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Decreased Bone Mineral Density Is Correlated with Increased Subclinical Atherosclerosis in Older, but not Younger, Mexican American Women and Men: The San Antonio Family Osteoporosis Study

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Abstract An association has been reported between cardiovascular disease (CVD) and osteoporosis, perhaps attributable to the presence of common risk factors. To assess this possibility, we measured areal bone mineral density (BMD) and carotid artery intimal medial thickness (IMT), a measure of preclinical atherosclerosis, in 535 women and 335 men from the San Antonio Family Osteoporosis Study. Variance decomposition methods were used to determine whether cross-sectional measures of areal BMD (measured by dual-energy X-ray absorptiometry) of the total hip, spine, and forearm were correlated with IMT, serum lipids, and/or C-reactive protein (CRP), a marker of inflammation, after accounting for known environmental factors. We observed significant inverse correlations of IMT and BMD at all bone sites in women >60 years of age ($P < 0.001$) and modest positive correlations (not significant) of IMT on hip BMD ($P < 0.1$) in women <60 years of age. Similarly, we observed negative correlations between IMT and forearm BMD in men >60 years of age ($P < 0.001$) and positive correlations in men <60 years of age ($P = 0.05$). Variation in risk factors for CVD, including serum levels of low- and high-density lipoprotein cholesterol, low-density lipoprotein particle size, triglycerides, paraoxonase 1 activity, and CRP did not account for the relationship between BMD and IMT in either older or younger men or women. In summary, our results demonstrate that decreased BMD is correlated with increased IMT in older (but not younger) Mexican American men and women, independent of serum CVD risk factors.

Keywords Osteoporosis · Atherosclerosis · Cardiovascular disease · Bone mineral density · Arterial intimal medial thickness

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Osteoporosis and cardiovascular disease (CVD) are maladies of major health concern, together responsible for a large proportion of morbidity and mortality among the elderly. Low bone mineral density (BMD), especially that of postmenopausal women, is associated with increased mortality due to osteoporotic fractures [1], stroke, and CVD [2]. The relationship between osteoporosis and CVD in women has been recognized for over 30 years [3]; however, it has often been described as an artifact of aging, despite studies showing the persistence of this relationship after adjusting for age [3, 4]. In fact, it has been estimated that women with bone mass in the lowest quartile (as measured in early postmenopause) are at twice the risk of CVD death as those in the highest quartile [2]. Likewise, women with lower metacarpal cortical area, a measure of

osteoporotic risk, have greater incidence of coronary heart disease [5]. Several additional studies have linked the progression of arterial calcification with concurrent bone loss [6–9]. Much of the evidence supporting a relationship between osteoporosis and CVD is drawn from studies of older women; few studies have been carried out in men. Large epidemiological studies of older men have shown that low BMD is associated with increased severity of calcified carotid plaques (n = 2,543 Caucasians, aged 55–74 years) [10] and CVD (n = 552 African Americans and 939 Caucasians, aged 68–80 years) [11]. A smaller study demonstrated lower BMD in patients (including 20 men) with peripheral vascular calcification compared to controls [12].

There is some speculation that the mechanism(s) linking osteoporosis and atherosclerosis acts through shared risk factors. For example, the importance of lipids in both atherosclerosis and bone disease has recently been investigated [13]. Several epidemiological studies [14–16], but not all [17], have reported associations between lipid profiles and BMD. Furthermore, lipid-lowering agents such as hepatic hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors have been shown to increase BMD [18] and reduce the risk of osteoporotic fractures in older women [19], as well as reduce intimal medial thickening (IMT) [20] and arterial calcification [21], two subclinical measures of atherosclerosis. One category of lipids, oxidized lipids, may promote atherosclerosis by inducing mineralization in the artery wall. However, in bone, oxidized lipids inhibit mineralization and osteoblastic differentiation, leading to decreased bone mass [8]. Variation in the gene coding for paraoxonase 1 (PON1) enzyme, which is strongly implicated in atherosclerosis and thought to be involved in conferring antioxidant properties to high-density lipoprotein (HDL), has been associated with BMD in Japanese women [22]. Thus, the role of lipids has been implicated in the pathogenesis of both bone and vascular disease.

The role of inflammation in vascular and bone health has also been studied. Atherosclerosis is thought to be coupled with an ongoing inflammatory response [23], and decreased BMD has been reported for individuals with various conditions of chronic inflammation including rheumatic diseases [24], lupus erythematosus [25, 26], periodontal disease [27], pancreatitis [28–30], and inflammatory bowel disease [31], as well as individuals with compromised immune function such as that due to human immunodeficiency virus [32, 33]. Therefore, inflammation may also influence concomitant changes in vascular and bone health.

We have previously reported that the cross-sectional relationship between BMD and preclinical atherosclerosis differs by age in Mexican American women [34]. For example, after controlling for the effects of body mass index, age, and age squared, greater hip areal BMD was correlated with increased IMT in women aged 20–40 but decreased IMT in women over 60 [34]. The age-dependent relationship between BMD and IMT is further compounded by menopausal status and the role of estrogen deficiency as a risk factor for both osteoporosis and artery disease [35]; however, the age-dependent relationships were significant even after controlling for menopausal status, leading us to conclude that the factors jointly affecting the development of osteoporosis and atherosclerosis may vary with age [34].

