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Calcified Atherosclerotic Plaque and Bone Mineral Density in Type 2 Diabetes:

The Diabetes Heart Study

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Abstract

The purpose of the present study was to determine the relationships between atherosclerotic calcified plaque (CP) and bone mineral density (BMD) in subjects with type 2 diabetes mellitus (DM2). CP in the coronary arteries, carotid bifurcation, and abdominal aorta was measured using computed tomography (CT) in 1,023 diabetic subjects from 453 families. Trabecular volumetric BMD in thoracic (T-vBMD) and lumbar (L-vBMD) spine was measured with quantitative CT (QCT), while areal BMD (aBMD) in the lumbar spine and hip was measured by dual X-ray absorptiometry (DXA). Correlation coefficients were computed to assess the magnitude of associations and generalized estimating equations (GEE1) were used to make statistical inferences while accounting for familial correlation. Subjects were 53.8% female, 85% European American (EA) and 15% African American (AA). After adjustment for age, significant inverse associations between CP and vBMD persisted in EA men (correlations between -0.11 and -0.16, all $p < 0.05$ with the exception of carotid CP vs. T-vBMD, $p=0.076$) and in AA women, excluding aortic CP, (correlations between -0.16 and -0.25, all $p < 0.05$). Estrogen use in AA but not EA women was consistently associated with an inverse relation between CP and vBMD. Significant inverse relationships between CP and vBMD were observed in EA men and AA women with DM2 after adjusting for age and other covariates. QCT determined vBMD was more strongly related to CP than aBMD by DXA. The relation between CP and BMD in diabetes is influenced by age, sex, and ethnicity, with further effect modification by hormone replacement therapy.

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Keywords

atherosclerosis; type 2 diabetes mellitus; osteoporosis; trabecular bone mineral density; calcium; computed tomography

Introduction

In 1964, Anderson, Barnett and Nordin first suggested that aortic calcification and low bone mineral content were related disorders(1). This prescient observation was initially met with skepticism. The common and often co-existing processes of osteoporosis and atherosclerosis were previously considered to be independent degenerative phenomena associated with aging. Cross-sectional studies in post-menopausal women have documented the coincident occurrence of low bone mineral density (BMD) and atherosclerosis, and low bone mass has been linked to cardiovascular disease (CVD) incidence (1bc), as well as morbidity and mortality. In the placebo arm of the Multiple Outcomes of Raloxifene Evaluation (MORE) trial, post-menopausal women with low bone mass and no vertebral fractures had 8.3 incident CVD events per 1000 patient years, compared to 15.1 such events in women with prevalent vertebral fractures(2). An incremental increase in CVD events was detected for each degree of decline in BMD(3). Similarly, a 1.31-fold increase in risk of stroke was found for each standard deviation decline in BMD in the Study of Osteoporotic Fractures(4).

Longitudinal studies also demonstrate relationships between bone mass and aortic calcified plaque. Twenty five year follow-up data from the Framingham Heart study demonstrated that in women, but not men, the percentage decline in cortical bone in the second metacarpal was significantly associated with the increase in aortic calcified plaque assessed from a lateral lumbar radiograph(5). In 2348 healthy postmenopausal women, Schulz et al. reported that aortic calcified plaque assessed by computed tomography (CT) was inversely related to BMD and directly related to fracture risk(6). A significant percentage (26.1%) of the variance observed in BMD was accounted for by aortic calcified plaque after adjustment for age and other confounders. In a subgroup analysis of women with longitudinal data, the increase in aortic calcium explained 47% of the observed decline in BMD. Women in the highest quartile for gain in aortic calcified plaque had four times the annual bone loss of those in the lowest quartile(6). The inverse relationship between these variables was consistently observed regardless of the initial BMD or vascular calcium score. In another large study of 2,662 generally healthy postmenopausal women, aortic calcification was associated with lower BMD, accelerated bone loss in the proximal femur, and future risk of fracture in the hip (7).

Diabetes may influence the relationships between CP and BMD. Prior studies have shown that older diabetic subjects have higher dual X-ray absorptiometry (DXA)-measured bone mass than non-diabetic subjects. Despite this observation, the fracture risk in diabetic subjects was 1.64 times higher after adjustment for hip BMD and other fracture risk factors(8-11). Our prior work with the Diabetes Heart Study (DHS) families indicates that BMD (areal BMD by DXA as well as volumetric BMD of vertebral trabecular bone by Quantitative CT [QCT]) is not independently associated with the presence of diabetes after adjustment for body mass index (BMI) and other covariates (12). Available data suggests that DM2 does not lead to lower BMD by either DXA or QCT in the spine, yet diabetics have increased CVD risk and a higher burden of CP.

In the present study we evaluated whether the inverse relationship often observed between CP and BMD is also present in subjects with DM2, specifically with respect to BMD in various skeletal compartments (e.g. vertebral trabecular bone, areal measures of BMD in the hip and spine) in diabetic DHS participants.

Methods

Study Population

The DHS recruited a bi-racial cohort of families containing $\geq$ two siblings with DM2. Probands were identified through community advertising and local clinics in northwestern North Carolina. Diabetic index cases diagnosed after the age of 34 years, in the absence of historical evidence of diabetic ketoacidosis and having one additional type 2 diabetic sibling were recruited. This report is limited to observations in type 2 diabetic participants, defined as above and receiving clinical treatment for hyperglycemia (oral agents and/or insulin) or having a fasting blood glucose > 126 mg/dl. The study was approved by the Institutional Review Board at the Wake Forest University School of Medicine and all participants provided written informed consent.

