/d)(^{2} )</td>
<td>812.6 ± 40.7</td>
<td>725.1 ± 51.9</td>
<td></td>
</tr>
<tr>
<td>Age at menopause (y)(^{2} )</td>
<td>48.7 ± 0.83</td>
<td>49.2 ± 0.81</td>
<td></td>
</tr>
<tr>
<td>Serum PTH (% change)(^{2} )</td>
<td>35.15 ± 6.33</td>
<td>19.67 ± 6.71(^{1} )</td>
<td></td>
</tr>
<tr>
<td>Serum 25(OH)D (% change)(^{2} )</td>
<td>–28.70 ± 5.00</td>
<td>–21.10 ± 5.25</td>
<td></td>
</tr>
<tr>
<td>Serum osteocalcin (% change)(^{2} )</td>
<td>–1.45 ± 6.32</td>
<td>9.44 ± 6.68</td>
<td></td>
</tr>
<tr>
<td>Urine NTx:Cr (% change)(^{2} )</td>
<td>15.08 ± 9.07</td>
<td>11.10 ± 9.55</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Spine (% change)(^{2} )</td>
<td>1.19 ± 1.08</td>
</tr>
<tr>
<td>Femoral neck (% change)(^{2} )</td>
<td>1.50 ± 1.18</td>
</tr>
<tr>
<td>Trochanter (% change)(^{2} )</td>
<td>–0.21 ± 1.66</td>
</tr>
<tr>
<td>Total body (% change)(^{2} )</td>
<td>–1.40 ± 0.69</td>
</tr>
<tr>
<td>Total femur (% change)(^{2} )</td>
<td>–0.62 ± 1.17</td>
</tr>
</tbody>
</table>

\(^{1} \)PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; NTx, N-telopeptide; Cr, creatinine.

\(^{2} \)\( \bar{x} \) ± SEM.

\(^{1} \)Adjusted \( \bar{x} \) ± SEM.

\(^{1} \)Significantly different from the low-caffeine group, \( P < 0.05 \) (analysis of covariance with smoking, alcohol intake, and caffeine intake as fixed factors and calcium intake, baseline BMD measures, and other significant correlated variables as covariates).
Calcium intake, which was an average of the baseline and the 3-y values, did not differ significantly between the 2 caffeine groups.

In the placebo group, the frequency of distribution of the VDR genotypes were as follows: low-caffeine group, TT = 42%, Tt = 47%, and tt = 11%; high-caffeine group, TT = 47%, Tt = 37%, and tt = 17%. As shown in Table 3, age, height, and weight did not differ significantly by VDR genotype in both the low- and high-caffeine groups. In the high-caffeine group, the average total calcium intake tended to be lower in the Tt and tt groups than in the TT group, but this difference was not significant. In both caffeine groups, the age at menopause did not differ significantly by VDR genotypes.

Calcitropic hormones and bone markers

At baseline, no significant differences existed in serum parathyroid hormone, serum 25-hydroxvitamin D, serum osteocalcin, and urinary N-telopeptide between the low- and high-caffeine groups (Table 1). In the longitudinal study, the percentage of change in serum parathyroid hormone concentrations was significantly lower in the high-caffeine group than in the low-caffeine group (Table 2). However, no significant differences existed in the percentage of change in serum 25-hydroxvitamin D, serum osteocalcin, and urinary N-telopeptide. The percentage changes in serum parathyroid hormone, serum 25-hydroxvitamin D, serum osteocalcin, and urinary N-telopeptide in the low- and high-caffeine groups did not differ significantly by VDR genotype (Table 3).

Bone mineral density

There were no significant differences between the 2 caffeine groups in baseline BMD at any of the sites measured (Table 1). In the longitudinal study, the rate of bone loss at the spine was higher in the high-caffeine group than in the low-caffeine group (Table 2).

Results of the interaction between caffeine intake, VDR genotype, and BMD are reported in Table 4 and Figure 1. The rate of bone loss was significantly greater at spine in subjects with the tt genotype than in those with the TT genotype when caffeine intake was >300 mg/d. Even at femoral neck, a similar trend was observed ($P = 0.069$). In the low-caffeine group, rates of bone loss did not differ significantly at any of the skeletal sites measured between women with the TT and tt genotypes.