In the present study, we have expanded on our previous work by evaluating in both men and women the contributing roles of serum CVD risk factors (lipids and a marker of inflammation) as potential mediators of the association between BMD and IMT. Because lipids appear to participate in the pathogenesis of bone and vascular diseases [8, 13], we analyzed BMD in relation to serum levels of low-density lipoprotein (LDL) cholesterol, HDL cholesterol, and triglycerides. Likewise, we included in our analyses the effects of median LDL particle diameter because the size of LDL particles has been shown to affect their affinity for oxidative modification, binding to the artery wall, and duration of residence in the plasma [36]. We also investigated the potential effects of serum PON1 activity, which is implicated in atherosclerosis [37] and may mediate the effect of lipids on disease pathogenesis. Additionally, in order to explore the role of inflammation acting in parallel on vascular and bone tissue, we included in our analyses serum C-reactive protein (CRP), a marker of inflammation that may be associated with BMD [38]. The goals of our study were to assess whether the relationship between BMD and IMT previously reported in Mexican American women is also apparent in men and to determine if this association is mediated, in part, by serum lipid-related factors or inflammation.

Experimental Subjects and Methods

Those enrolled in this study were individuals at least 18 years of age from large Mexican American families in the San Antonio area. All participants provided informed consent for their involvement in this project, and all procedures were approved by the Institutional Review Board at the University of Texas Health Science Center, San Antonio.

Population Recruitment and Data Collection

Recruitment and data collection for the San Antonio Family Osteoporosis Study (SAFOS) have been previously

described in detail [39]. In brief, 34 probands between 40 and 60 years of age and all first-, second-, and third-degree relatives were invited to participate in the study (535 women, 335 men). Medical examinations were conducted between 1997 and 2000 and included anthropometric measurements, a blood sample collection, a glucose tolerance test, and an interview regarding social, behavioral, and lifestyle factors relating to cardiovascular risk. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2) to assess adiposity and log-transformed to reduce skewness. Diabetes was diagnosed according to the World Health Organization's plasma glucose criteria (fasting plasma glucose concentration >7.0 mmol/L and/or 2-hour post-glucose load concentration >11.0 mmol/L) and/or self-report of current use of diabetes medications. Menopausal status was also assessed by self-report, whereby women having an elapsed time since most recent menstrual period of ≥1 year or having undergone bilateral oophorectomy were defined as "postmenopausal."

Physical Measurements

Areal BMD (g/cm^2) was measured for the lumbar spine (L1–L4), total hip, one-third radius (measured one-third radius length from distal end), and ultradistal radius using dual-energy X-ray absorptiometry (DXA) (Hologic 1500W; Hologic, Bedford, MA) as previously described [39]. Two radius sites were included due to differing compositions of cortical and trabecular bone: one-third radius, like total hip, is largely comprised of cortical bone, whereas ultradistal radius, like total spine, is primarily trabecular bone. IMT (mm) of the far wall of the extracranial carotid artery was measured according to the standard protocol by B-mode ultrasound as previously described [34]. IMT measurements were inverse-transformed to reduce skewness and standardized (mean = 0, standard deviation [SD] = 1) prior to analysis. We then multiplied the standardized inverse IMT value by -1 for ease of interpretation so that positive and negative values of $-IMT^{-1}$ would correspond to artery walls that are thicker and thinner than the mean, respectively.

Biochemical Measurements

Cholesterol and triglyceride levels were measured on a Ciba-Corning (Medfield, MA) Express Plus clinical chemistry analyzer by enzymatic methods using reagents supplied by Boehringer-Mannheim Diagnostics (Indianapolis, IN) and Stanbio (San Antonio, TX), respectively. Apolipoprotein B–containing lipoproteins were precipitated by use of dextran sulfate-Mg^{2+}, and HDL cholesterol (mmol/L) was determined in the supernatant [40]. LDL cholesterol levels (mmol/L) were estimated on the basis of triglyceride (mmol/L) and non-HDL cholesterol levels as described [41, 42]; HDL, LDL, and triglyceride levels were log-transformed to reduced skewness. Coefficients of variation in these assays were 2.1% for plasma cholesterol, 4.6% for HDL cholesterol, and 3.7% for triglycerides. LDL size phenotypes (nm) were estimated by nondenaturing acrylamide gradient gel electrophoresis using composite gradient gels and methods described previously [43]. Sudan black B was used to stain cholesterol in LDL particles, and we derived an estimate of LDL size distributions from which median LDL particle diameter was defined as the diameter where half the LDL absorbance (i.e., particles between 21 and 29 nm) is on larger and half is on smaller particles [43]. The coefficient of variation (CV) for a control sample run on each gel was 0.6%. Serum CRP levels (mg/L) were measured using an ultrasensitive competitive immunoassay (antibodies and antigens from Calbiochem, La Jolla, CA) and natural log-transformed to reduce skewness. The interassay coefficient of determination was 8.9% [44, 45]. Arylesterase activity of PON1 was assayed at 30°C by adding serum to 100 mM Tris buffer (pH 8.5) containing 2 mM $CaCl_2$ and 2 mM phenyl acetate as substrate. Rate of phenol formation was monitored at 270 nm in the linear phase using a Molecular Devices (Palo Alto, CA) SpectraMax M2 microplate reader in kinetic data acquisition mode and an extinction coefficient of 1310/M*cm. Samples were run in duplicate, and the average CV was 1.1% for these samples; a low-level control run on each plate gave a between-assay CV of 7.8%. PON1 (but not other serum CVD risk factors) was assayed on serum samples collected 5.2 years prior to BMD and IMT measurements.