The examination included interviews for medical history, current medications and health behaviors. We did not adjust CP for use of anti-resorptive agents (i.e., bisphosphonates, calcitonin, selective estrogen receptor modulators) since fewer than 2% of DHS participants took these agents. We also did not adjust for glucocorticoid usage, taken by 6% of DHS participants, since they did not impact BMD in these analyses (data not shown). Measurements of body size, resting blood pressure, 12-lead electrocardiogram, fasting blood draw and urine collection were conducted in the General Clinical Research Center. History of CVD was provided by participant self-report. We excluded subjects with elevated serum creatinine concentrations (≥ 1.6 mg/dl in men, ≥ 1.4 mg/dl in women) due to potential effects of kidney failure on BMD and CP. Stratified analyses were performed in those reporting prior coronary artery bypass surgery or coronary artery stents (however, these procedures did not significantly alter associations).

Vascular Imaging

Calcified atherosclerotic plaque was measured in the coronary arteries using single and multidetector CT systems incorporating a standardized protocol based on those currently implemented in the National Heart Lung and Blood Institute's (NHLBI) Multi-Ethnic Study of Atherosclerosis (MESA) studies(13). From the initiation of CT exam in 1999, three versions of the General Electric CT system have been used (CTi, LightSpeed QXi & Pro16 system, General Electric Medical Systems, Waukesha, WI) capable of 520 msec, 520 msec and 244 msec temporal resolutions, respectively, when operating in the cardiac mode. The robustness of the coronary calcium score using various CT systems with differing temporal resolutions in this range has been established(13-15). Other technical factors were: 120 KVp, 106 mAs (weight < 220 lbs) or 133 mAs (weight ≥ 220 lbs) and 2.5 mm slice collimation (3 mm for the CTi system) and reconstruction with the standard kernel. Images were obtained during suspended respiration and with ECG gating at 50% of the RR interval. A calibration standard (Image Analysis, Columbia, KY) was placed underneath each participant during the scans. In addition to daily calibrations, twice a month calibration scans for measurement of calcium hydroxyapatite were performed and recorded to document stability of the CT systems measurement of CT numbers and calcium concentrations to document the absence of temporal drift. Calcified plaque measured in each of the epicardial coronary vessels was summed to create the total calcified plaque burden. Two cardiac scans were performed sequentially and the average of the two measurements was used in analysis. For the carotid exam, an un-enhanced CT scan was performed through the neck from C2-3 to the C6-7 disc levels covering the right and left carotid bifurcations using a helical acquisition with 2.5-mm or 3-mm slice collimation depending on CT scanner capability, a 120 kv, 280 mA, 0.8 second gantry rotation, 360 degree scan reconstruction and standard reconstruction kernel. The display field of view was 18 cm resulting in pixel dimensions of 0.35 mm $\times$ 0.35 mm. To measure calcified plaque in the abdominal aorta, an un-enhanced scan of the abdomen was performed. The technical

factors for this series were 120 kV, 250 mA, 0.8 second gantry rotation helical mode (7.5 mm/s), 2.5 mm slice thickness and standard reconstruction kernel. The display field of view was 35 cm, resulting in a pixel dimension of 0.68 by 0.68 mm.

CT exams in these vascular beds were analyzed by experienced analysts producing measures of calcified plaque the Agatston score corrected for slice thickness on a GE Advantage Windows Workstation using the SmartScores software package (General Electric Medical Systems, Waukesha, WI). The reproducibility of coronary and carotid calcified plaque scores obtained from the duplicate scans and inter and intra-observer variability were all > 0.96 . As previously reported by our group, helical CT scans yield comparable vascular calcium measurements as electron beam CT (EBCT)(15).

Bone Imaging

DXA scans of posterior-anterior spine and proximal femur obtained using a fan-beam scanner (Delphi A, Hologic, Waltham, MA, USA) to determine BMD(16). Quantitative computed tomography (QCT) trabecular BMD of the thoracic and lumbar vertebrae were measured using the same CT images obtained for measuring calcified plaque in the coronary and abdominal aorta using our previously published methods(17). Volumetric BMD (vBMD) was measured in the thoracic spine (T8-T11) and lumbar spine (T12-L3) using QCT-5000 software (Image Analysis, Columbia, KY). The software imports the CT data creating a three dimensional volume. The operator selected the appropriate mid-vertebral slice for BMD analysis by angling the analysis plane as appropriate for each vertebral level. The program calculated the BMD in mg/cm^3 on a level-by-level basis. After all the images were measured, these values were then averaged to provide a mean BMD for that entire location, either thoracic or lumbar spine.

