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Bone Mineral Density, Carotid Artery Intimal Medial Thickness, and the Vitamin D Receptor *Bsm*I Polymorphism in Mexican American Women

C. M. Kammerer,[1] A. A. Dualan,[2] P. B. Samollow,[3] A. R. S. Périssé,[2] R. L. Bauer,[4] J. W. MacCluer,[3] D. H. O'Leary,[5] B. D. Mitchell[2]

[1]University of Pittsburgh School of Public Health, Pittsburgh, PA, USA
[2]University of Maryland School of Medicine, Baltimore, MD, USA
[3]Southwest Foundation for Biomedical Research, San Antonio, TX, USA
[4]The University of Texas Health Science Center, San Antonio, TX, USA
[5]Tufts-NewEngland Medical Center, Boston, MA, USA

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Abstract. Low bone mineral density (BMD) is a predictor of cardiovascular mortality, suggesting that osteoporosis and cardiovascular disease may share common risk factors. We assessed the relationship between BMD and intimal medial thickening (IMT) of the common carotid artery, a marker of sub-clinical atherosclerosis, in 471 women examined as part of the San Antonio Family Osteoporosis Study, a population-based study of osteoporosis risk conducted in Mexican American families. Because of the documented role of vitamin D metabolism in bone metabolism and its possible role in cardiovascular function, we further evaluated whether allelic variation at the vitamin D receptor locus (*VDR*) influenced joint variation in BMD and IMT. The association of BMD with IMT depended on age, with low BMD being correlated with high IMT in older women, but with low IMT in younger women [age by IMT interaction effects significant at the spine ($P = 0.042$), radius ultradistal ($P = 0.010$), and hip ($P = 0.006$)]. In all women, the VDR *Bsm*I BB genotype was associated with significantly higher forearm BMD ($P = 0.005$ for both radius ultradistal and midpoint), higher IMT ($P = 0.05$), and higher spine BMD in older women ($P = 0.06$), but not with hip BMD. The association of the VDR genotype with IMT was independent of its association with BMD. Although a functional consequence of the *Bsm*I polymorphism on vitamin D metabolism has not been established, these findings support a possible biological relationship among *VDR*, bone metabolism, and atherosclerosis. We conclude that *VDR* polymorphisms may be one of multiple factors influencing the joint risk of atherosclerosis and osteoporosis.

Key words: Vitamin D receptor — Bone mineral density — Mexican Americans — Atherosclerosis — Epidemiology

Correspondence to: B. D. Mitchell; E-mail: bmitchel@medicine.umaryland.edu

Low bone mineral density (BMD) is associated with total non-traumatic mortality [1] and with mortality from cardiovascular diseases (CVD) [2]. In post-menopausal women, BMD is reported to be lower in those with aortic calcification compared to those without [3–7]. More recently, prospective studies have revealed a correlation between progression of bone loss and calcification of the aorta in women progressing through menopause [8, 9].

Why low BMD should be associated with cardiovascular risk is not known. A possible explanation is that the association could be attributed to common factors that influence both cardiovascular risk and bone metabolism. If so, a possible pathway that could be relevant is that involving vitamin D metabolism and calcium homeostasis. The importance of vitamin D to bone metabolism is well established through its role in mediating active calcium absorption in the intestine [10]. A possible effect of vitamin D metabolism on cardiovascular risk is less well established, but suggested on the basis of vitamin D′s role in modulating lipolysis and insulin secretion [11, 12]. Vitamin D receptors are also present in aortic endothelial and vascular smooth vessel cells [13, 14], where they help regulate cellular differentiation and proliferation [15].

These issues motivated us to explore the relationship between bone mineral density and pre-clinical atherosclerosis among Mexican American women characterized for osteoporosis and cardiovascular risk in a large population-based family study. Specifically, we sought to assess the relationship between BMD and intimal medial thickening of the common carotid artery, a marker of sub-clinical atherosclerosis in women spanning a large age range. We further evaluated whether the *Bsm*I polymorphism in the VDR, which has been associated with variation in BMD in many studies [16], is also associated with carotid artery wall thickness in our population of Mexican American women.