Covariates

The following covariates were considered in our study: medical variables including diabetes status (yes/no), menopausal status (pre-/post-), oral contraceptive use (yes/no), hormone replacement therapy (yes/no), and number of births (count); demographic variables including sex (male/female), age (years), and sex × age (years); anthropometric variables including BMI (m^2/kg); lifestyle variables including smoking status (ever/never), current alcohol consumption (yes/no), education (years), and total breastfeeding duration (months); and serum risk factors for CVD including LDL cholesterol (mmol/L), LDL median particle diameter (nm), HDL cholesterol (mmol/L), triglycerides (mmol/L), PON1 activity (μmol/min/mL), and CRP (mg/L).

Statistical Analyses

We assessed the relationship between lipid-related risk factors for CVD and BMD after removing the effects of known medical, demographic, anthropometric, and lifestyle covariates (as stated above, all variables were tested for nonnormality and transformed if necessary). In light of well-documented differences in BMD and CVD risk factors between men and women and because significant sex by IMT interactions were observed in preliminary analyses for BMD of the hip, spine, and forearm (P = 0.004, 0.001, and 0.06, respectively; results not shown), all analyses were performed for males and females separately. To identify an initial set of medical, lifestyle, demographic, and anthropometric covariates that affect measures of BMD, we performed bidirectional stepwise regression using a liberal significance threshold of $\alpha = 0.1$. These covariates were included in the genetic analyses described below.

Pedigree-based analyses were performed in a variance component framework as previously described [39] to account for the familial relationships in our sample. This approach partitions the variation of a trait into measured environmental (i.e., those due to covariates), additive genetic (polygenic), and residual environmental (error) effects. Our general model of BMD, y, for individual i was as follows: $y_i = \mu + \Sigma\beta_j X_{ij} + \Sigma\beta_k Y_{ik} + \Sigma\beta_l d_i Z_{il} + g_i + e_i$, where μ is the mean BMD; X_{ij} is the jth medical, demographic, anthropometric, and lifestyle covariate for the ith individual; Y_{ik} is kth CVD risk covariate for the the ith individual; d_i is a binary indicator of age (<60 or >60 years) for the ith individual; $d_i Z_{il}$ is the lth CVD risk covariate for individuals >60 years of age; β_j, β_k, and β_l are the corresponding regression coefficients; g_i is the additive genetic effect; and e_i is the residual error effect. This general model includes an age–interaction term to assess whether effects of CVD risk covariates differ by age cohort; previous work suggests they do [34, 46]. Maximum likelihood methods were used to estimate covariate effects on BMD while accounting for the familial relationships within the data, and significance was assessed by comparing nested models via the likelihood ratio test (i.e., comparing the likelihood of the model retaining the covariate of interest to that excluding it). To be certain of capturing any effects that mediate the relationship between BMD and IMT, covariates with possible effects at $P < 0.1$ were retained in our models. Details regarding the heritable component in these models are addressed in Mitchell et al. [39]. These analyses were performed using the Sequential Oligogenic Linkage Analysis Routines (SOLAR) software package [47].

To assess the correlates of BMD, including IMT and CVD-related traits, we analyzed four models of BMD variation: (1) the base model, in which the effects of significant demographic, anthropometric, medical, and lifestyle variables on BMD were tested; (2) the IMT model, in which the effects of IMT and all significant covariates from the base model were simultaneously tested; (3) the serum CVD risk factors model, in which the effects on BMD of serum risk factors for CVD and all significant covariates from the base model were simultaneously tested; and (4) the combined model in which serum CVD risk factors, IMT, and significant covariates from the base model were all tested. By comparing these models, we were able to estimate the amount of BMD variation attributable to IMT and CVD risk factors and determine if CVD risk factors accounted for the relationship between BMD and IMT. The IMT, serum CVD, and combined models were compared to the base model using the likelihood ratio test.

In our analyses, the effects of IMT and serum CVD risk factors were allowed to differ between individuals younger and older than 60 years of age for comparability to other studies, for ease of interpretation, and because previous reports suggest that the relationships between BMD and IMT [34] and between BMD and CVD risk factors [46] may change with age. In addition, regression by locally weighted scatterplot smoothing (LOWESS) was used to assist in selecting age ranges for age-specific effects, as well as to illustrate the nonlinear association between BMD and IMT in different age cohorts. Although we present results from our analyses by dichotomizing at age 60 years for the reasons given above, we also obtained similar results when we dichotomized at age 55 or menopausal status in women as well as when we allowed covariate effects to vary continuously with age (results not presented). Covariate screening, LOWESS regression, and plotting were performed in the R environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria).

