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Relationships Between Calcified Atherosclerotic Plaque and Bone Mineral Density in African Americans With Type 2 Diabetes

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Abstract

Inverse relationships have been reported between bone mineral density (BMD) and calcified atherosclerotic plaque (CP). This suggests these processes may be related. We examined relationships between BMD and CP in 753 African Americans with type 2 diabetes from 664 families, accounting for the effects of modifiable cardiovascular disease (CVD) risk factors. Association analyses were performed using generalized estimating equations (GEEs) to assess cross-sectional relationships between computed tomography–determined measures of thoracic and lumbar vertebral volumetric BMD (vBMD) and CP in the coronary and carotid arteries and infrarenal aorta. Significant inverse associations were seen between thoracic and lumbar vBMD and CP in all three vascular beds in unadjusted analyses. A fully adjusted model accounting for age, sex, body mass index, systolic blood pressure, low-density lipoprotein cholesterol, C-reactive protein, hemoglobin A_{1c}, smoking, and hormone-replacement therapy revealed significant inverse associations between thoracic vBMD and CP in coronary and carotid arteries and aorta, whereas lumbar vBMD was associated with CP in coronary artery and aorta. Inverse associations exist between vertebral BMD and calcified atherosclerotic plaque in African-American men and women with type 2 diabetes. This relationship was independent of conventional CVD risk factors and

Disclosures

All the authors state that they have no conflicts of interest.

supports the hypothesis that bone metabolism and atherosclerotic plaque mineralization are related processes.

Keywords

AFRICAN AMERICANS; ATHEROSCLEROSIS; CALCIFIED PLAQUE; BONE MINERAL DENSITY; DIABETES MELLITUS; OSTEOPOROSIS

Introduction

Osteoporosis or low bone mineral density (BMD) and atherosclerosis frequently coexist. Several lines of evidence suggest these are related disease processes because inverse relationships between BMD and calcified atherosclerotic plaque (CP) have been observed in postmenopausal women in a variety of population-based studies.^(1–8) The relationship between osteopenia (lower bone mass) and development of CP [with higher cardiovascular disease (CVD) morbidity and mortality] was noted in the placebo arm of the Multiple Outcomes of Raloxifene Evaluation (MORE),⁽⁴⁾ in postmenopausal Danish women,⁽⁵⁾ and in the Study of Osteoporotic Fractures (SOF).⁽⁶⁾ Two longitudinal studies also revealed inverse relationships between rates of change in BMD and CP.^(7,8) These effects appeared to be strongest in women, perhaps owing in part to the beneficial effects of endogenous estrogen on skeletal and cardiovascular health.

A prior Diabetes Heart Study (DHS) report revealed that CP was inversely related to BMD in European-American men and a subset of African-American women, with significance persisting after adjustment for age, sex, race, body mass index (BMI), smoking, and prevalent CVD in this type 2 diabetes (T2D)–enriched cohort.⁽⁹⁾ The inverse relationship between BMD and CP in the DHS was weaker in European-American women and was strongly affected by age and gender. BMD measured by dual-energy X-ray absorptiometry (DXA) or by quantitative computed tomography (QCT) was not independently associated with the presence of diabetes in these subjects.⁽¹⁰⁾ Potential benefits of using QCT-measured BMD include responsiveness of the trabecular compartment and reduced confounding effects of degenerative joint disease and body size. A subsequent report limited to the European-American subset with T2D from the DHS revealed that polymorphisms in the *bone morphogenetic protein 7 (BMP7)* gene were significantly associated with inverse relationships between QCT BMD and CP in the coronary and carotid arteries and abdominal aorta.⁽¹¹⁾

Relative to European Americans, African Americans generally ingest less dietary calcium yet manifest higher BMD values (lower rates of osteoporosis), lower levels of vascular CP, and reduced formation of calcium-containing kidney stones.^(12–14) Therefore, African Americans represent a unique population to test for associations between BMD and CP. The relationship between bone mineralization and vascular disease has not been examined in large numbers of African Americans with T2D. Here these relationships were analyzed in the full sample of 753 African Americans with T2D recruited in the original DHS⁽¹⁵⁾ and follow-up African-American DHS.⁽¹⁶⁾ Analyses assessed whether effects of conventional

CVD risk factors potentially mediated observed relationships between volumetric BMD (vBMD) and CP.