Methods

The San Antonio Family Osteoporosis Study (SAFOS) was designed to investigate the genetic and environmental determinants of BMD in large Mexican American families. Families enrolled into the SAFOS were selected because of their concurrent participation in a follow-up examination of the San Antonio Family Heart Study (SAFHS), a population-based prospective family study of atherosclerosis and its risk factors. Details of the sampling and recruitment procedures for the SAFHS and SAFOS have been previously described [16]. Briefly, probands for the 34 SAFOS families were originally identified from a low-income neighborhood in the San Antonio area using a house-to-house recruitment procedure. Eligibility criteria for study probands were that they be 40–60 years of age and have large families. All first, second, and third degree relatives of the proband and the proband's spouse were invited to participate; the invitation was extended regardless of the probands' (or relatives') medical history.

Participating subjects received a medical examination in our clinic in the morning following a 12-hr fast. Fasting blood samples were collected for biochemical analysis and a 2-hr glucose tolerance test was then performed following ingestion of a 75-g glucose equivalent load. Diabetes was diagnosed using the plasma glucose criteria of the World Health Organization [17] and self-report of current use of antidiabetic medications. Relevant to this report, the basic medical examination also included measurement of height and weight (without shoes), from which body mass index was calculated as weight (in kilograms) divided by height (in meters) squared. Women were considered to be menopausal if more than one year had elapsed since last menstrual period or if they had undergone surgical menopause, defined as having both ovaries removed. All procedures were approved by the Institutional Review Board at the University of Texas Health Science Center at San Antonio and all participants gave informed consent.

Bone mineral content was measured at the spine (L1-L4), hip, and forearm (radius ultradistal and radius midpoint) using a dual energy x-ray absorptiometer (DEXA) (Hologic 1500W, Hologic, Inc., Bedford, MA). The areal bone mineral density (BMD; g/cm^2) was determined by dividing the bone mineral content (BMC; g) by the projected area of the region scanned (cm^2). Total hip BMD was defined as the sum of the BMC at the neck, trochanter, and intertrochanter sites divided by the total area of these three sites. All measurements were obtained and analyzed using standard protocols provided by the manufacturer. The short-term *in vivo* precision of the BMD was determined for our technician on 27 subjects who were examined twice on the same day. The precision of the lumbar spine was 0.009 g/cm^2 (CV% = 1.0%) and precision of the total hip was 0.007 g/cm^2 (CV% = 0.87%). The precision of the manufacturer's spine phantom was $0.0017 g/cm^2$ (CV% = 0.17%).

B-mode ultrasound evaluations of atherosclerosis were completed on bilateral segments of the extracranial carotid arteries using a standardized protocol [18, 19]. A lateral view of the common carotid artery was obtained by a trained sonographer. The view was obtained for the 10 mm segment of the carotid artery immediately proximal to the origin of the bulb, where the near and far walls were parallel. Ultrasound images were recorded on super-VHS tapes and sent in three separate batches to the central Carotid Ultrasound Reading Center where they were scored by trained readers. The ultrasound readers measured the thickness of the near and far wall of the common carotid artery. Intimal-medial thickness (IMT) was defined as the mean of two far wall measurements. Prior to analysis, IMT measurements were transformed using the inverse function (i.e., 1/IMT) to reduce skewness. To remove batch (reader) effects, 1/IMT was standardized within batches (mean = 0 and SD = 1). These standardized 1/IMT values were used in all analyses. Therefore, negative values of 1/IMT correspond to artery walls that are thicker than the mean (e.g., atherosclerosis-associated) and positive values of 1/IMT correspond to artery walls that are thinner than the mean (i.e., CV protective).