Results

The study population (n = 870) contained 34 extended Mexican American families including 535 females and 335 males. Tables 1 and 2 summarize baseline population characteristics for women and men. As expected, means of BMD for all bone sites were greater in men compared to women and generally decreased with age. An exception was spine BMD, which did not decrease with age in men, possibly due to the high prevalence of osteoarthritis and aortic calcification in older men, which can lead to inflated densitometric values [48–50]. Also as expected, mean IMT increased with age in both men and women.

Table 1 Mean (range) population characteristics in women and men

	Women (n = 535)	Men (n = 335)
Demographic		
Age (years)	43.2 (18.6–89.2)	42.1 (18.1–96.7)
Anthropometric		
BMI (kg/m^2)	31.6 (16.3–65.6)	29.8 (17.6–53.1)
Medical		
Postmenopausal (%)	27.4	—
Diabetic (%)	20.4	19.9
Lifestyle		
Current/ever smoker (%)	17.1	31.8
Current alcohol consumer (%)	29.4	60.2
Oral contraceptive user (%)	12.9	—

To determine the proportion of BMD variation explained by measured environmental covariates while accounting for the familial relationships in our sample, we modeled the variance components of BMD sites in men and women separately. Analyses of covariates revealed that serum CVD markers are noncollinear (pairwise R^2 ranging 0–0.23 for all covariates except triglyceride levels and LDL particle diameter, for which R^2 was 0.54 in men and 0.34 in women). As described in detail above, four models were constructed to assess the correlates of BMD. In the initial base model, only age, BMI, menopausal status (in women), and diabetes status (in men) were significantly associated with BMD. As expected, BMD was inversely associated with age and directly associated with BMI at all bone sites in both women and men. Postmenopausal women had lower BMD at all bone sites, and diabetic men had lower BMD at both sites of the radius. Altogether, these covariates accounted for 29–40% of variation in BMD in women and 3–22% of BMD variation in men. As previously reported [39], genetic factors accounted for much of the variation in BMD in both men and women, with heritability (i.e., the proportion of variation due to genes) of BMD estimated as 23–52% for bone sites in women and 13–51% for bone sites in men.

Table 3 shows beta coefficients and significance for covariates in the IMT model, which builds on the base model by additionally including the possible effect of IMT, which was allowed to differ for participants younger and older than 60 years. IMT was inversely associated with BMD at all bone sites in women >60 years of age and at the radius sites in men >60 years of age ($P < 0.001$ for all). In contrast, increased IMT was marginally correlated with higher hip BMD in women <60 years of age ($P = 0.05$) and with higher ultradistal radius BMD in men <60 years of age ($P = 0.016$). While the exact interpretation of beta values (i.e., the expected change in BMD [mg/cm^2] per 1 SD increase in -IMT^{-1}) is somewhat obscured by our transformation and standardization of IMT values, the relative slopes show a dramatic decline in BMD in older individuals and a modest incline in BMD in younger individuals with increasing IMT. This changing relationship between BMD and IMT with age is illustrated in Figure 1. Compared to the base model, an additional 4–6% of BMD variation in women ($P < 0.00001$ for all) and 5–15% of radius BMD variation in men ($P < 0.001$ for both) were explained by including the effects of IMT.

To determine whether serum risk factors for CVD (LDL cholesterol, LDL median particle diameter, HDL cholesterol, triglycerides, PON1 activity, CRP) were associated

Table 2 Mean (SD) BMD, IMT, and serum CVD risk factors across age strata (years) for women and men

	Women <40 (n = 246)	Women 40–60 (n = 206)	Women >60 (n = 83)	Men <40 (n = 172)	Men 40–60 (n = 113)	Men >60 (n = 50)
BMD (g/cm^2)						
Hip	1.03 (0.14)	1.04 (0.14)	0.89 (0.16)	1.13 (0.15)	1.11 (0.15)	1.07 (0.15)
Spine	1.04 (0.10)	1.03 (0.11)	0.85 (0.15)	1.05 (0.12)	1.07 (0.14)	1.05 (0.16)
One-third radius	0.68 (0.04)	0.67 (0.05)	0.56 (0.08)	0.78 (0.05)	0.79 (0.07)	0.73 (0.10)
Ultradistal radius	0.47 (0.05)	0.47 (0.06)	0.38 (0.08)	0.56 (0.06)	0.54 (0.07)	0.49 (0.07)
CVD risk factors						
LDL cholesterol (mmol/L)	2.52 (0.65)	2.80 (0.82)	2.78 (0.76)	2.66 (0.81)	2.94 (0.81)	2.53 (0.94)
Med. LDL (nm)	26.8 (0.54)	26.7 (0.64)	26.8 (0.59)	26.6 (0.66)	26.5 (0.73)	26.4 (0.59)
HDL cholesterol (mmol/L)	1.24 (0.29)	1.32 (0.34)	1.36 (0.31)	1.18 (0.33)	1.22 (0.38)	1.18 (0.40)
PON1 (umol/min/mL)	77.9 (20.1)	78.3 (19.0)	73.5 (21.2)	78.3 (18.1)	76.2 (18.1)	66.8 (18.7)
Triglycerides (mmol/L)	1.33 (0.74)	1.74 (1.44)	1.63 (0.73)	1.49 (1.10)	1.68 (0.93)	1.69 (0.85)
CRP (mg/L)	2.99 (3.08)	4.61 (6.44)	4.67 (6.29)	1.99 (3.02)	3.02 (4.56)	1.86 (1.58)
-IMT^{-1} (standardized)	–0.63 (0.75)	0.15 (0.73)	0.95 (0.81)	–0.43 (0.90)	0.40 (0.80)	1.39 (0.87)

