Caffeine intake increases the rate of bone loss in elderly women and interacts with vitamin D receptor genotypes[1–4]

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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. *Am J Clin Nutr* 2001;74:694–700.

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.

See corresponding editorial on page 569.

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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 D_3 (Rocaltrol; 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 *Taq*I 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 Aktinson, 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 mmol/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/cm^2) 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 *Taq*I 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 *Taq*I restriction sites of *VDR* was performed with the forward oligonucleotide primer 5′-CCAAGACTACAAGTACCGCG-3′ and the reverse oligonucleotide primer 5′-TGAGGAGGGCTGCTGAGTAC-3′. The PCR product (≈2035 base pairs) was digested with *Taq*I (New England Biolabs, Beverly, MA) according to the manufacturer's instructions. The digested products were fractionated by gel electrophoresis

TABLE 1
Baseline characteristics, biochemical indexes, and bone mineral density (BMD) data of the study population (all ethnic groups) in the cross-sectional study[1]

	Low caffeine: ≤300 mg/d (n = 265)	High caffeine: >300 mg/d (n = 178)
Age (y)[2]	71.6 ± 0.22	71.2 ± 0.26
Height (cm)[2]	159.3 ± 0.40	158.9 ± 0.49
Weight (kg)[2]	68.9 ± 0.76	67.1 ± 0.94
Total calcium intake (mg/d)[2]	777.5 ± 21.1	704.6 ± 19.30[3]
Age at menopause (y)[2]	48.2 ± 0.4	46.9 ± 0.50[3]
Serum PTH (ng/L)[4]	35.55 ± 1.19	36.12 ± 1.25
Serum 25(OH)D (pmol/L)[4]	75.80 ± 2.2	72.5 ± 2.25
Serum osteocalcin (μg/L)[4]	3.65 ± 0.13	4.01 ± 0.13
Urine NTx:Cr (nmol BCE/mmol Cr)[4]	52.31 ± 2.86	54.72 ± 2.72
BMD		
Spine (g/cm^2)[4]	0.994 ± 0.014	0.982 ± 0.014
Femoral neck (g/cm^2)[4]	0.763 ± 0.007	0.760 ± 0.007
Trochanter (g/cm^2)[4]	0.686 ± 0.010	0.683 ± 0.013
Total body (g/cm^2)[4]	1.003 ± 0.008	0.996 ± 0.010
Total femur (g/cm^2)[4]	0.813 ± 0.010	0.821 ± 0.013

[1] PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; NTx, *N*-telopeptide; Cr, creatinine; BCE, bone collagen equivalents.
[2] $\bar{x}$ ± SEM.
[3] Significantly different from the low-caffeine group, $P < 0.05$ (Student's t test).
[4] Adjusted $\bar{x}$ ± SEM.

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 *Taq*I was genotyped as the *t* allele and the absence of the restriction site was genotyped as the *T* allele. The absence of the *Taq*I site resulted in a single 2035–base pair band. The presence of the restriction was seen as 2 bands of 1917 and 118 base pairs. The presence or absence of the *Taq*I polymorphic site is in linkage with the absence (*B*) or presence (*b*), respectively, of the *Bsm*I polymorphic site.

Statistical analysis

All analyses were done with the SPSS statistical package for WINDOWS (version 10.0; SPSS Inc, Chicago). Baseline characteristics of the cross-sectional and longitudinal study populations were compared between caffeine groups by using Student's t test and between *VDR* genotypes within caffeine groups by using one-way analysis of variance. For the cross-sectional analysis, biochemical indexes and BMD measurements in the low- and high-caffeine groups were compared by using analysis of covariance (ANCOVA) after adjustment for the fixed effects of smoking and alcohol use, calcium intake, and other significant covariates (ie, age, height, weight, and 25-hydroxyvitamin D concentrations) identified from the correlation analysis. For the longitudinal analysis, the percentage of change in BMD and biochemical indexes over 3 y was compared between the 2 caffeine groups by using ANCOVA after correction for the fixed effects of baseline smoking and alcohol intake, the average total calcium intake, each respective baseline BMD value, and other significant covariates identified from the correlation analysis.