The *VDR* genotypes were determined from *Bsm*I restriction fragment patterns of polymerase chain reaction (PCR) products amplified from high molecular weight genomic DNA isolated from the SAFOS study participants using *VDR*-specific primers [20]. Alleles were classified according to whether the *Bsm*I cleavage site was absent (allele *B*) or present (allele *b*) [21]. The *B* and *b* alleles have sometimes been designated as *D1* and *D2*, respectively.

Our major analytic goals were to estimate the correlations between BMD and IMT, and to determine whether mean values of these traits differed by *VDR* genotype. Although these cross-sectional analyses are conceptually straightforward, the actual conduct of the analysis is more complex because data obtained on family members are not independent. Failure to incorporate the dependent structure of the data may result in an underestimate of the variance associated with the effect measure and inflated *P*-values. We therefore carried out all statistical analyses using a variance component framework that enabled us to take into account the correlations among family members in this sample. Briefly, this approach involves partitioning the variation in a quantitative trait into components attributable to individual-specific covariates (e.g., linear and nonlinear (i.e., quadratic) age effects, and diabetes status), an additive genetic (polygenic) component, and a residual nonmeasured environmental component. The additive genetic component is modeled as a random effect from the covariance matrix, which is a function of the coefficient of relatedness between all pairs of individuals. For example, a parent and offspring share 1/2 of their genes in common, and thus have a coefficient of relatedness equal to 1/2. Thus, the effects of all independent variables on BMD or 1/IMT are estimated conditional on the correlations among related individuals. The significance of a particular independent variable (e.g., diabetes status) was assessed by the likelihood ratio test, which compares the likelihood of a full model (e.g., age, age^2, BMI, and diabetes status) to that of a nested model (e.g., age, age^2, and BMI only, with the diabetes status effect constrained to be zero). The likelihood ratio statistic is distributed asymptotically as a chi-square statistic with degrees of freedom equal to the difference in number of parameters in the two models being compared [22]. Analyses were conducted using the SOLAR software program [23].

We first performed a series of analyses to determine which of several covariates were associated with BMD or IMT in our population. These potential covariates (for which previous studies have reported associations with BMD or IMT) included age, nonlinear age (modeled as age^2), body mass index (BMI), diabetes status, and menopausal status. We conservatively incorporated all covariates that were significant at $P < 0.1$ in our models, prior to evaluating the effects of *VDR* on BMD and IMT and the relationship between BMD and IMT. Because most previous studies describing the relationship between subclinical atherosclerosis and BMD and between *VDR* and BMD have been performed in older women, we also allowed for the possibility of age-specific effects in our models by the use of interaction terms.

Results

Phenotypic and VDR genotype data were available on 471 women, including 306 pre-menopausal and 165 post-menopausal women, from 28 families ranging in size from 2 to 43 individuals. Characteristics of these subjects are shown in Table 1 for women in three age cohorts: < 40 yr, 40–60 yr, and > 60 yr. These groupings are provided for descriptive purposes only; no analyses were performed using these groups. Mean BMI increased from 30.7 kg/m^2 in women < 40 to 32.7 kg/m^2

Table 1. Clinical characteristics of women according to age (mean ± SD)

Trait	< 40 yo (n = 221)	41 – 60 yo (n = 182)	> 60 yo (n = 68)
Age, years	26.7	48.9	69.2
BMI, kg/m^2	30.7 ± 7.9	32.7 ± 7.6	31.4 ± 6.49
Diabetes (%)	7.8	28.6	35.3
Smoking (%)	23.0	10.0	8.8
Oral contraceptives (%)	23.0	2.7	0.0
Post-menopausal (%)	0.1	52.2	100.0
1/IMT (standardized)	0.57 ± 0.83	−0.10 ± 0.74	−0.83 ± 0.83
Radius midpoint BMD, g/cm^2	0.596 ± 0.045	0.587 ± 0.046	0.497 ± 0.073
Radius ultradistal BMD, g/cm^2	0.468 ± 0.053	0.463 ± 0.053	0.383 ± 0.079
Hip BMD, g/cm^2	1.028 ± 0.139	1.041 ± 0.140	0.891 ± 0.165
Spine BMD, g/cm^2	1.041 ± 0.104	1.023 ± 0.137	0.860 ± 0.157