Table 3 Relationship (beta coefficients, [*P* values]) of IMT with BMD in older (>60 years) and younger (<60 years) women and men (IMT model)

	Hip		Spine		One-third radius		Ultradistal radius	
Women								
Sample size, *n*	445		447		444		455	
LRT *P* value[a]	1×10^{-6}		0.0002		9×10^{-13}		4×10^{-8}	
-IMT^{-1} (standardized)								
<60 years	15.3	[0.05]	—		—		—	
>60 years	–73.0	[3×10^{-10}]	–62.4	[1×10^{-6}]	–43.9	[9×10^{-13}]	–44.1	[4×10^{-13}]
Men								
Sample size, *n*	327		323		285		288	
LRT *P* value	—		—		4×10^{-11}	0.0007		
-IMT^{-1} (standardized)								
<60 years	—		—		—		11.3	[0.016]
>60 years	—		—		–56.9	[4×10^{-11}]	–27.0	[8×10^{-6}]

[a] LRT *P* value from the likelihood ratio test comparing the IMT and base models. Models also include significant effects of age, BMI, menopausal status, and diabetes status (not shown in table). Coefficients shown in "<60 years" and/or ">60 years" rows indicate the magnitude of the relationship differs significantly by age cohort

with BMD, we modeled the effects of these covariates on BMD, allowing them to differ by age group (<60 or >60 years) jointly with those from the base model. Table 4 summarizes the beta coefficients and *P* values for the serum CVD risk factors model. In women >60 years, PON1 activity was positively associated ($P < 0.03$ for all) and HDL cholesterol was modestly negatively associated ($P = 0.08$ to 0.03) with BMD at all bone sites. In contrast, HDL cholesterol was positively associated with spine BMD in men ($P = 0.03$). The correlations of triglycerides and CRP with BMD were inconsistent across bone sites and sexes. Compared to the base model, serum CVD risk factors accounted for an additional 1–3% of BMD variation in women ($P < 0.01$ for all) and 0–3% of BMD variation in men (no effect for hip, $P < 0.02$ for spine and radius sites).

Finally, to assess whether the observed association between BMD and IMT could be explained by serum CVD risk factors, we extended the base model to simultaneously include the effects of IMT and serum CVD risk factors, once again allowing (but not requiring) effects to differ for individuals younger and older than 60 years of age. Mirroring the IMT model, age-specific IMT effects in the combined model were retained for all bone sites in women and radius sites in men (Table 5). Lipid-related risk factors for CVD, including LDL cholesterol, LDL median particle diameter, HDL cholesterol, PON1, triglycerides, and CRP, were associated with BMD at some sites in women or men, though such associations were not consistent across bone sites or sexes ($P = 0.1$–0.001). Specifically, LDL cholesterol was weakly inversely related to hip and spine BMD, and LDL particle size was weakly inversely related to spine BMD in women of all ages ($P < 0.1$). PON1 activity was positively correlated with hip and ultradistal radius BMD in women >60 years of age. In men, the effects of HDL cholesterol and triglycerides mirrored those of the serum CVD risk factors model. CRP was inversely associated with BMD at radius sites in women <60 years of age but directly associated in women >60 years ($P < 0.01$ for all). Based on comparison of parameters and *P* values from the IMT and combined models (Tables 3 and 5), we conclude that the inclusion of serum CVD risk factors did not account for the relationship between BMD and IMT; instead, the effects of lipids, PON, and CRP on BMD were independent of the effects of IMT. The IMT variables were retained in the combined model, and their respective slopes remained nearly constant despite incorporation of lipid, PON1, and CRP covariates. The cumulative effect of all significant serum CVD risk factors accounted for an additional 2–3% of BMD variation in women and 0–2% in men beyond that of the IMT model. Altogether, covariates included in the combined model explained significantly ($P < 0.05$) more variation in BMD than the base model for all sites except hip in men.