In the analysis of the interaction between *VDR* genotypes, caffeine intake, and BMD, the percentage of change in biochemical indexes and BMD over 3 y between *VDR* genotypes in the low- 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 subjects in 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 2
Baseline characteristics, biochemical indexes, and bone mineral density (BMD) data of the study population (all ethnic groups) in the longitudinal study[1]

	Low caffeine: ≤300 mg/d (n = 63)	High caffeine: >300 mg/d (n = 33)
Age (y)[2]	71.2 ± 0.49	70.2 ± 0.56
Height (cm)[2]	160.4 ± 0.83	159.3 ± 1.01
Weight (kg)[2]	69.7 ± 1.7	68.1 ± 2.5
Average calcium intake (mg/d)[2]	812.6 ± 40.7	725.1 ± 51.9
Age at menopause (y)[2]	48.7 ± 0.83	49.2 ± 0.81
Serum PTH (% change)[3]	35.15 ± 6.33	19.67 ± 6.71[4]
Serum 25(OH)D (% change)[3]	−28.70 ± 5.00	−21.10 ± 5.25
Serum osteocalcin (% change)[3]	−1.45 ± 6.32	9.44 ± 6.68
Urine NTx:Cr (% change)[3]	15.08 ± 9.07	11.10 ± 9.55
BMD		
Spine (% change)[3]	1.19 ± 1.08	−1.90 ± 0.97[4]
Femoral neck (% change)[3]	1.50 ± 1.18	−0.55 ± 1.07
Trochanter (% change)[3]	−0.21 ± 1.66	−2.13 ± 1.52
Total body (% change)[3]	−1.40 ± 0.69	−2.65 ± 0.62
Total femur (% change)[3]	−0.62 ± 1.17	−1.22 ± 1.12

[1] PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; NTx, *N*-telopeptide; Cr, creatinine.
[2] $\bar{x}$ ± SEM.
[3] Adjusted $\bar{x}$ ± SEM.
[4] 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).

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]

	Low caffeine: ≤300 mg/d			High caffeine: >300 mg/d		
	TT (*n* = 24)	*Tt* (*n* = 27)	*tt* (*n* = 6)	*TT* (*n* = 14)	*Tt* (*n* = 11)	*tt* (*n* = 5)
Age (y)[2]	71.1 ± 0.73	71.2 ± 0.74	70.2 ± 2.28	69.8 ± 0.84	71.0 ± 0.94	67.9 ± 1.01
Height (cm)[2]	161.8 ± 1.55	159.9 ± 1.03	161.2 ± 1.18	161.3 ± 1.19	160.2 ± 1.35	157.7 ± 3.34
Weight (kg)[2]	70.1 ± 3.01	69.1 ± 2.39	70.8 ± 5.67	71.1 ± 4.51	63.0 ± 2.77	72.7 ± 8.12
Average calcium intake (mg/d)[2]	848.1 ± 76.4	791.4 ± 52.6	768.5 ± 142.4	870.4 ± 88.8	586.7 ± 64.1	659.6 ± 141.2
Age at menopause (y)[2]	48.7 ± 1.37	49.1 ± 1.27	46.6 ± 1.40	51.2 ± 0.77	47.7 ± 1.48	47.4 ± 2.58
Serum PTH (% change)[3]	27.62 ± 9.55	26.51 ± 9.17	12.35 ± 15.40	13.24 ± 8.23	19.84 ± 8.57	21.28 ± 12.65
Serum 25(OH)D (% change)[3]	−14.39 ± 7.57	−22.74 ± 7.12	−19.78 ± 16.3	−25.48 ± 10.25	−12.48 ± 10.93	−27.97 ± 16.12
Serum osteocalcin (% change)[3]	14.84 ± 11.04	−0.44 ± 10.61	−8.63 ± 17.60	−0.45 ± 8.62	5.12 ± 8.96	10.85 ± 13.42
Urine NTx:Cr (% change)[3]	14.99 ± 16.72	24.62 ± 16.33	−3.46 ± 31.71	6.39 ± 13.35	13.31 ± 14.97	30.95 ± 21.49

[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] $\bar{x}$ ± SEM.
[3] Adjusted $\bar{x}$ ± SEM.