BMI = body mass index; BMD = bone mineral density; IMT = intimal medial thickness

Table 2. Coefficients of the effects (± se) of age, age^2, body mass index, menopause, and diabetes on BMD and IMT in Mexican American women

	Radius midpoint BMD (g/cm^2) (n = 451)	Radius ultradistal BMD (g/cm^2) (n = 451)	Hip BMD (g/cm^2) (n = 443)	Spine BMD (g/cm^2) (n = 444)	1/IMT (standardized) (n = 448)
Mean	0.597 ± 0.003	0.470 ± 0.004	1.045 ± 0.010	−1.050 ± 0.010	0.138 ± 0.047
Age (10 yr difference)	−0.014 ± 0.001**	−0.012 ± 0.002**	−0.020 ± 0.004**	−0.019 ± 0.005**	−0.34 ± 0.03**
Age^2 (10 yr difference)	−0.0008 ± 0.0001**	−0.0007 ± 0.0001**	−0.0011 ± 0.0002**	−0.0011 ± 0.0002**	−0.0006 ± 0.0010
BMI (2 unit difference)	0.003 ± 0.001**	0.006 ± 0.001**	0.021 ± 0.002**	0.012 ± 0.001**	−0.006 ± 0.005
Menopause (post- vs .pre-)	−0.007 ± 0.006	−0.010 ± 0.007	−0.014 ± 0.017	−0.051 ± 0.012**	−0.03 ± 0.12
Diabetes (present vs absent)	−0.009 ± 0.006	−0.003 ± 0.006	0.011 ± 0.015	0.020 ± 0.014	−0.192 ± 0.100*
Smoking (yes/no)	−0.0004 ± 0.0057	0.008 ± 0.006	−0.003 ± 0.015	−0.004 ± 0.014	0.131 ± 0.103
Residual h^2	0.441 ± 0.093	0.493 ± 0.104	0.70 ± 0.11	0.68 ± 0.12	0.17 ± 0.09

BMD = body mass index; IMT = intimal medial thickness; BMI = body mass index; h^2 = heritability
*$P < 0.10$; **$P < 0.001$

in women aged 40–60 yr, and then slightly decreased in the older cohort. The percentage of women with diabetes increased with increasing age (as expected), from 7.8% to 35.3%, whereas the percent of women reporting that they currently smoked decreased from 23.0% in the youngest group to 8.8% in the oldest group.

Approximately 23% of younger women reported current use of oral contraceptives. Also as expected, mean thickness of arterial walls increased with increasing age (i.e., 1/IMT decreased with increasing age), and BMD decreased with increasing age.

Table 2 summarizes the effects of selected covariates on variation in BMD and IMT. Age was significantly associated with decreasing BMD at each of the four sites and with increasing IMT (or decreasing 1/IMT) ($P < 0.0001$). In addition, a non-linear (quadratic) effect of age was significantly associated with variation in BMD, but not IMT. Increasing BMI was associated with increasing BMD at all four sites ($P < 0.0001$), although it was not associated with IMT. Post-menopausal women had significantly higher BMD at the spine ($P = 0.001$), even after accounting for the linear and quadratic effects of age. Diabetes was weakly associated with increased pre-clinical atherosclerosis (or decreased 1/IMT) ($P = 0.06$), while smoking was associated with neither BMD nor IMT. There was a significant ($P < 10^{-6}$) heritable component to the residual BMD levels, ranging from 44% for radius midpoint BMD to 70% for hip BMD, whereas the heritability of 1/IMT was very modest (17%, $P = 0.01$).