Statistical Methods

The sample means, standard deviations and medians were computed for the continuous characteristics, BMD and CP. The proportions were calculated for the discrete demographic characteristics. To compare the study population characteristics between ethnicity and sex groups, the generalized estimating equation (GEE1) procedure (18) was used to account for the correlated data structure inherent in a study using siblings. Each characteristic was treated as the dependent variable. To test for the main effects, ethnicity and sex were treated as the covariates; to test for the interaction between these two variables, additional interaction term was fitted in the model. Sex-stratified analysis was performed to test for the main effect of ethnicity; ethnicity-stratified analysis was performed to test for the main effect of sex. Spearman correlation coefficients were computed to estimate the magnitude of the association between BMD (using QCT in the thoracic and lumbar spine, DXA in the hip and spine) and vascular calcium. Partial correlation coefficients were calculated to adjust for the potential common effects of age, ethnicity, sex, BMI, smoking and prevalent CVD. Criteria used to define prevalent CVD included Q-wave abnormalities on examination, self-reported evidence for clinical CVD (angina, myocardial infarction, stroke), or prior vascular procedures (i.e. coronary artery bypass graft, percutaneous transluminal angioplasty, endarterectomy). The partial correlation coefficients were obtained via computing the Spearman's rank correlation coefficient between the residuals from models regressing vascular calcium and BMD, respectively, onto the covariates. Analyses were performed in the entire sample, separately by sex and ethnicity, and in post-menopausal women (defined as age ≥ 55 years) by estrogen use. Simple correlation coefficient tests were deemed invalid due to the correlated data structure and were re-evaluated using the GEE1 procedure. In order to better approximate the distributional assumptions of conditional normality and homogeneity of variance, the natural log of CP values plus a constant of one were analyzed. Standard regression diagnostics for collinearity and influence were computed for each model. All statistical analyses were considered significant at $p < 0.05$. SAS software (Cary, NC) was used for the statistical analyses.

Results

The characteristics of the sex- and ethnicity-stratified study population are presented in Table 1. There are 1,023 DHS participants with DM2 from a total of 453 families. Overall, participants had a mean age of 62.4 years, mean BMI 32.7 kg/m², mean diabetes duration 11.7 years and sub-optimal control of fasting blood sugar, HbA1c, systolic blood pressure, triglycerides and LDL cholesterol.

Systolic blood pressure, diastolic blood pressure, hemoglobin A1c, LDL-cholesterol, HDL-cholesterol and spine vBMD were all higher in AAs compared to EAs, despite similar durations of DM2 and anti-hypertensive medication treatment (Table 1). AA men had the highest prevalence of current smokers, EA men had the highest level of past smoking. Serum triglyceride concentrations were higher in EA women than EA men, and higher in EA than in AA counterparts.

Calcified atherosclerotic plaque, reported as the total calcium score using Agatston Units, averaged 705 for the coronary arteries, 111 for the carotid bifurcations, and 3765 for the infra-renal abdominal aorta. When stratified by ethnicity and sex, EA men had a significantly higher burden of CP in all three vascular territories, EA women had the second highest burden of CP (after EA men) in the carotid bifurcation and abdominal aorta, while AA men had the second highest burden of coronary CP (Table 1). Overall, AA women had the lowest burden of CP in the three vascular territories. The differences in CP plaque were significant in all ethnicity sex comparisons with a significant interaction for both coronary and aortic CP. Carotid CP was not significantly different in AA by sex or between AA & EA women and no interaction was present (Table 1b).

BMD measured by DXA and QCT demonstrated significant differences by race and sex and no significant race by sex interactions were observed (Table 1b). For the vBMD measured by QCT, AA women had the highest trabecular vBMD in the spine, followed by AA men, EA women and then EA men. For the DXA measures of the spine and hip aBMD, AA men had the highest aBMD, followed by EA men, AA women, and then EA women.

Correlations between CP in the coronary, carotid and abdominal aorta territories and BMD using QCT (thoracic and lumbar spine) and DXA (hip and spine) are presented in Table 2 as unadjusted (model 1), adjusted for age (model 2), and adjusted for age, sex, ethnicity, BMI, smoking and prevalent CVD (model 3). Inverse relations were present between CP and BMD across multiple vascular beds in all ethnic and sex groups with the QCT measure of vBMD and to a limited extent with the DXA measures of aBMD. After adjustment for age, significant inverse correlations between CP and vBMD in multiple sites were observed primarily in EA men and AA women, although there was a tendency for the relation in EA women as well.

In EA men an inverse association was present between the vascular sites (coronary, carotid and abdominal aorta) and the two sites of vBMD (thoracic and lumbar) before and after age adjustment (model 1 with 6 of 6 associations demonstrating inverse correlations with $p < 0.05$; model 2 with 5 of 6 associations demonstrating inverse correlations with $p < 0.05$ and 1 borderline $p = 0.076$) and in the expanded model adjusted for age, sex, ethnicity, BMI, smoking and prevalent CVD (model 3 with 3 of 6 associations demonstrating inverse correlations with $p < 0.05$ and highest borderline $p = 0.078$). In AA women an inverse association is present between the vascular sites (coronary, carotid and abdominal aorta) and the two sites of vBMD (thoracic and lumbar) before adjustment (model 1 with 6 of 6 associations demonstrating inverse correlations with $p < 0.05$). After age adjustment, the relation between aortic CP and vBMD remains negative but is no longer significant. However, AA women continue to have a significant inverse relation between coronary and carotid CP and vBMD after age adjustment and in the expanded model adjusted for age, sex, ethnicity, BMI, smoking and prevalent CVD

(model 2 with 4 of 4 associations demonstrating inverse correlations with $p < 0.05$; model 3 with 3 of 4 associations demonstrating inverse correlations with $p < 0.05$ and the fourth $p = 0.10$). In EA women, significant associations were observed only in the unadjusted models, once accounting for age (or other covariates) no associations between CP and BMD are observed although the relation between thoracic vBMD and aortic CP approached significance ($p = 0.062$). In AA men, this study had limited power to detect an association secondary to the smaller sample size. Prior to age adjustment significant inverse associations between CP and vBMD were observed (model 1, 3 of 6 $p < 0.05$ and 2 of 6 $p < 0.11$). All of these associations become non-significant after adjusting for age.