DISCUSSION

Caffeine intake of >300 mg/d was associated with a higher rate of bone loss in postmenopausal elderly women at most of the skeletal sites studied and significantly so at the spine. We also report here for the first time the influence of caffeine intake

### TABLE 3
Characteristics and biochemical indexes of the study population treated with placebo: interaction of caffeine intake and vitamin D receptor gene (VDR) genotype (whites only)\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Low caffeine: ≤300 mg/d</th>
<th>High caffeine: &gt;300 mg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT (n = 24)</td>
<td>Tt (n = 27)</td>
</tr>
<tr>
<td>Age (y)(^2)</td>
<td>71.1 ± 0.73</td>
<td>71.2 ± 0.74</td>
</tr>
<tr>
<td>Height (cm)(^2)</td>
<td>161.8 ± 1.55</td>
<td>159.9 ± 1.03</td>
</tr>
<tr>
<td>Weight (kg)(^3)</td>
<td>70.1 ± 3.01</td>
<td>69.1 ± 2.39</td>
</tr>
<tr>
<td>Average calcium intake (mg/d)(^2)</td>
<td>848.1 ± 76.4</td>
<td>791.4 ± 52.6</td>
</tr>
<tr>
<td>Age at menopause (y)(^2)</td>
<td>48.7 ± 1.37</td>
<td>49.1 ± 1.27</td>
</tr>
<tr>
<td>Serum osteocalcin (% change)(^4)</td>
<td>14.84 ± 11.04</td>
<td>−0.44 ± 10.61</td>
</tr>
</tbody>
</table>

\(^1\) Analyses were done separately for the low- and high-caffeine groups. There were no significant differences by genotype. PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; NTx, N-telopeptide; Cr, creatinine.

\(^2\) Adjusted $\bar{x}$ ± SEM.

\(^3\) Admitted $\bar{x}$ ± SEM.

\(^4\) Significantly different from subjects in the high-caffeine group with the TT genotype, $P = 0.05$ (analysis of covariance with smoking, alcohol intake, caffeine intake, and VDR genotype as fixed factors and calcium intake, baseline BMD measures, and other significantly correlated variables as covariates).

### TABLE 4
Association of caffeine intake, vitamin D receptor gene (VDR) genotype, and rate of bone loss in postmenopausal elderly women (whites only)\(^1\)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Spine</th>
<th>Femoral neck</th>
<th>Trochanter</th>
<th>Total body</th>
<th>Total femur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low caffeine: ≤300 mg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (n = 24)</td>
<td>1.45 ± 1.83</td>
<td>3.81 ± 1.87</td>
<td>−1.21 ± 2.57</td>
<td>−1.23 ± 1.24</td>
<td>−0.56 ± 1.82</td>
</tr>
<tr>
<td>Tt (n = 27)</td>
<td>1.92 ± 1.75</td>
<td>−1.87 ± 1.85</td>
<td>−0.70 ± 2.48</td>
<td>−2.50 ± 1.19</td>
<td>−0.73 ± 1.76</td>
</tr>
<tr>
<td>tt (n = 6)</td>
<td>−0.02 ± 2.60</td>
<td>−1.34 ± 2.09</td>
<td>0.44 ± 4.09</td>
<td>−2.06 ± 1.77</td>
<td>1.54 ± 2.89</td>
</tr>
<tr>
<td>High caffeine: &gt;300 mg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (n = 14)</td>
<td>−0.34 ± 1.42</td>
<td>0.79 ± 1.49</td>
<td>−2.38 ± 1.75</td>
<td>−3.13 ± 0.95</td>
<td>−1.42 ± 1.08</td>
</tr>
<tr>
<td>Tt (n = 11)</td>
<td>−1.98 ± 1.22</td>
<td>−0.40 ± 1.56</td>
<td>−3.04 ± 1.60</td>
<td>−2.31 ± 0.81</td>
<td>−1.09 ± 1.10</td>
</tr>
<tr>
<td>tt (n = 5)</td>
<td>−8.14 ± 2.62</td>
<td>−5.42 ± 2.25</td>
<td>−3.16 ± 2.04</td>
<td>−6.40 ± 1.75</td>
<td>−3.86 ± 1.84</td>
</tr>
</tbody>
</table>

\(^1\) Adjusted $\bar{x}$ ± SEM.
on the association between BMD and VDR genotype as defined with use of the TaqI restriction enzyme. Women with the tt genotype were more susceptible to the negative effect of caffeine, losing more bone over time.