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-hydroxyvitamin 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-hydroxyvitamin D, serum osteocalcin, and urinary *N*-telopeptide. The percentage changes in serum parathyroid hormone, serum 25-hydroxyvitamin 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 4
Association of caffeine intake, vitamin D receptor gene (*VDR*) genotype, and rate of bone loss in postmenopausal elderly women (whites only)[1]

Genotype	Spine	Femoral neck	Trochanter	Total body	Total femur
			% change in BMD		
Low caffeine: ≤300 mg/d					
TT (*n* = 24)	1.45 ± 1.83	3.81 ± 1.87	−1.21 ± 2.57	−1.23 ± 1.24	−0.56 ± 1.82
Tt (*n* = 27)	1.92 ± 1.75	−1.87 ± 1.85	−0.70 ± 2.48	−2.50 ± 1.19	−0.73 ± 1.76
tt (*n* = 6)	−0.02 ± 2.60	−1.34 ± 2.09	0.44 ± 4.09	−2.06 ± 1.77	1.54 ± 2.89
High caffeine: >300 mg/d					
TT (*n* = 14)	−0.34 ± 1.42	0.79 ± 1.49	−2.38 ± 1.75	−3.13 ± 0.95	−1.42 ± 1.08
Tt (*n* = 11)	−1.98 ± 1.22	−0.40 ± 1.56	−3.04 ± 1.60	−2.31 ± 0.81	−1.09 ± 1.10
tt (*n* = 5)	−8.14 ± 2.62[2]	−5.42 ± 2.25	−3.16 ± 2.04	−6.40 ± 1.75	−3.86 ± 1.84

[1] Adjusted $\bar{x}$ ± SEM.
[2] 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).

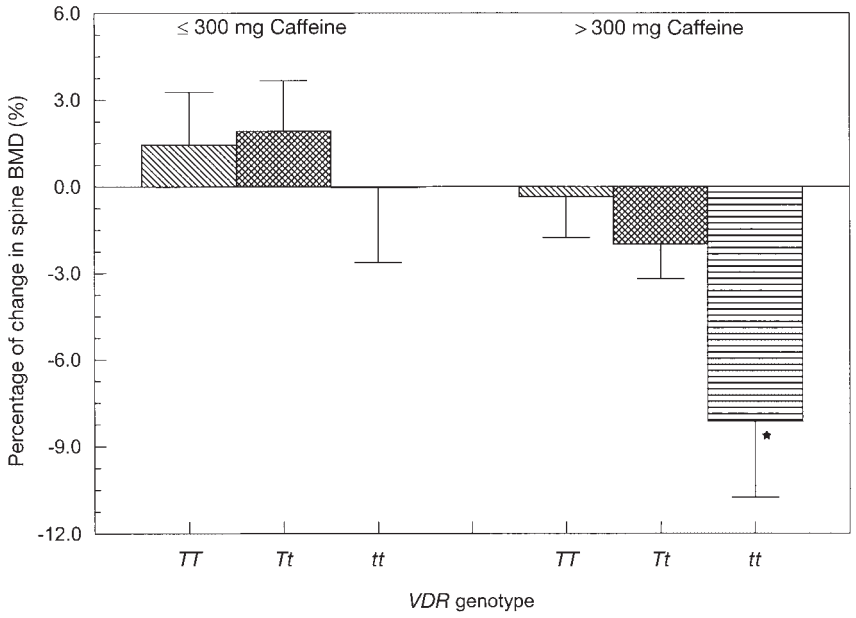

FIGURE 1. Interaction of caffeine intake, vitamin D receptor gene (*VDR*) genotype, and percentage of change in spine bone mineral density (BMD) in postmenopausal elderly women. Values are adjusted means ± SEMs. Comparison between *VDR* genotypes within caffeine groups was done by analysis of covariance. The fixed factors included smoking, alcohol intake, and *VDR* genotype; average calcium intake, baseline BMD, and other significantly correlated variables were used as covariates. Multiple comparisons were done by using Bonferroni's post-hoc test. *Significantly different from the *TT* genotype in the high-caffeine group, $P = 0.05$.

on the association between BMD and *VDR* genotype as defined with use of the *Taq*I 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

indexes 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 association 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 significant, 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 susceptible 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 extensively investigated the influence of another environmental factor, calcium intake, on the association between *VDR* genotype and BMD. Kiel et al (31) reported an association between dietary calcium intake and BMD only in elderly women with the *bb* genotype. 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 accelerated 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 suggest that women with the *BB* or *tt* genotype [the *Bsm*I allele *B* is strongly concordant with the *Taq*I allele *t* (22)] are more susceptible 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 deleterious effect of caffeine consumption than were those with the *TT* genotype. In addition to the *VDR* genotypes defined by *Taq*I, we also studied the interaction between caffeine intake, rate of bone loss, and *VDR* genotype defined by *Bsm*I. 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 studied the influence of caffeine intake on the association between the *VDR* genotypes defined by both *Taq*I and *Bsm*I and BMD at baseline. 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 important 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.

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