DXA-derived aBMD measures were less well correlated with CP. Significant relationships between aBMD and CP were observed in AA women and EA men. In AA women, the inverse associations between spine aBMD and coronary artery CP were present in the unadjusted and age adjusted models, but not in the fully adjusted model. In EA men, unadjusted and age adjusted associations between spine aBMD and coronary CP were positive, but again not present in the fully adjusted model. In EA women, hip aBMD was negatively associated with coronary, carotid, and aortic CP only in the unadjusted model. In AA men, DXA aBMD of the hip is inversely associated with coronary CP prior to age adjustment and with abdominal aortic CP only in model 3, observations based on a smaller number of individuals.

Stratified analyses were performed in post-menopausal women based upon estrogen use, since this factor is known to influence BMD and atherogenesis (Table 3). AA women reporting use of estrogen had significant inverse correlations between coronary, carotid and abdominal aortic CP with thoracic vBMD after adjustment for age, and these remained significant after further adjustment for BMI, smoking and prevalent CVD. Coronary artery CP and abdominal aorta CP were also inversely associated with L-vBMD in AA women taking estrogen. Among EA estrogen users, inverse relationships between carotid CP with T-vBMD and L-vBMD and hip aBMD were observed before, but not after adjustment for age, although a significant relationship was observed with L-vBMD in the fully adjusted model. Among estrogen non-users, inverse relationships between CP and BMD at multiple sites were accounted for by adjustment for age, except that coronary CP and spine aBMD remained significantly associated in AA women.

Discussion

Type 2 diabetes mellitus, osteoporosis and CVD are major causes of morbidity and mortality worldwide. Calcified plaque and bone mineralization can be precisely quantified, and their relationships analyzed non-invasively, to provide insight for disease prevention and therapy. This analysis is the first to define the relationship between BMD and CP using extensive phenotyping of both traits in a large high risk population that included men and subjects with DM2. A particular strength of this report was our ability to measure BMD using two complimentary approaches. DXA measurement of aBMD is the de facto standard for determining BMD in both clinical practice and the research setting. QCT provides a measurement of vBMD specific for the metabolically active trabecular bone in the vertebrae. Our findings indicate that the relationship between calcified atherosclerotic plaque and bone mineralization in diabetic individuals is complex and influenced by a variety of factors. In addition, vertebral trabecular bone measured by QCT (vBMD) but not the more commonly measured and reported combined cortical and trabecular bone of the spine or hip (aBMD measured by DXA) demonstrated significant associations with CP at multiple vascular sites. The strongest and most consistent inverse associations between vBMD and CP were observed in diabetic EA men. EA men had the highest calcified atherosclerotic plaque burden at all three vascular sites and the lowest vertebral trabecular vBMD of all sex-ethnic groups (all contrasts highly significant - see Table 1b). AA women also demonstrated significant inverse

associations between CP and BMD, and AA women had the highest average vertebral trabecular bone (vBMD by QCT) and the lowest calcified plaque burden. Stratified analyses in AA women taking estrogen demonstrated significant inverse association between coronary, carotid and aortic CP and thoracic vBMD and between coronary and aortic CP and lumbar vBMD (all $p < 0.05$). Significant age-independent associations between BMD and CP in the larger sample of EA women, even when stratified by estrogen use, were largely absent except for the carotid bifurcation and lumbar spine. The complex association between BMD and CP in women with potential effect modification by ethnicity and estrogen use is interesting and requires further study. Estrogen use is associated with reduced atheromatous plaque formation in animal models and with reduced incidence of clinical CHD and plaque progression in younger women (19), while being a potent bone protectant known to increase bone mass in women (20). Recent results from the Women's Health Initiative demonstrated a significant reduction in coronary artery calcified plaque burden among women 50-59 years of age when randomized to estrogen compared to placebo(20a) Thus, estrogen use may strengthen the inverse relation between bone density and CP. It is not clear why such an effect would be observed in AA women and not in EA women in this study. We have insufficient prescribing and compliance data to carefully examine the influence of dose, duration of treatment, or type of estrogen or progestogen on the relation between bone and CP. Skeletal status determined using aBMD (DXA) of the hip and spine captures the combined cortical and trabecular BMD, whereas vBMD by QCT of the thoracic and lumbar vertebra provides measures of trabecular vBMD while avoiding interference from cortical bone, osteoarthritic changes or adjacent vascular (or other) calcifications. Our results demonstrated stronger associations between CP and trabecular vBMD measured using QCT, than with aBMD using DXA. This may be related to the fact that the trabecular bone compartment is more metabolically active than the cortical compartment due to its larger surface area and exposure. In this population of relatively overweight or obese subjects, biomechanical influences on bone remodeling may have an exaggerated influence on site-specific vBMD as well as aBMD, which may alter relationships between BMD and CP.

Atherosclerosis was assessed by measuring CP at three important vascular territories directly associated with the clinical expression of CVD: the carotid bifurcation, abdominal aorta and coronary arteries. Coronary artery CP measured by cardiac CT has been demonstrated to add significantly to traditional risk factors in the prediction of near-term coronary artery disease events, CVD events and all-cause mortality in adults. We detected significant negative associations between CP in the coronary, carotid and aortic territories with vBMD in EA men and AA women. Associations between CP in these vascular beds and CVD risk factors have been demonstrated(21). Carotid bifurcation plaques are the proximate cause of embolic stroke and aorto-iliac atherosclerosis is associated with the clinical expression of symptomatic peripheral arterial disease and risk of future coronary heart disease events(22).