Prior to formally evaluating the relationships among *VDR* genotypes, BMD, and IMT, we assessed the relationships among these traits using unadjusted data. Initially, we compared mean BMD and 1/IMT across the *VDR* genotypes. The frequency of the B allele in this Mexican American population was 23.5%. Genotype frequencies were consistent with those expected under Hardy Weinberg equilibrium ($\chi^2 = 2.99$; $P = 0.08$). As can be seen in Table 3, individuals with the *BB* genotype had higher mean BMD at both forearm sites and the spine, but not at the hip. In addition, mean (unadjusted) 1/IMT was lower for the *BB* genotype (indicating that mean IMT was higher). We then estimated the regression coefficients between unadjusted 1/IMT and unad-

Table 3. Unadjusted mean BMD and 1/IMT (± SE) according to VDR genotype and unadjusted correlations between 1/IMT and BMD

	Radius midpoint BMD (g/cm^2)	Radius ultradistal BMD (g/cm^2)	Hip BMD (g/cm^2)	Spine BMD (g/cm^2)	l/IMT (standardized)
VDR genotypic means					
BB	0.596 ± 0.008	0.477 ± 0.009	1.015 ± 0.020	1.044 ± 0.022	−0.123 ± 0.110
Bb	0.573 ± 0.005	0.449 ± 0.006	1.011 ± 0.013	1.005 ± 0.013	0.131 ± 0.087
bb	0.577 ± 0.004	0.452 ± 0.004	1.010 ± 0.012	1.003 ± 0.008	0.112 ± 0.059
Correlation between 1/IMT and BMD					
Beta coefficient*	0.016 ± 0.003	0.015 ± 0.003	0.016 ± 0.007	0.039 ± 0.006	NA
$r^{2\dagger}$	0.06	0.05	0.01	0.05	

NA = not applicable
VDR = vitamin D receptor; BMD = bone mineral density; IMT = intimal medial thickness
* Correlation coefficient for effect of l/IMT on BMD
† Percent of variance in BMD explained by variation in l/IMT

Table 4. Beta coefficients (± SE) for the effects of VDR genotype on BMD and 1/IMT and for the effects of 1/IMT on BMD in Mexican American women, adjusted for covariate effects#

	Radius (midpoint) BMD (g/cm^2)	Radius (ultradistal) BMD (g/cm^2)	Hip BMD (g/cm^2)	Spine BMD (g/cm^2)	l/IMT (standardized)
VDR genotype (BB vs Bb/bb)	0.023 ± 0.008**	0.024 ± 0.009**	0.0135 ± 0.0207	0.027 ± 0.020	−0.270 ± 0.138*
VDR * age	0.005 ± 0.005	0.0010 ± 0.0005	0.0018 ± 0.0012	0.0022 ± 0.0012†	0.008 ± 0.008
l/IMT	−0.001 ± 0.003	0.003 ± 0.003	−0.0026 ± 0.0069	0.0132 ± 0.0066*	NA
l/IMT * age	0.0002 ± 0.0002	0.0005 ± 0.0002**	0.0012 ± 0.0004***	0.00080 ± 0.00041*	NA

# Effects adjusted for all significant covariates (in Table 2)
NA = not applicable
† $P = 0.06$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

justed BMD. Consistent with expectations, the correlations were statistically significant ($P < 0.01$ for all) and positive in direction (see Table 3), reflecting that people with low BMD (who are likely to be older) are also more likely to have high IMT (or low l/IMT).

Following these unadjusted analyses, we conducted a more formal analysis of the relationships among *VDR* genotype, BMD and IMT, while also accounting for the potentially confounding effects of age, sex, and other external covariates. In the first set of these analyses we constructed models to estimate the effects of *VDR* genotype on BMD and l/IMT. With these models, we simultaneously estimated the effects of the covariates (e.g., age, BMI, menopause, diabetes) on BMD and/or l/IMT, and then evaluated whether the residual variation in BMD or l/IMT could be explained by the differing *VDR* genotypes. We also allowed the possible relationship between *VDR* genotype and BMD or l/IMT to vary by age. That is, we included *VDR* genotype by age interaction effects into the model (Table 4). Because initial comparisons revealed no evidence for meaningful differences in BMD and IMT between women carrying one or two copies of the 'b' allele (see Table 3), women with the Bb and bb genotypes were combined into a single category for these analyses.