Several studies have examined the influence of caffeine intake on BMD, taking into account a variety of confounding factors. Most of these studies found no association between caffeine intake and BMD (12-18). A significant inverse relation between caffeine consumption and BMD, however, was reported by some researchers, mostly in studies of elderly women. Daniell (37) first observed a greater prevalence of high caffeine intake in white women aged 60-69 y with low bone mass than in those with normal bone mass. The association disappeared, however, after adjustment for smoking and obesity. A negative correlation between current caffeine intake and forearm BMD was reported by Yano et al (38) in an elderly Hawaiian Japanese population. Cooper et al (19) found that high caffeine intake may predispose elderly women, whose calcium balance is impaired, to cortical bone loss from the proximal femur. Bauer et al (2) studied the factors associated with appendicular bone mass in older women. They reported that lifetime caffeine intake was associated with low bone mass at the radius. Barrett-Connor et al (4) observed in the Rancho Bernardo Study that in postmenopausal white women, lifetime caffeinated coffee intake had an inverse association with BMD at the hip and spine after adjustment for a variety of confounding factors. An intake of 2 cups of coffee daily was associated with lower bone density in older women who did not drink milk daily.

The results of prospective studies conducted to study the effect of caffeine on the rate of bone loss are also conflicting. Harris and Dawson-Hughes (3) found that caffeine intake accelerated bone loss from the spine and total body in elderly postmenopausal women with low calcium intakes. On the other hand, Slemenda et al (39) and Hansen et al (15) detected no significant effect of caffeine on the rate of bone loss.

In the present study, we observed that a caffeine intake of >300 mg/d increased the rate of bone loss significantly at the spine in subjects studied longitudinally. Even at the other skeletal sites measured (eg, femoral neck, trochanter, and total body), women with caffeine intakes of >300 mg/d lost more bone than did those with intakes ≤300 mg/d. This association existed even after adjustment for smoking, alcohol intake, calcium intake, age, and other significant covariates.

Harris and Dawson-Hughes (3) reported that daily consumption of 2–3 cups of brewed coffee may accelerate bone loss from the spine and total body in nonsmoking postmenopausal women with low calcium intakes (<800 mg Ca/d). In their population, they reported a statistically significant inverse relation between caffeine intake and calcium intake. Heaney and Recker (40) also found an inverse relation between caffeine intake and calcium intake. Contrary to this, Kiel et al (7) found no significant correlation between dietary calcium intake and caffeine intake in the Framingham Study, in which higher caffeine intake was associated with a greater risk of hip fracture. Meyer et al (5) also investigated a possible interaction between calcium and caffeine intake and found none. In our study population, we found a significant negative correlation between caffeine intake and calcium intake in the cross-sectional population ($r = -0.116, P = 0.014$), but not in the longitudinal population ($r = -0.176, P < 0.093$). We included calcium intake as a covariate in the ANCOVA, but this had no significant effect on the results. Our results therefore suggest that high caffeine consumption per se has a negative effect on BMD, which may be further accentuated by low calcium intakes. However, we could not gain insight into the mechanism of how caffeine exerts its negative effect because we found no significant changes in any of the biochemical

![Figure 1](image-url)
indexing measured. In fact, the percentage of change in serum parathyroid hormone with age was significantly less in high-
caffeine group than in the low-caffeine group.

We then examined whether the significant negative associa-
tion between caffeine intake and the rate of bone loss observed in
our prospective study was influenced by VDR genotype. Both
genetic and lifestyle factors are considered important for bone
mass and risk of osteoporosis. As shown in Table 4 and Figure 1,
women with the tt genotype had a higher rate of bone loss at all
the skeletal sites measured than did those with TT genotype
when their caffeine intake was > 300 mg/d. The effect was signif-
icant, however, only at the spine. Although the rate of bone loss
at the total body and total femur was more than twice as high in
women with the tt genotype than in those with the TT genotype
in the high-caffeine group, these differences were not significant.

Note that the numbers of subjects with the tt genotype were only
6 and 5 in the low- and high-caffeine groups, respectively. These
results suggest that women with the tt genotype are more sus-
cceptible to the deleterious effect of caffeine.