Our results show the relations between sub-clinical atherosclerosis and BMD vary by an individual's ethnicity and sex. Significant ethnicity and sex differences in coronary CP have previously been reported in the Multi-Ethnic Study of Atherosclerosis (MESA) population. In the MESA study population, recruited at 6 sites across North America and selected based upon the absence of clinical CVD, the relative risk of an AA having coronary calcification was 0.78 compared to EA participants(23). Among MESA participants with coronary CP, EAs had the greatest CP burden, followed by Chinese, Hispanic and AA participants. Similar lower prevalence of coronary CP was observed in the subset of diabetic MESA participants(24), a report from Los Angeles(25), and previously reported by us in our Diabetes Heart Study families(26), where the most marked ethnic differences in CP were observed among men.

Evidence for a systematic and possibly genetic process controlling the process of calcification in arteries and bone is supported by the marked ethnic variation in the prevalence of

osteoporosis(27) and calcified vascular plaque (23-26). African Americans, who typically ingest less dietary calcium than European Americans, nonetheless manifest increased BMD, skeletal resistance to the effects of parathyroid hormone and lower levels of calcified coronary artery plaque. African Americans appear to preferentially deposit calcium in the skeletal system. The pathologic condition of atherosclerotic calcified plaque is less prevalent and extensive in both AA men and women. Given equal access to medical care, African Americans have a reduced risk of bone fractures(28) and myocardial infarction(29-31), relative to European Americans.

The Study of Osteoporotic Fractures demonstrated that BMD using DXA in the heel, femoral neck and forearm was higher in older diabetic women (mean age 72 years), compared to non-diabetic women(8). However, diabetic women in this report had higher fracture rates related to factors that increased the risk of fractures. In the Health ABC study, older diabetic subjects (age 70-79 years) had higher baseline hip BMD using DXA(11). Longitudinal data from Health ABC indicated that the 4 year change in femoral neck BMD (a site of trabecular bone measured using DXA) declined significantly only in EA diabetic women. No decline in trabecular BMD was observed in AA women, AA men or EA men. Previously identified variation in BMD and atherosclerosis between sexes and ethnic groups support differential relationships between BMD and CP based upon sex and ethnicity.

We have previously demonstrated the independent heritability of BMD and calcified atherosclerotic plaque(32) in Diabetes Heart Study families. It is likely that genetic determinants influence the association between calcified atherosclerotic plaque and BMD. Several biologic pathways, particularly those involving mineralization inhibitors, may participate in the propensity for development of osteoporosis with atherosclerosis. For example, fetuin-A, matrix GLA protein and pyrophosphates all inhibit calcium deposition and are present or active in blood vessel walls(33). It is possible that polymorphisms in these mineralization inhibitor genes may account for a portion of the risk of atherosclerosis and osteoporosis. In the Coronary Artery Disease in Young Adults (CARDIA) study, single nucleotide polymorphisms (SNPs) in the osteopontin and matrix GLA protein genes were not associated with BMD and calcified atherosclerotic plaque(34), although CARDIA was perhaps hampered by a relatively young study population with limited expression of calcified atherosclerotic plaque or osteoporosis. In rodents, statins activate the expression of bone morphogenic protein 2 (BMP-2)(35) and variable results on BMD have been observed in humans following statin therapy (36).

There are several limitations in the interpretation of our results. First, the DHS is a genetic study, by design based on a family sampling strategy. To prevent overstating statistical significance, we present only a conservative definition of significance to account for the inherent correlation that is present within family members. Second, the observations are limited to individuals with DM2. It is possible and perhaps even likely that the observed relations in diabetics would be different in non-diabetic subjects. The complexity of the relations between bone mineralization and calcified atherosclerotic plaque, as well as the ethnic-specific and sex-specific findings, support the contention that genetic causes likely impact the relations between bone and atherosclerotic calcified plaque. Replication of our results in a large non-diabetic population would be informative as to whether these relations are specific to diabetes. Furthermore, evaluating the relations between CP and BMD in non-diabetic populations will likely provide greater insight to the biologic pathways involved and whether they are unique to diabetes. Third, although we report the largest study evaluating the relation of BMD to CP in AA and EA men and women to date, our inferences related to AA men and the stratified analyses on the effects of estrogen in women are limited by relatively small sample sizes but document the importance of including minorities and both sexes in clinical studies in this area of science.

In summary, our results indicate that a complex but significant relation is present between atherosclerotic calcification and bone mineralization in type 2 diabetics. The observation of this relation in humans supports the hypothesis that metabolic, cellular and/or genetic processes related to both atherosclerosis and bone metabolism play a role in osteoporosis and atherosclerosis.

Furthermore, we have shown the relation between vascular calcification and bone density is strongly influenced by age, sex and ethnicity, with further effect modification in women receiving supplemental estrogen. EA men and AA women with diabetes have a significant inverse associations between vertebral trabecular bone and CP at multiple vascular sites, effects that remained significant after adjustment for age and other covariates. Thus, inverse relationships between bone and vascular calcification exist in a population of overweight and obese diabetic subjects at high risk for atherosclerotic vascular diseases. Subjects with obesity and diabetes are often advised to lose weight, and weight loss is usually paralleled by loss of bone. The existence of an independent inverse relationship between the skeletal and vascular calcium fluxes could lead to increased vascular calcification. It would be interesting, but technically challenging, to assess whether the calcium released from bone is preferentially relocated to the arterial wall over other calcium pools. Such an effect might occur if released skeletal calcium circulates in a mineral form or complexed with phospholipids or peptides which might be more readily incorporated into the vascular mineralization process. Whether these events occur or lead to increases or decreases in clinical events is a largely unexplored area worthy of investigation. These results support the performance of additional studies designed to better understand interactions between bone mineralization and atherosclerotic calcified plaque, as well as the influences of genes and hormone replacement therapy on this interaction.