Women with the BB genotype at *VDR* ($n = 37$) had significantly higher residual BMD at both forearm sites than women with the Bb/bb genotypes ($n = 394$) ($P = 0.005$ for each, Table 4). The *VDR* genotype was not significantly associated with hip ($P = 0.52$) or spine ($P = 0.18$) residual BMD. Although the magnitude of the effect was similar for both forearm sites and the spine, the variation within each genotype was larger for the spine (Tables 3 and 4). We did detect a weak *VDR* genotype by age interaction effect ($P = 0.06$) on spine residual BMD. Subsequent investigation revealed that mean spine BMD did not differ between premenopausal women having the *BB* and those having the *Bb*/*bb* genotypes (mean BMD ± standard error (s.e.) = 1.045 ± 0.007 vs. 1.051 ± 0.019 g/cm^2, respectively). However, post-menopausal women with the *BB* genotype had significantly lower mean (± s.e.) spine BMD than did their counterparts with the Bb/bb genotype (0.926 ± 0.013 vs. 1.031 ± 0.053 g/cm^2, respectively; $P = 0.037$), although this result obtained from a subgroup should be interpreted cautiously.

We next examined the relationship between *VDR* and IMT (while simultaneously accounting for the effects of age and diabetes status). Women with the *BB* genotype had significantly thicker vessel walls (or lower l/IMT)

compared to women with either the *Bb* or *bb* genotypes ($P = 0.05$), but there was no evidence that this association differed by age (Table 4).

The final set of analyses addressed the relationship between BMD and 1/IMT. Specifically, we evaluated whether variation in BMD could be explained by variation in IMT, while simultaneously estimating the effects of potential covariates on BMD, including age, BMI, diabetes, menopausal status, and the *VDR* genotype. We also allowed the relationship between IMT and BMD to vary by age. The results of these analyses are also presented in Table 4. At three of the four BMD sites (all sites except radius midpoint), we detected significant ($P < 0.05$) age by 1/IMT interaction effects, implying that the correlations between BMD and IMT differed by age. Specifically, we found that 1/IMT decreased with increasing BMD at the younger ages, but decreased with decreasing BMD at older ages (i.e., thicker artery walls were associated with higher BMD at younger ages, but with lower BMD at older ages). For illustrative purposes only, we plotted the residuals for hip BMD (after removing effects of age, age^2, and BMI) against 1/IMT in each of three age categories (see Fig. 1). As can be seen, 1/IMT decreases with increasing BMD in the youngest age group (Fig, 1a) and increases with increasing BMD in the oldest age group (Fig. 1c). Although this interaction effect is strongest on hip BMD ($P = 0.006$), it also achieves statistical significance for BMD of the radius midpoint ($P = 0.010$) and spine ($P = 0.042$).