Discussion

Mounting evidence supports the concept that vascular and skeletal health are interrelated, with several epidemiological studies demonstrating a relationship between BMD and measures of atherosclerosis/CVD, although the mechanisms mediating this relationship are not understood. Our results, showing an inverse relationship between BMD and IMT (a measure of subclinical atherosclerosis) in older

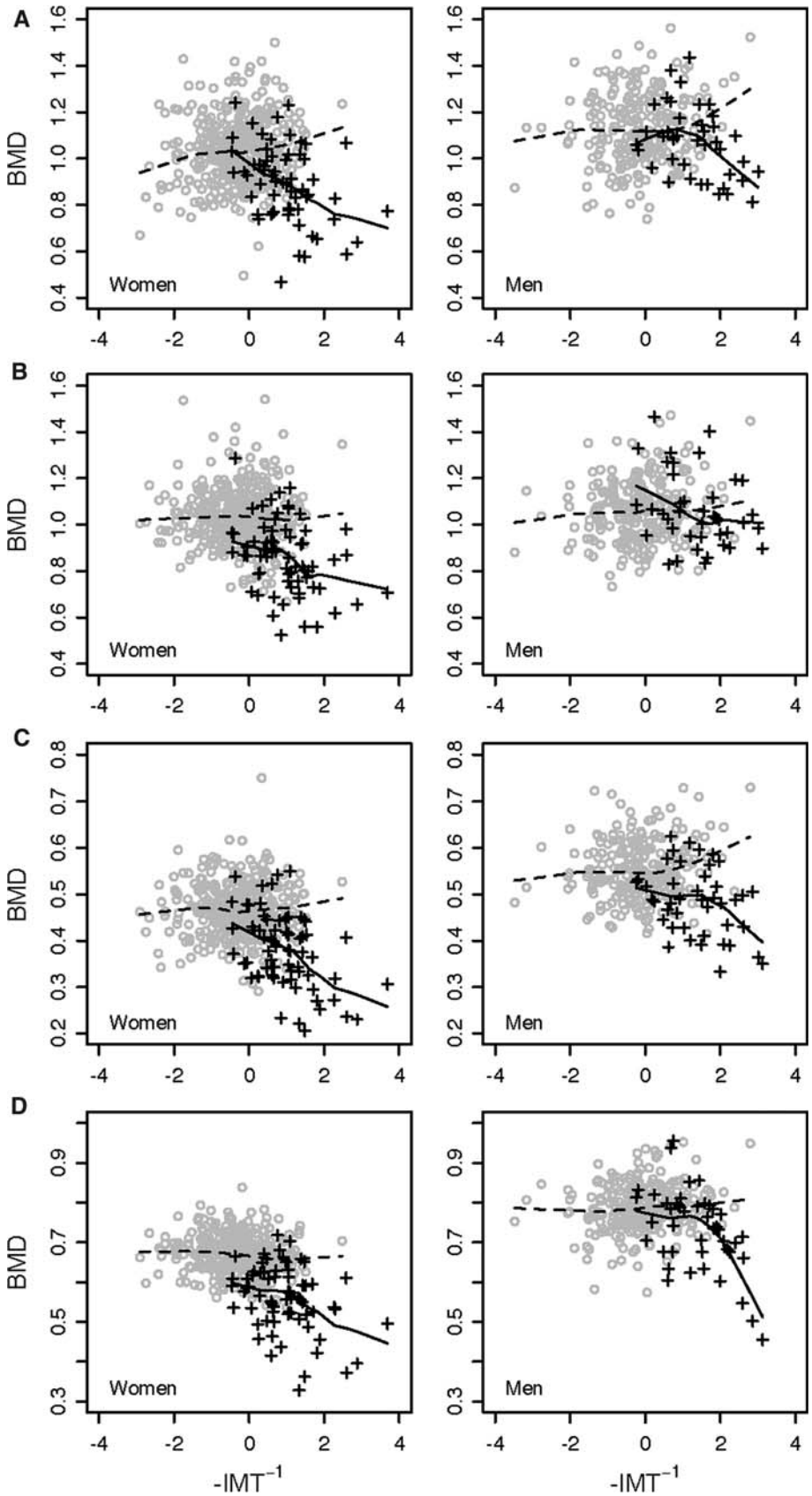

Fig. 1 Relationship (plotted as local regression LOWESS curves) between (**a**) hip, (**b**) spine, (**c**) one-third radius, and (**d**) ultradistal radius BMD and IMT for women and men aged <60 (*gray circles and dashed line*) and >60 (*plus signs and solid line*)

Table 4 Relationship (beta coefficients, [*P* values]) of lipid-related risk factors for CVD with BMD in women and men (serum CVD risk factors model)

	Hip		Spine		One-third radius		Ultradistal radius	
Women								
Sample size, *n*	507		510		491		502	
LRT *P* value[a]	0.005		0.01		0.0004		$3x10^{-5}$	
PON1 activity (μmol/min/mL)								
>60 years	—		241	[0.009]	149	[0.0008]	172	[5×10^{-5}]
HDL cholesterol (LN[mmol/L])								
>60 years	–259	[0.08]	–269	[0.06]	–134	[0.03]	–111	[0.07]
Triglycerides (LN[mmol/L])								
>60 years	—		—		–87.4	[0.01]		
CRP (LN[mg/L])								
All ages	—		—		–15.4	[0.02]	–11.8	[0.08]
Men								
Sample size, *n*	327		316		300		305	
LRT *P* value	—		0.03		0.01		0.007	
PON1 activity (μmol/min/mL)					161	[0.02]		
>60 years								
HDL cholesterol (LN[mmol/L])								
>60 years			305	[0.03]				
Triglycerides (LN[mmol/L])								
All ages					27.2	[0.08]	31.0	[0.03]
CRP (LN[mg/L])								
>60 years							–71.6	[0.02]