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Abbreviations

aBMD, Areal BMD
 CP, Atherosclerotic calcified plaque
 BMD, Bone mineral density
 CVD, Cardiovascular disease
 CT, Computed tomography
 DXA, Dual X-ray absorptiometry
 GEE1, Generalized estimating equations
 L-vBMD, Lumbar vertebrae vBMD
 QCT, Quantitative CT
 T-vBMD, Thoracic vertebrae vBMD
 vBMD, Trabecular volumetric BMD
 DM2, Type 2 diabetes mellitus

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Table 1

1a: Characteristics of Study Population by Ethnicity and Sex
1b:Tests for Differences in Population Characteristics by Ethnicity and Sex¹

African America (AA)										European American (EA)										
Variable	Women					Men					Women					Men				
	N	Mean	SD	Median	N	Mean	SD	Median	N	Mean	SD	Median	N	Mean	SD	Median	N	Mean	SD	Median
Age, yrs	110	59.6	9.1	58.5	53	59.9	9.1	60.9	440	62.5	9.5	62.4	420	63.4	8.9	64				
DM Duration, yrs	110	11.6	7.9	9.5	53	12.3	8.6	10.2	431	11.3	7.1	9.7	410	12.0	7.7	10.2				
HbA1c, yrs	110	8.8	2.7	8.1	53	8.4	2	8	440	7.6	1.8	7.2	419	7.5	1.4	7.2				
BMI, kg/m ²	110	35.2	7.6	34.4	53	31.2	6.4	30.8	439	33.7	7.6	32.8	417	31.3	5.9	30.5				
SBP, mm Hg	110	146.6	24.1	143.5	53	142.5	19.4	143	439	140.7	19.2	139	417	138.8	19.1	137.5				
DBP, mm Hg	110	76.5	11.3	75.8	53	76.1	10.7	78	439	70.5	9.9	69.5	417	72.6	9.9	72.5				
Total chol, mg/dl	108	196	45	192	52	178	33	174	438	196	46	190	416	176	40	173				
HDLc, mg/dl	108	52	16	50.5	52	45	12	44.5	438	46	13	44	415	38	10	37				
LDLc, mg/dl	106	116	41	112.5	49	107	29	105	404	107	35	104	391	100	32	97				
non-HDLc, mg/dl	108	144	44	142	52	133	36	128.5	438	150	45	143.5	415	137	39	132				
Triglycerides, mg/dl	108	141	81	120.5	52	135.8	119	100	438	220.3	150	187	416	194	124	165				
Calcified Plaque																				
Coronary	105	282	631	12.5	49	509	1016	72		371	630	68	392	1194	1434	659.8				
Carotid	102	67	185	3.8	50	69	181	6		88	169	16.5	389	151	212	69.5				
Abdominal Aorta	97	1687	2720	491	48	1757	3953	418.5		3062	3809	1394	361	5303	5772	3663				
Bone																				
OCT Spine T-vBMD	103	180	51	174	50	160	40	163		146	45	140.9	392	135	37	134.9				
OCT Spine L-vBMD	95	163	47	156.6	48	152	37	156.5		131	43	129.5	359	122	35	119.2				
DXA Spine L-aBMD	86	1.07	0.19	1.08	45	1.10	0.17	1.10		1.02	0.18	1.01	345	1.09	0.17	1.08				
DXA-Total Hip aBMD	85	0.99	0.17	0.99	43	1.07	0.18	1.06		0.95	0.17	0.93	344	1.02	0.16	1.02				
Smoking (%)																				
Current	18	16.5%			22	41.5%							66	15%						
Past	34	31.2%			24	45.3%							124	28.2%						
Medications (%)																				
Lipid Lowering	49	44.5%			18	34.0%							201	46.0%						
Estrogen	16	15.4%				-							111	26.2%						
Anti-hypertensive	69	82.1%			36	80.0%							261	82.1%						
Thiazide Diuretic	30	28.3%			15	28.8%							115	27.3%						
Main Effects																				
Variable	Face	sex	Interaction		AA	E/A	Men	Women												
Age, yrs	0.0001	0.14	0.95		Asex	Asex	Arace	Arace												
DM Duration, yrs	0.79	0.19	0.99		0.62	0.16	0.01	0.0014												
HbA1c, yrs	0.0001	0.07	0.14		0.65	0.22	0.89	0.81												
BMI, kg/m ²	0.09	0.0001	0.10		0.0003	0.0001	0.70	0.03												
SBP, mm Hg	0.0001	0.15	0.20		0.16	0.38	0.15	0.0001												
DBP, mm Hg	0.0001	0.0018	0.0364		0.50	0.0002	0.02	0.0001												
Total chol, mg/dl	0.83	0.0001	0.72		0.0112	0.0001	0.65	0.98												
HDLc, mg/dl	0.0001	0.0001	0.70		0.0029	0.0001	0.0001	0.0001												
LDLc, mg/dl	0.0019	0.0019	0.62		0.13	0.0060	0.13	0.01												
non-HDLc, mg/dl	0.13	0.0001	0.79		0.13	0.0001	0.47	0.20												
Triglycerides, mg/dl	0.0001	0.0014	0.24		0.89	0.0012	0.0013	0.0001												
Calcified Plaque																				
Coronary	0.0006	0.0001	0.0053		0.0472	0.0001	0.0013	0.0574												
Carotid	0.0053	0.0001	0.09		0.95	0.0001	0.0061	0.17												
Abdominal Aorta	0.0001	0.0001	0.0203		0.78	0.0001	0.0001	0.0003												
Bone																				