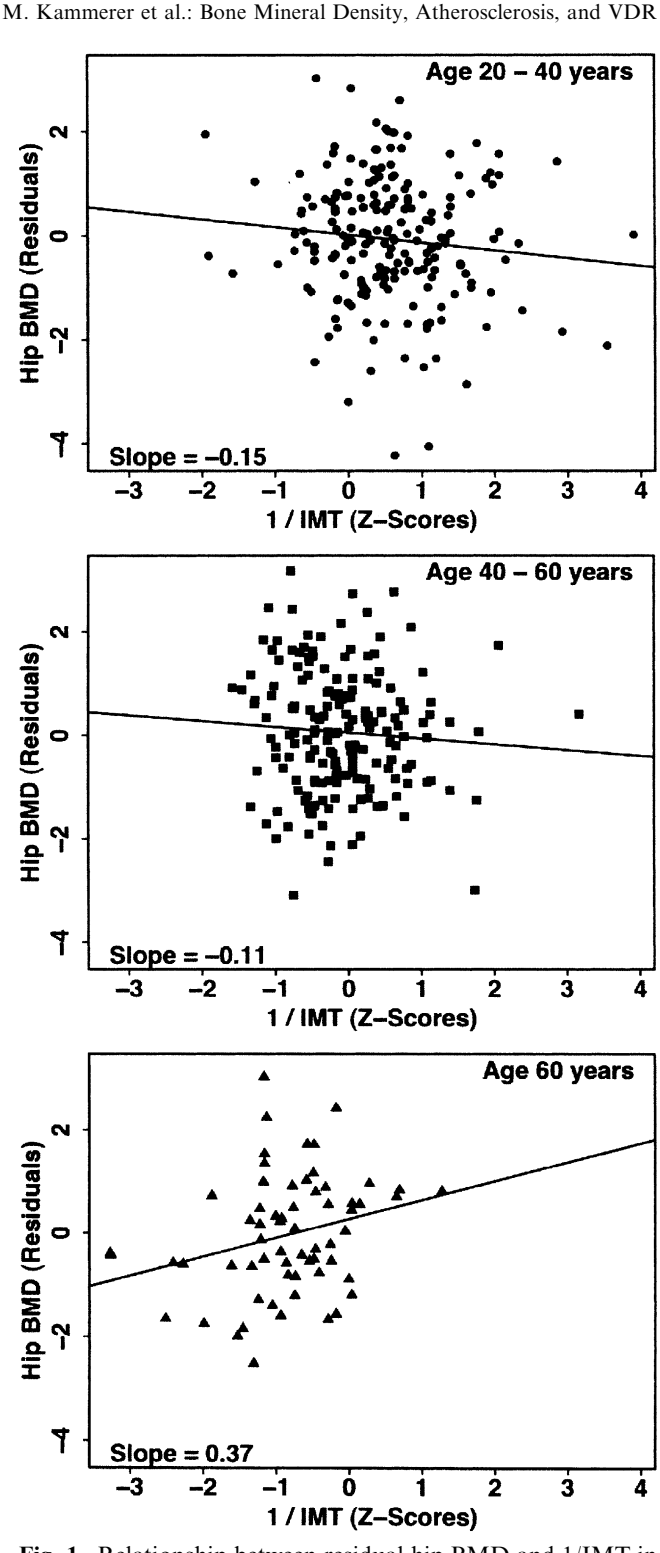

Fig. 1. Relationship between residual hip BMD and 1/IMT in Mexican American women age 20–40 yrs (Fig. 1a), 41–60 yrs (Fig. 1b), and >60 yrs (Fig. 1c).

Discussion

Our data support the growing body of evidence for an association between low bone mineral density and atherosclerosis. Interestingly, the association between preclinical atherosclerosis and BMD observed in our study was highly dependent on age. This pattern was true across three of the four BMD sites, the spine, hip, and radius midpoint, but not at the radius ultradistal site. In younger Mexican American women, there was a positive relationship between increasing BMD and increasing IMT, whereas in older women there was a negative relationship, with decreased BMD being associated with increasing IMT. Our results in older women are similar to those of other investigators who have reported a relationship between BMD and carotid plaque [28] and/or aortic calcification [27], between aortic or coronary calcification and metacarpal bone loss BMD [4, 8, 9], and between BMD and CHD mortality [24]. With the exception of Kiel et al. [9], who also studied older men, most previous studies have been performed predominantly in older post-menopausal women.