[a] LRT *P* value from the likelihood ratio test comparing the serum CVD risk factors and base models. Models also include significant effects of age, BMI, menopausal status, and diabetes status (not shown in table). Coefficients shown in "<60 years" and/or ">60 years" rows indicate the magnitude of the relationship differs significantly by age cohort. Coefficients shown in "All ages" rows indicate the magnitude of the relationship does not significantly differ by age cohort

individuals, corroborate previous studies and support the hypothesis that common etiological factors are involved in bone and vascular disease. However, efforts to identify factors responsible for the mechanistic link between BMD and atherosclerosis and CVD have, as of yet, been unsuccessful [9, 51, 52].

Parhami et al. [8, 13] have suggested that lipid levels may act as an etiological factor for both diseases and proposed that a biological mechanism involving lipid oxidation is the link between the atherosclerotic and osteoporotic risks. One process by which oxidized lipids may contribute jointly to osteoporosis and atherosclerosis is by inducing osteoblastic differentiation in vascular tissue (as shown in vitro) and inhibiting such differentiation in bone cells [8, 13, 53], leading to the buildup of calcium in arteries and simultaneous loss from bone. PON1 interacts with lipids, catalyzing the reduction of oxidized LDL and conferring antioxidant properties on HDL, and therefore is mechanistically a strong candidate gene for modulating the lipid oxidation pathway. Another potential mechanism by which oxidized lipids may affect both BMD and atherosclerosis is by mimicking chronic inflammation. By stimulating an immune response, the accumulation of lipids in vascular tissue may result in mineralization to confine potential infectious agents, whereas a similar accumulation of lipids in bone tissue may result in osteolysis to remove substrate for infectious growth [53].

These two mechanisms may explain the paradox of concurrent low BMD and arterial mineralization and atherosclerosis; however, epidemiological data in support of the oxidized lipid hypothesis are lacking. One study looking at the relationship between lipids (including HDL cholesterol, LDL cholesterol, and triglycerides), atherosclerosis, and osteoporosis failed to find significant associations between serum lipids and BMD at the hip and spine (though serum triglycerides differed in women with and without vertebral fractures) [52]. Similarly, no association between serum PON1 (which metabolizes oxidized lipids) and BMD at the hip and spine was observed by Verit et al. [51]. Likewise, the relationship between CRP and BMD in the third National Health and Nutrition Examination Survey (NHANES) sample was not

Table 5 Relationship (beta coefficients, [*P* values]) of IMT and serum CVD risk factors with BMD in women and men (combined model)

	Hip		Spine		One-third radius		Ultradistal radius	
Women								
Sample size, *n*	436		444		442		450	
LRT *P* value[a]	1×10^{-8}		1×10^{-6}		3×10^{-13}		8×10^{-16}	
LDL cholesterol (LN[mmol/L])								
All ages	–82.1	[0.08]	–77.6	[0.09]	—		—	
LDL particle diameter (nm)								
All ages	—		–20.4	[0.04]	—		—	
HDL cholesterol (LN[mmol/L])								
>60 years	–269	[0.1]	—		–106	[0.1]	—	
PON1 activity (μmol/min/mL)								
>60 years	204	[0.04]	—		—		138	[0.001]
CRP (LN[mg/L])								
<60 years	—		—		–22.3	[0.003]	–18.9	[0.01]
>60 years	—		—		20.9	[0.005]	31.6	[0.001]
-IMT^{-1} (standardized)								
<60 years	19.3	[0.01]	—		—		—	
>60 years	–59.2	[7×10^{-8}]	–62.0	[1×10^{-6}]	–46.1	[3×10^{-13}]	–44.7	[8×10^{-13}]
Men								
Sample size, *n*	327		316		283		281	
LRT *P* value[a]	—		0.032		5×10^{-11}		2×10^{-5}	
HDL cholesterol (LN[mmol/L])								
>60 years	—		305	[0.03]	—		—	
Triglycerides (LN[mmol/L])								
All ages	—		—		30.3	[0.04]	25.5	[0.07]
CRP (LN[mg/L])								
>60 years	—		—		—		–58.1	[0.05]
-IMT^{-1} (standardized)								
<60 years	—		—		—		11.8	[0.01]
>60 years	—		—		–56.5	[5×10^{-11}]	–24.3	[2×10^{-5}]

[a] LRT *P* value from the likelihood ratio test comparing the combined and base models. Models also include significant effects of age, BMI, menopausal status, and diabetes status (not shown in table). Coefficients shown in "<60 years" and/or ">60 years" rows indicate the magnitude of the relationship differs significantly by age cohort. Coefficients shown in "all ages" columns indicate the magnitude of the relationship does not significantly differ by age cohort

significant after adjusting for demographic, anthropometric, and lifestyle covariates [38]. In contrast, in the present study, we identified weak associations between BMD and lipids, PON1, and CRP; however, when analyzed jointly with IMT, the effects were inconsistent across bone sites and sexes and clearly did not account for the same variation in BMD as did IMT. The inconsistency of the relationship between lipids and BMD across bone sites may be due to (1) inadequate power to identify effects of small magnitude, (2) bone site-specific differences in composition or metabolism [54, 55], or (3) serum lipids not accurately reflecting levels of oxidized lipids. However, we have approximately 96% power in women and 84% power in men to detect a covariate effect explaining at least 2% of variation in BMD; thus, we have power to detect any meaningful effects [56]. Nonetheless, while lipid levels and/or inflammation may be responsible for part of the link between these two diseases via a common oxidative mechanism, the serum traits measured in this study did not account for the relationship between low BMD and preclinical atherosclerosis.