Variable	Main Effects		Interaction	EA		Men		Women	
	race	sex		Δsex	Δsex	Δrace	Δrace	Δrace	Δrace
OCT Spine T-vBMD	0.0001	0.0001	0.16	0.0082	0.0001	0.0001	0.0001	0.0001	0.0001
OCT Spine L-vBMD	0.0001	0.0001	0.53	0.07	0.0005	0.0001	0.0001	0.0001	0.0001
DXA Spine L-aBMD	0.0340	0.0001	0.14	0.75	0.0001	0.93	0.01	0.01	0.01
DXA-Total Hip aBMD	0.0037	0.0001	0.72	0.0323	0.0001	0.0365	0.04	0.04	0.04
Smoking (%)									
Current	0.0127	0.0137	0.0037	0.0002	0.33	0.0001		0.98	
Past	0.40	0.0001	0.09	0.06	0.0001	0.06	0.68		
Medications (%)									
Lipid Lowering	0.08	0.35	0.10	0.24	0.14	0.0182	0.63		
Estrogen			-	-	-	-	0.0081		
Anti-hypertensive	0.76	0.11	0.73	0.77	0.11	0.65	0.99		
Thiazide Diuretic	0.20	0.0001	0.06	0.97	0.0001	0.0244	0.95		

DM - diabetes mellitus; HbA1c - glycosylated hemoglobin; BMI - body mass index; SBP - systolic blood pressure; DBP - diastolic blood pressure; chol and c - cholesterol.

/ Please see text for statistical methods

Table 2
Spearman Correlation Coefficients for Arterial Calcified Plaque versus Bone Mineral Density by Ethnicity and Sex

Artery Measure	Bone Measure	Model	African America			European American		
			Women		Men	Women		Men
			N	R*	GEE p-value	N	R*	GEE p-value
Coronary	QCT Spine T-vBMD	1	108	-0.306	<i>0.0001</i>	50	-0.224	0.2260
		2	108	-0.192	<i>0.0400</i>	50	0.008	0.6158
		3	96	-0.193	0.1065	42	-0.031	0.7891
	QCT Spine L-vBMD	1	100	-0.364	<i>0.0001</i>	48	-0.333	<i>0.0425</i>
		2	100	-0.250	<i>0.0116</i>	48	-0.078	0.8834
		3	88	-0.262	<i>0.0138</i>	40	-0.077	0.6091
	DXA Spine L-aBMD	1	90	-0.292	<i>0.0013</i>	44	-0.048	0.6891
		2	90	-0.264	<i>0.0054</i>	44	0.001	0.9112
		3	79	-0.222	0.1020	39	0.088	0.8782
	DXA Hip aBMD	1	89	-0.119	0.2192	42	-0.355	<i>0.0396</i>
		2	89	0.000	0.7732	42	-0.231	0.1870
		3	78	-0.074	0.9363	37	-0.100	0.8075
Carotid Bifurcation	QCT Spine T-vBMD	1	105	-0.350	<i>0.0001</i>	51	-0.161	0.0944
		2	105	-0.164	<i>0.0183</i>	51	0.001	0.6174
		3	94	-0.184	<i>0.0063</i>	43	0.054	0.5600
	QCT Spine L-vBMD	1	97	-0.357	<i>0.0001</i>	49	-0.197	0.2679
		2	97	-0.159	<i>0.0493</i>	49	-0.027	0.9988
		3	86	-0.182	<i>0.0339</i>	41	0.080	0.8442
	DXA Spine L-aBMD	1	87	-0.084	0.6203	45	0.104	0.2015
		2	87	-0.049	0.4097	45	0.148	0.1558
		3	77	-0.036	0.6214	40	0.206	0.3377
	DXA Hip aBMD	1	86	-0.187	0.1499	43	-0.064	0.9256
		2	86	-0.074	0.9276	43	0.040	0.7532
		3	76	-0.079	0.6255	38	0.275	<i>0.0001</i>
Abdominal Aorta	QCT Spine T-vBMD	1	100	-0.348	<i>0.0069</i>	49	-0.253	<i>0.0350</i>
		2	100	-0.119	0.6689	49	-0.074	0.5597
		3	88	-0.136	0.3982	41	-0.102	0.6149
	QCT Spine L-vBMD	1	100	-0.383	<i>0.0009</i>	49	-0.242	0.1054
		2	100	-0.148	0.4544	49	-0.031	0.8963
		3	88	-0.171	0.2511	41	0.006	0.5632
	DXA Spine L-aBMD	1	90	-0.122	0.4096	45	-0.020	0.6349
		2	90	-0.066	0.8400	45	0.032	0.8892
		3	79	-0.018	0.7030	40	0.137	0.6380
	DXA Hip aBMD	1	89	-0.146	0.0663	43	-0.222	0.2389
		2	89	0.008	0.7277	43	-0.106	0.9861
		3	78	0.042	0.6893	38	0.219	<i>0.0032</i>

Models: 1) unadjusted; 2) adjusted for age; 3) adjusted for age, smoking, BMI, and prevalent cardiovascular disease.