Research Design and Methods

Study Populations

The DHS recruited siblings concordant for T2D from internal medicine clinics and community advertising.⁽¹⁷⁾ Diabetes was diagnosed after age 34 years in the absence of historical evidence of ketoacidosis. Additional unrelated African Americans with diabetes were recruited in the African-American DHS using the same diagnostic criteria, except that T2D was diagnosed after age 30 years.⁽¹⁶⁾ Measures of CP in subjects who had undergone prior coronary artery bypass graft surgery or carotid endarterectomy were excluded because the CP mass score in relevant arteries would be affected by these procedures. Participants with prior myocardial infarction or stroke were included. Four female subjects were receiving bisphosphonates; no other antiresorptive agents were reported except for hormone-replacement therapy (HRT). We included these four subjects because they comprised only 0.53% (4 of 753) of the sample and did not significantly affect the analyses. The study was approved by the Institutional Review Board at the Wake Forest University School of Medicine (WFUSM), and participants provided written informed consent. Examinations were conducted in the General Clinical Research Center, including completion of medical questionnaires and measurement of vital signs and clinical chemistries. Fasting glucose, hemoglobin A_{1c}, and lipid profiles were performed at LabCorp (Burlington, NC, USA; methods: www.labcorp.com). High-sensitivity C-reactive protein was measured in the Wake Forest Hypertension Center Core Laboratory using an enzyme-linked immunosorbent assay (Alpco Immunoassays, Salem, NH, USA). History of prior CVD was provided by self-report and chart review.

Vascular imaging

CP was measured in the coronary (CorCP) and carotid arteries (CarCP) and infrarenal abdominal aorta (AorCP) using single- and multidetector computed tomography (CT) systems incorporating a standardized scanning protocol based on those currently implemented in the National Heart Lung and Blood Institute's Multi-Ethnic Study of Atherosclerosis (MESA), as reported previously.⁽¹⁸⁾ The traditional Agatston unit or calcium score has been used often to report results, but when compared with volume-based measures of CP, this scoring system adds noise to CT measurements.⁽¹⁹⁾ This report uses the calcium mass score based on the milligrams of calcium hydroxyapatite (SmartScore, GE Healthcare, Waukesha, WI, USA) within the plaque and is calculated from the plaque volume score calibrations of the CT number to calcium mass on a pixel-by-pixel basis. In this report, the mass score provides a direct comparison of the amount of calcified plaque in each of the vascular territories.⁽¹⁶⁾ Additional scoring parameters included a 90 Hounsfield unit (HU) threshold and two adjacent pixels to define the minimum calcified lesion size. The reproducibility of coronary and carotid CP measures obtained from sequential scans and the inter- and intraobserver variability in performing the analysis are all greater than 0.96. In addition, we documented the comparability and accuracy of current-generation multidetector CT systems and demonstrated the comparability in a large multicenter study with a variety

of CT systems.^(19–21) Figure 1 contains an image from a cardiac CT scan with extensive coronary artery CP.

Bone imaging

Quantitative CT (QCT) for trabecular vBMD (mg/cm^3) in the thoracic spine (T_8 – T_{11}) and lumbar spine (T_{12} – L_3) was measured using QCT-5000 volumetric software (Image Analysis, Columbia, KY, USA) with an external calibration phantom from the same CT images used to measure CP in the coronary arteries and aorta, respectively. Coefficients of variation for these measures have been reported previously and were less than 1% for thoracic and lumbar BMD, and in sequential studies performed in the same individual, the precision error was 2.3%.^(9,10,22) Figure 2 shows measures of trabecular vBMD using QCT in the thoracic and lumbar spine.