Though more work is needed to better elucidate the role of lipids in the relationship between BMD and atherosclerosis, this study makes an important contribution to the growing body of literature in that we identified the relationship between BMD and atherosclerosis risk factors across a broad range of ages (18–97 years) in both women and men. In contrast to most previous studies, we were able to assess the relationship between osteoporosis and CVD risk factors specifically in younger individuals. Weak

positive associations between IMT and BMD were observed for bone sites in both men and women <60 years of age. Positive associations may be spurious or may be due to some unknown mechanism or confounder. One possibility is that our adjustment for BMI may not entirely account for the effects of obesity, which has strong opposite effects on bone and vascular health. The residual effect of obesity not explained by BMI may drive the marginal, yet significant, positive relationship between BMD and IMT in younger individuals at some skeletal sites. In contrast, significant negative associations were observed for all bone sites in women >60 years and radius sites in men >60 years (though nonsignificant bone sites in older men graphically suggest a similar negative trend). These observations suggest that the joint etiology between BMD and IMT is most apparent in older individuals. We speculate that years of exposure to oxidized lipids and/or inflammation in young and middle-aged adults may impact future bone and vascular health, leading to the observed association in older individuals. Longitudinal study designs incorporating measures of oxidized LDL (rather than surrogates) and/or other measures of inflammation (such as interleukin-6) could be used to better investigate the association between bone and vascular disease if a time lag between effect and outcome exists.

A complementary hypothesis, that reduced blood flow to bone resulting from impaired vascular function accounts for the mechanistic link between atherosclerosis and osteoporosis, has also been proposed [57]. This idea is supported by epidemiological evidence [11, 57, 58], though blood flow did not fully explain associations between vascular disease and BMD and does not explain the modest positive association demonstrated in individuals <60 years in this study. However taken together, the oxidized lipids and reduced blood flow hypotheses may act as parallel processes that explicate part of a more complicated joint etiology of atherosclerosis and osteoporosis. Though the mechanisms linking bone and vascular diseases are unknown, one protein that is certainly involved in their joint pathogenesis is osteoprotegerin (OPG), a soluble decoy receptor that intercepts the receptor activator of nuclear factor κB (RANK) ligand, a bone resorption signaling molecule [58, 59]. OPG knockout mice develop severe osteoporosis [60], and human OPG gene polymorphisms are associated with BMD in postmenopausal women [61]. Interestingly, OPG knockout mice also experience arterial calcification [60], and human OPG gene promoter polymorphisms are associated with both IMT and blood flow [62], clearly implicating OPG and the RANK ligand as regulators of both bone metabolism and vascular calcification. Another protein possibly involved in the link between BMD and IMT is klotho, a general antiaging factor, which, when deficient in mice, leads to both severe osteoporosis and progressive atherosclerosis [6]. Other unknown factors may also act in parallel on both BMD and IMT, contributing toward the indirect relationship between osteoporosis and atherosclerosis observed in older individuals.

While this study has several strengths that contributed to our detection of the associations between BMD, IMT, and CVD risk factors, including our well-characterized population sample and assessment of multiple serum traits associated with CVD, specific limitations also require consideration. First, our sample size is relatively small, especially for male subjects >60 years of age, and limits our power to detect small effects, possibly explaining the inconsistent effects of serum lipids, PON1, and CRP on BMD across bone sites and sexes. Second, estrogen deficiency, which has large effects on BMD, was indirectly assessed via age and menopausal status; and adjustment for these covariates may not have completely accounted for the effects of estrogen levels. Third, PON1 levels were measured approximately 5 years prior to DXA bone measurements; thus, our results reflect possible effects of serum PON1 levels on future BMD. The elapsed time between PON1 and BMD measurements, however, should not seriously affect our analyses as both diseases of interest, osteoporosis and atherosclerosis, are conditions that progress over the course of many years.

In summary, we have identified an association between subclinical indicators of osteoporosis and cardiovascular health in both Mexican American men and women and shown that this relationship changes with age. In the young and middle-aged, BMD is modestly positively associated with IMT, whereas in older individuals, BMD is acutely inversely associated with IMT. Moreover, we show that serum CVD risk factors, including LDL cholesterol, HDL cholesterol, LDL particle size, triglycerides, PON1, and CRP, do not account for the concurrent relationship between BMD and IMT. Still, the effects of lipid-related risk factors in younger individuals may be manifest in older individuals. Our findings provide insight into the pathomechanism(s) connecting these diseases; however, complete understanding of how atherosclerosis and osteoporosis are biologically interrelated requires deeper investigation.

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