Table 3
Spearman Correlation Coefficients for Arterial Calcified Plaque versus Bone Mineral Density in Post-Menopausal Women by Ethnicity and Estrogen Use

Artery Measure	Bone Measure	Model	African American Women						European American Women					
			Estrogen (Yes)			Estrogen (No)			Estrogen (Yes)			Estrogen (No)		
			N	R*	GEE p-value	N	R*	GEE p-value	N	R*	GEE p-value	N	R*	GEE p-value
Coronary	QCT Spine T-vBMD	1	17	-0.399	0.0012	77	-0.168	0.0685	120	-0.241	0.2573	280	-0.186	0.0041
		2	17	-0.402	0.0001	77	-0.092	0.3056	120	-0.03	0.3981	280	-0.066	0.2938
		3	16	-0.639	0.0020	70	-0.064	0.4552	105	0.055	0.1949	262	-0.049	0.4557
	QCT Spine L-vBMD	1	16	-0.203	0.0003	70	-0.216	0.0677	106	-0.154	0.4358	254	-0.192	0.0304
		2	16	-0.159	0.0001	70	-0.143	0.2739	106	0.09	0.4343	254	-0.067	0.6644
		3	15	-0.628	0.0005	63	-0.113	0.5124	93	0.162	0.1418	235	-0.061	0.7519
	DXA Spine L-aBMD	1	15	-0.131	0.1875	65	-0.242	0.0317	102	0.08	0.3721	246	0.001	0.8384
		2	15	-0.179	0.0706	65	-0.225	0.0329	102	0.169	0.2449	246	0.08	0.1912
		3	14	-0.753	0.0005	58	-0.054	0.7826	90	0.226	0.1191	228	0.043	0.2931
	DXA Total Hip aBMD	1	15	0.155	0.7922	64	-0.104	0.4364	102	-0.104	0.818	248	-0.143	0.143
		2	15	0.200	0.0840	64	0.001	0.8717	102	0.081	0.1683	248	-0.034	0.9645
		3	14	-0.468	0.1673	57	0.059	0.4221	90	0.236	0.0338	229	-0.031	0.7219
Carotid Bifurcation	QCT Spine T-vBMD	1	17	-0.764	0.0358	75	-0.236	0.0002	119	-0.309	0.0002	280	-0.115	0.0081
		2	17	-0.833	0.0949	75	-0.035	0.1047	119	-0.146	0.2307	280	0.075	0.8453
		3	16	-0.851	0.0110	68	-0.053	0.3322	104	-0.147	0.1231	262	0.06	0.9646
	QCT Spine L-vBMD	1	16	-0.522	0.0576	68	-0.259	0.0004	106	-0.257	0.0161	254	-0.096	0.1491
		2	16	-0.499	0.2022	68	-0.081	0.0609	106	-0.077	0.716	254	0.117	0.345
		3	15	-0.594	0.0285	61	-0.053	0.5059	93	-0.102	0.0148	235	0.085	0.9954
	DXA Spine L-aBMD	1	15	-0.193	0.5769	63	-0.029	0.7168	102	-0.039	0.6423	246	0.08	0.3423
		2	15	-0.361	0.1270	63	0.003	0.1993	102	0.022	0.8388	246	0.193	0.0276
		3	14	-0.423	0.0066	56	0.122	0.5879	90	-0.034	0.3964	228	0.146	0.1234
	DXA Total Hip aBMD	1	15	-0.211	0.2845	62	-0.161	0.169	102	-0.238	0.0033	248	-0.155	0.0552
		2	15	-0.140	0.2835	62	-0.031	0.6653	102	-0.103	0.5779	248	-0.005	0.7392
		3	14	-0.329	0.1458	55	0.018	0.6164	90	-0.177	0.1968	229	-0.037	0.3203
Abdominal Aorta	QCT Spine T-vBMD	1	16	-0.407	0.0002	70	-0.243	0.0895	106	-0.439	0.0234	254	-0.279	0.0001
		2	16	-0.404	0.0001	70	-0.005	0.8701	106	-0.222	0.8593	254	-0.037	0.502
		3	15	-0.624	0.0001	63	0.044	0.6338	93	-0.212	0.2267	236	0.009	0.9526
	QCT Spine L-vBMD	1	16	-0.247	0.0003	70	-0.289	0.1241	106	-0.335	0.0602	255	-0.29	0.0016
		2	16	-0.186	0.0001	70	-0.056	0.758	106	-0.064	0.8742	255	-0.031	0.9699
		3	15	-0.667	0.0001	63	0.02	0.3131	93	-0.094	0.6593	236	-0.011	0.8261
	DXA Spine L-aBMD	1	15	-0.043	0.0001	65	-0.084	0.5477	102	-0.04	0.5956	247	-0.069	0.8624
		2	15	-0.112	0.0001	65	-0.04	0.7094	102	0.059	0.3876	247	0.073	0.0992
		3	14	-0.564	0.0036	58	0.23	0.1545	90	0.025	0.7098	229	0.058	0.1185
	DXA Total Hip aBMD	1	15	0.094	0.0888	64	-0.178	0.3849	102	-0.296	0.1932	249	-0.219	0.1338
		2	15	0.160	0.0001	64	-0.002	0.5246	102	-0.107	0.3226	249	-0.019	0.6042
		3	14	-0.445	0.0145	57	0.176	0.1634	90	-0.042	0.5871	230	0.021	0.2188

Post-menopausal is defined as age > 55 years. Models: 1) unadjusted; 2) adjusted for age; 3) adjusted for age, smoking, BMI, and prevalent cardiovascular disease.