The above studies suggest that reduced BMD or osteoporosis and atherosclerotic disease may be influenced by common genes [25] or environmental factors, including dietary exposures [25], inflammation [26], and hormones/drugs [27]. One gene that may influence BMD and development of atherosclerotic disease is *VDR* because the hormonal form of vitamin D controls a number of processes essential to bone metabolism, in-

cluding calcium absorption in the gut, bone mineralization, and secretion of parathyroid hormone. Furthermore, vitamin D receptors are present in aortic endothelial [14] and vascular smooth muscle cells [3]. The *Bsm*I polymorphism of *VDR*, which is located in the intron between exons 8 and 9, has been associated with BMD in numerous studies, with most reporting the B allele to be associated with slowed calcium absorption, lower BMD, and faster bone loss whereas others have reported associations in either the opposite direction or not at all (reviewed by [15]). Gong et al. [28] performed a qualitative meta-analysis using information from 75 studies (14,000 individuals) and concluded that the B allele, in addition to two other polymorphisms, was associated with lower BMD. They also concluded that non-genetic factors (such as age or dietary status), as well as genetic heterogeneity (such as ethnicity) interfere with the detection of effects of *VDR* genotypes on BMD. In contrast to some, but not all, previous studies, we observed that the BB genotype was associated with increased BMD in both forearm sites and spine BMD in post-menopausal women, but not hip BMD.

Because *Bsm*I and other polymorphisms that have been associated with BMD are not known to be functional, the fact that our associations with BMD differ in direction from reports in other populations is not surprising, especially because our studies were conducted using data from a different ethnic group. More generally, our results are consistent with the underlying hypothesis that variants in *VDR* affect BMD.

The relationship between *VDR* polymorphisms and atherosclerosis is controversial. On the one hand, the frequency of the *Bsm*I B allele has been reported to be higher in patients with calcific aortic stenosis [29] and in those with angiographic evidence coronary artery disease, although on the other hand, an association of the b allele with severity of coronary artery disease has also been reported [30]. In more recent study of 3,441 patients no relationship was found between this polymorphism and CAD [31]. Our results are consistent with several of the initial studies in which we found that the BB genotype was associated with lower mean 1/IMT, or increased wall thickening, a phenotype associated with increased CAD. Interestingly, the direction of the relationship between *VDR* genotypes and IMT was contrary to our initial expectation since the BB genotype was associated with increased BMD. However, because *VDR* genotypes and IMT were both significantly and independently associated with BMD, depending upon skeletal site, we conclude that *VDR* may be one of multiple factors influencing the joint risk of atherosclerosis and osteoporosis.

Our results were obtained from a Mexican American population. Although the families included in our study were not ascertained on the basis of any particular disease phenotype, they were selected on the basis of their being relatively large in size. It is possible that there might be rare mutations and/or shared familial exposures unique to these families that jointly influence variation in BMD and CVD susceptibility.

Biological mechanisms linking low BMD and CVD have been proposed but have not been well studied. Several researchers have suggested a role for hyperlipidemia and lipid oxidation [32], and inflammation [26]. *In vitro* studies have shown that oxidized lipids promote osteoblastic differentiation of vascular cells and inhibit such differentiation in bone cells (see [31]). One possible mechanism by which this may occur is through accumulation of oxidized lipids in tissue so as to mimic chronic infection, thereby stimulating an immune response that promotes the hardening of soft tissue (to wall off infectious agents) and the softening of hard tissue (to dissolve a substrate for growth of infectious agents) [26]. Although such a mechanism could explain the inverse relationship between BMD and CVD observed in older women (and men) in our study and in others, it does not explain the positive relationship between BMD and pre-clinical atherosclerosis that we observed in younger women. Furthermore, our results suggest that the relationship between BMD and CVD may impact trabecular versus cortical bone differently, given our results that radius midpoint BMD was not associated with IMT, whereas radius ultradistal BMD was. In conclusion, our results support the hypothesis that a common set of factors jointly affect the development of osteoporosis and atherosclerosis, but that these factors may change with age and may impact different skeletal sites unequally.

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