Statistical methods

A series of generalized estimating equations (GEEs) assuming exchangeable correlation and using the empirical estimate of the variance to adjust for familial correlation was computed to test for associations between CP and thoracic and lumbar vBMD.⁽²³⁾ The Box-Cox method was applied to identify the appropriate transformation of each outcome variable that would best approximate the distributional assumptions of conditional normality and homogeneity of variance of the residuals.⁽²⁴⁾ This method suggested taking the natural log of $(\text{CorCP} + 1)$, $(\text{CarCP} + 1)$, and $(\text{AorCP} + 1)$ to minimize the influence of extremely large covariate values on parameter estimates in the models. Age, sex, and body mass index (BMI) adjustments were incorporated after an initial unadjusted analysis. The final analysis further adjusted for low-density lipoprotein (LDL) cholesterol, systolic blood pressure, hemoglobin (Hb) A_{1c} , highly sensitive C-reactive protein (hsCRP), smoking, and HRT in women. Standard regression diagnostics for collinearity and influence were computed for each model reported.

Results

The 753 participants consisted of 196 individuals from 87 families in the DHS and 557 additional unrelated subjects in the African-American DHS. Demographic characteristics of the study group are listed in Table 1. Relative to women, men had significantly lower mean BMI and were more likely to be current and former smokers. Approximately 22% of women were receiving HRT. Table 2 contains the results of laboratory and imaging tests. Relative to women, men had significantly lower mean total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, hsCRP, calcium, phosphorus, and intact parathyroid hormone (iPTH) levels. As expected, men had significantly higher mean serum creatinine concentrations, estimated glomerular filtration rates, diastolic blood pressures, and CorCP mass scores, with a tendency toward higher CarCP and AorCP.

Across all subjects, coronary artery CP and infrarenal aorta CP were inversely associated with thoracic and lumbar vBMD in unadjusted, partially adjusted (age, sex, and BMI), and fully adjusted models (Table 3). Carotid artery CP was inversely associated with thoracic vBMD in unadjusted and fully adjusted models but was inversely associated with lumbar

vBMD only in the unadjusted model; the effect disappeared after age and sex adjustment. We targeted an overall type 1 error rate of 0.05. However, owing to the strong correlations between CP across the three vascular beds and vBMD across the two vertebral regions, a strict Bonferroni correction [p value = $.05 \div (2 \times 3) = 0.008$] was felt likely to be too stringent. The observed relationship between CorCP and thoracic vBMD and AorCP and thoracic vBMD remain significant, even after strict Bonferroni correction.

In sex-stratified analyses using fully adjusted models (Table 4), relationships between coronary CP and thoracic and lumbar vBMD appear to be similar in men and women. Thoracic vBMD was more highly associated with aortic CP in men and with carotid CP in women.

Discussion

This report evaluated cross-sectional relationships between computed tomography–determined thoracic and lumbar vertebral vBMD and CP in three vascular beds in a large sample of African Americans with T2D. As in nondiabetic subjects, inverse associations between CP and BMD were observed consistently in unadjusted and fully adjusted models, except for a nonsignificant association between lumbar vBMD and carotid artery CP. The inverse relationships between bone mineralization and atherosclerosis in African Americans with T2D did not appear to be mediated by conventional CVD risk factors because they remained significant after adjustment for these factors. To address clinical relevance of this association, we defined dichotomized levels of CP in each vascular bed at their 75th percentile denoting severe disease (levels corresponded to 613 for CorCP, 100 for CarCP, and 6366 for AorCP) and also created an indicator variable to identify individuals with significant bone disease defined as either osteopenia or osteoporosis (thoracic vertebrae QCT BMD < 120 mg/cm³; approximately 6% of our study sample had bone disease using this definition). A chi-square statistic was computed to test for association between each of the dichotomized CP variables and the binary measure of BMD. The minimum chi-square value was 15.5, 1 degree of freedom (p value = 8.2×10^{-5}). Based on this, we believe that these are clinically important relationships.