To our knowledge, this is the first report of an association of
VDR genotype and caffeine consumption. Earlier studies exten-
sively investigated the influence of another environmental factor,
calcium intake, on the association between caffeine intake and
BMD. Kiel et al (31) reported an association between dietary cal-
cium intake and BMD only in elderly women with the bb geno-
type. They observed that BMD was higher in women with the
bb genotype than in women with the BB genotype only when the
calcium intake was >800 mg/d. Krall et al (32) reported acceler-
ated rates of bone loss from the hip in postmenopausal women
with the BB genotype when their calcium intakes were low, which
was attributed to reduced calcium absorption (29, 30). Salamone
et al (41) also described an interaction between VDR genotype
and calcium intake in healthy premenopausal women in whom
the BB and Bb genotype groups showed an association between
higher calcium intake and greater femur BMD. These studies sug-
gest that women with the BB or tt genotype [the BsmI allele B is
strongly concordant with the TaqI allele r (22)] are more sus-
cceptible to the modulatory effect of calcium intake.

As seen in the interaction of calcium intake and VDR genotype
reported previously, we found that postmenopausal elderly
women with the tt genotype were more influenced by the dele-
terious effect of caffeine consumption than were those with the
TT genotype. In addition to the VDR genotypes defined by TaqI,
we also studied the interaction between caffeine intake, rate of
bone loss, and VDR genotype defined by BsmI. Women with the
BB genotype lost more bone than did those with the bb genotype
when their caffeine intake was >300 mg/d (data not shown). As
described above, it is well established that the tt genotype is
strongly concordant with the BB genotype. Furthermore, we stud-
ied the influence of caffeine intake on the association between
the VDR genotypes defined by both TaqI and BsmI and BMD at base-
line. We found no significant interaction between caffeine intake
and VDR genotype on BMD at baseline (data not shown).

In summary, we found a higher rate of bone loss at the spine
in postmenopausal elderly women with caffeine intakes >300 mg/d
(≈514 g, or 18 oz, brewed coffee/d) than in those with intakes
≤300 mg/d. In addition, we identified caffeine intake as an impor-
tant dietary factor that alters one's genetic predisposition for bone
remodeling. Postmenopausal elderly women with the tt genetic
variant of VDR appear to be more susceptible to the negative
effect of caffeine as evidenced by higher rates of bone loss. A
clear understanding of the interaction between VDR alleles,
BMD, and lifestyle factors will aid in identifying those at greater
risk of osteoporosis. This information will also be instrumental in
suggesting appropriate lifestyle changes to conserve BMD.

We thank Karen A Rafferty for her help in collecting and analyzing the
food diary data and Kurt E Bulhorn for the laboratory analysis.

REFERENCES

1. US Department of Agriculture, Human Nutrition Information Ser-
vice. Food and nutrition intakes by individuals in the United States,
1 day, 1987–88. Nation-wide consumption survey 1987–88. Wash-
ington, DC: US Department of Agriculture, Human Nutrition Infor-
mation Service, 1993. (NFCS report no. 87-11.)

appendicular bone mass in older women. The study of Osteoporotic

3. Harris SS, Dawson-Hughes B. Caffeine and bone loss in healthy

4. Barrett-Connor E, Chang JC, Edelstein SL. Coffee-associated
osteoporosis offset by daily milk consumption. The Rancho

5. Meyer HE, Federsen JI, Loken EB, Tverdal A. Dietary factors and
the incidence of hip fracture in middle-aged Norwegians—a

fracture in white women. Study of Osteoporotic Fractures Research


Willett WC. Caffeine, moderate alcohol intake, and risk of fractures
of the hip and forearm in middle-aged women. Am J Clin Nutr

9. Lloyd T, Schaeffer JM, Walker MA, Demers LM. Urinary hormonal
concentrations and spinal bone densities of premenopausal vegetar-

10. Massey LK, Whiting SJ. Caffeine, urinary calcium, calcium metab-

11. Hasling C, Sondergaard K, Charles P, Mosekilde L. Calcium metab-
olism in postmenopausal osteoporotic women is determined by

a minor risk factor for bone loss and fractures. Age Ageing 1992;

13. Lloyd T, Rollings N, Eggli DF, Kiesellhorst K, Chinchilli VM.
Dietary caffeine intake and bone status of postmenopausal women.

14. Hansen MA. Assessment of age and risk factors on bone density
and bone turnover in healthy premenopausal women. Osteoporos Int

15. Hansen MA, Overgaard K, Riis BJ, Christiansen C. Potential risk
factors for development of postmenopausal osteoporosis—exam-

16. Reid IR, Ames RW, Evans MC, Sharpe SJ, Gamble GD. Determin-
ants of the rate of bone loss in normal postmenopausal women.

longitudinal bone loss in elderly men and women: the Framingham

18. Packard PT, Recker RR. Caffeine does not affect the rate of gain in