It is likely that inherited factors in concert with environmental exposures mediate the inverse associations between vBMD and CP. Investigators have been searching for unifying biologic factors that might underlie bone and vascular disease as a single and related phenotype. It is unclear whether loss of bone mineralization and development of CP are biologically linked processes in African Americans without kidney disease. It is possible that CP and osteoporosis develop coincidentally with aging in Western societies as a result of alterations in circulating hormonal (estrogens) and other factors that influence both processes or through perturbations in one system subsequently influencing the other system. Examples of the latter process includes the possibility that atherosclerotic vascular disease, the prime determinant of CP formation, results in altered blood flow to the skeleton and a net loss of bone. It is also possible that elevated bone resorption increases circulating ions and other molecular species that promote vascular calcification.

The most direct potential mechanism linking bone loss to vascular calcification involves an elevated release of calcium (Ca^{2+}) and phosphate (P_i) from bone, perhaps in the form of CaP_i complexes, via enhanced osteoclast-mediated resorptive processes. These mineral ions and/or complexes then may localize to vascular sites and provide a nidus for future mineralization or lead to locally elevated Ca^{2+} and/or P_i levels that promote CaP_i precipitation in the artery.^(25,26) Alternatively, in vitro studies suggest that under conditions such as locally elevated P_i concentrations, vascular smooth muscle cells may assume an osteoblastic phenotype and deposit calcium hydroxyapatite.^(27–31) Alkaline phosphatase may play a role in this process, as was shown in endochondral calcification.^(32,33) Estrogen also may play a role in this inverse relationship because estrogen has atheroprotective effects^(34,35) and inhibits vascular mineralization⁽³⁶⁾ while simultaneously preventing bone loss in women as well as men.⁽³⁷⁾ Metabolically, liberation of calcium from the skeleton during accelerated bone turnover and subsequent mineral deposition at sites of atherosclerosis and necrosis in blood vessel walls provides an attractive mechanism potentially linking loss in bone density with CVD.

Genetic abnormalities associating bone turnover with atherosclerotic plaque calcification strengthen the link as the mechanism of association between osteoporosis and risk of CVD. Polymorphisms in genes involved in both osteoporosis and vascular disease are associated with these phenotypes, including *KLOTHO* (the coreceptor for fibroblast growth factor 23), *osteoprotegerin* (*OPG*), and *bone morphogenetic protein 7* (*BMP7*), supporting the link between bone turnover and vascular calcification.^(11,38–43)

There are limitations to this report. Conclusions were derived from a cross-sectional analysis in those with T2D. Replication in studies with longitudinal measures of BMD and CP remain to be performed, but few such studies exist in African Americans. The population-based MESA is reevaluating participants, although MESA is not enriched for T2D.⁽⁴⁴⁾ DXA was not used to assess BMD in African-American DHS participants. Instead, we used QCT to precisely quantify trabecular bone in the thoracic and lumbar vertebrae. QCT has been applied to the diagnosis of osteoporosis. The American College of Radiology (ACR 2008 Guidelines) suggests that spinal trabecular QCT vBMD values below 80 mg/m^3 represent diagnostic criteria for osteoporosis, with osteopenia defined as a vBMD between 80 and 120 mg/m^3 . Based on these criteria, 3 study participants had osteoporosis (2 males and 1 female) and 41 had osteopenia (15 males and 26 females); we believe the low frequency of osteoporosis was strongly related to African-American race and relative obesity. Therefore, our results link lower levels of vBMD with presence of CP in subjects with low likelihoods of developing osteopenia or osteoporosis.

We conclude that an inverse relationship exists between bone mineralization and atherosclerosis in African Americans with T2D, an effect that is not mediated by the presence or severity of conventional CVD risk factors. A number of biologic factors may link bone health with susceptibility to CP. It remains possible that exposure to novel CVD risk factors differentially affects bone and blood vessel health in members of different races based on genetic predisposition.

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J Divers and TC Register contributed equally to this article.

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Fig. 1. Cardiac CT scan, low dose and without intravenous contrast material, demonstrating extensive calcified plaque within the walls of the left anterior descending (*arrows*) and circumflex (*) coronary arteries.

Fig. 2. Measurement of trabecular bone mineral density (BMD) using quantitative CT in the thoracic and lumbar spine. The CT images are re-formatted and a region of interest defined within the vertebral bodies to measure the trabecular BMD calibrated to an external phantom positioned behind the participant's spine (*dashed rectangle*).

Table 1

Demographic Characteristics of the Study Group

Variable	Measure	Women (n = 450)		Men (n = 303)		Full sample (n = 753)		p Value ^a
		Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median	
Age	Years	56.3 (9.5)	56.0	56.7 (9.6)	56.0	56.4 (9.6)	56.0	.6442
BMI	kg/m ²	37.0 (8.6)	36.0	32.2 (7.2)	31.0	35.0 (8.4)	33.7	<.0001
Age T2D onset	Years	46.0 (9.9)	46.0	46.6 (10.0)	46.0	46.3 (9.9)	46.0	.5087
T2D duration	Years	10.3 (7.7)	8.0	10.6 (8.6)	8.0	10.4 (8.1)	8.0	.8379
DBP	mmHg	76.4 (11.7)	76.0	78.6 (10.9)	79.0	77.3 (11.4)	77.0	.0086
SBP	mmHg	136.2 (20.8)	134.0	133.5 (18.5)	132.0	135.1 (19.9)	133.0	.1337
Variable	Measure	n ^b	%	n ^b	%	n ^b	%	p Value ^a
ACE	Yes	176	40.4	129	43.9	305	41.8	.3456
ARB	Yes	61	14.0	32	10.9	93	12.7	.2170
HRT	Yes	99	21.2					
Smoking	Past	151	32.5	129	41.9	280	36.2	<.0001
Smoking	Current	86	18.5	95	30.8	181	23.4	<.0001
Statins	Yes	211	45.1	140	45.5	351	45.2	.9195
AorCP	>0	335	80.3	250	86.8	585	83.0	.0246
CarCP	>0	246	55.8	176	59.5	422	57.3	.3225
CorCP	>0	354	79.7	237	80.1	591	79.9	.9106

BMI = body mass index; T2D = type 2 diabetes; DBP = diastolic blood pressure; SBP = systolic blood pressure; ACE = use of angiotensin-converting enzyme inhibitors; ARB = use of angiotensin-receptor blockers; HRT = hormone-replacement therapy; AorCP = infrarenal aorta calcified plaque; CarCP = carotid calcified plaque; CorCP = coronary calcified plaque.

^a p Value for differences between the sexes.

^b Among those with available data.

Table 2

Laboratory and Imaging Results of Study Sample

Variable	Units	Women (n = 450)			Men (n = 303)			Combined (n = 753)			p Value ^a
		Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	
Total cholesterol	mmol/L	4.85	1.13	4.71	4.53	1.28	4.43	4.72	1.20	4.61	<.0001
LDL cholesterol	mmol/L	2.89	0.98	2.77	2.70	0.99	2.67	2.82	0.99	2.73	.0067
HDL cholesterol	mmol/L	1.31	0.38	1.27	1.14	0.33	1.11	1.24	0.37	1.19	<.0001
Triglycerides	mmol/L	1.43	1.05	1.18	1.54	1.75	1.19	1.47	1.37	1.18	.9779
High-sensitivity CRP	nmol/L	10.48	17.14	5.71	6.67	9.52	2.86	8.57	15.24	4.76	<.0001
Fasting glucose	mmol/L	8.34	3.67	7.44	8.76	4.14	7.71	8.51	3.87	7.55	.2285
Hemoglobin A _{1c}	Proportion	0.083	0.023	0.077	0.082	0.020	0.078	0.083	0.022	0.077	.9639
Coronary calcified plaque	Mass score	612.0	1611.0	26.8	1002.8	1994.0	115.3	768.3	1783.0	52.0	.0014
Carotid calcified plaque	Mass score	147.6	463.0	3.0	210.0	614.3	9.3	172.7	529.5	4.5	.0708
Aorta calcified plaque	Mass score	5003.8	9124.0	828.0	5802.7	11507.0	1198.0	5330.2	10165.0	1025.0	.0695
Thoracic vBMD	mg/cm ³	200.5	55.8	198.4	193.5	50.2	190.8	197.7	53.7	195.7	.1319
Lumbar vBMD	mg/cm ³	174.8	49.9	170.5	173.7	44.2	172.2	174.3	47.6	170.7	.8787

LDL = low density lipoprotein; HDL = high density lipoprotein; CRP = C-reactive protein; vBMD = volumetric bone mineral density.

^a p Value for differences between the sexes.

Table 3

Relationships Between Vertebral vBMD and CP

Vascular Bed	Adjustment	Thoracic vBMD			Lumbar vBMD		
		Estimate	SD	p	Estimate	SD	p
Coronary artery calcified plaque	Unadjusted	-0.0125	0.002	<.0001	-0.0158	0.0022	<.0001
	Age and sex	-0.0038	0.0019	.0487	-0.0043	0.0023	.0659
	Age, sex, and BMI	-0.0045	0.002	.0202	-0.005	0.0024	.0348
Infrarenal aorta calcified plaque	Full ^a	-0.0061	0.0021	.0031	-0.0059	0.0024	.0153
	Unadjusted	-0.0191	0.0024	<.0001	-0.0238	0.0028	<.0001
	Age and sex	-0.0070	0.0022	.0015	-0.0075	0.0028	.0075
Carotid artery calcified plaque	Age, sex, and BMI	-0.0061	0.0022	.0062	-0.0068	0.0029	.0183
	Full ^a	-0.0065	0.0023	.0056	-0.0067	0.003	.0239
	Unadjusted	-0.0114	0.0018	<.0001	-0.013	0.0021	<.0001
	Age and sex	-0.0040	0.0018	.0244	-0.0026	0.0022	.2372
	Age, sex, and BMI	-0.0035	0.0018	.0471	-0.0021	0.0022	.3344
	Full ^a	-0.0036	0.0018	.0416	-0.0016	0.0022	.4498

^aFull adjustment included age, sex, BMI, systolic blood pressure, LDL cholesterol, CRP, HbA1c, smoking, and HRT.

Table 4
Relationships Between Vertebral vBMD and CP: Fully Adjusted Models Stratified by Sex

Outcome	Predictor	Men				Women			
		Estimate	SD	Upper, lower confidence interval	p Value	Estimate	SD	Upper, lower confidence interval	p Value
Coronary artery calcified plaque	Thoracic vBMD	-0.0081	0.0032	-0.0144, -0.0019	.011	-0.0048	0.0026	-0.0099, 0.0002	.062
	Lumbar vBMD	-0.0068	0.0036	-0.014, 0.0003	.062	-0.0055	0.0032	-0.0116, 0.0007	.083
Aorta calcified plaque	Thoracic vBMD	-0.0099	0.0037	-0.0171, -0.0026	.008	-0.0035	0.003	-0.0093, 0.0023	.235
	Lumbar vBMD	-0.0087	0.0047	-0.0179, 0.0005	.065	-0.0041	0.0039	-0.0118, 0.0035	.290
Carotid artery calcified plaque	Thoracic vBMD	-0.0093	0.0193	-0.0471, 0.0286	.632	-0.0036	0.0021	-0.0077, 0.0005	.088
	Lumbar vBMD	0.0234	0.0146	-0.0052, 0.0519	.109	-0.002	0.0025	-0.0069, 0.003	.436