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# Systemic atherosclerosis and bone density

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requirements for the degree Doctor of Philosophy

in

Public Health (Epidemiology)

by

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2006

DEDICATION

With thanks to my parents,

J. A. H.

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The dissertation author was the primary researcher and author.

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ABSTRACT OF THE DISSERTATION

Systemic atherosclerosis and bone density

by

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Doctor of Philosophy in Public Health (Epidemiology)

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Molecular and cell biology studies have demonstrated an association between bone and arterial wall disease, but epidemiologic evidence of an independent bone-artery association has been inconclusive. We tested the cross-sectional associations of volumetric trabecular lumbar bone mineral density (vBMD) with multiple measures of structural and functional cardiovascular disease in the context of shared determinants including inflammatory markers and sex hormones. Participants were 946 women and 963 men free of clinical cardiovascular disease upon enrollment in the Multi-Ethnic Study of Atherosclerosis between 2000 and 2002.

In women, vBMD was inversely and significantly associated with presence and extent of coronary calcium, the extent of aortic calcium, and greater carotid artery plaque echogenicity but not with the ankle-brachial index or carotid artery intima-media thickness. In men, lower vBMD was significantly associated with presence of aortic calcium, lower ankle-brachial index, and greater internal carotid intima-media thickness but not with common carotid intima-media thickness. In all models, adjustment for standard and novel cardiovascular disease and osteoporosis risk factors did not materially affect results. These findings suggest that bone loss and atherosclerosis may be related processes and that low bone density may reflect cardiovascular disease risk.

We further demonstrated in a sub-sample of 423 women and 670 men naïve to lipid-modifying medications that dyslipidemia, defined by total cholesterol to high density lipoprotein cholesterol ratio, significantly modified the associations between bone density and coronary and aortic calcified atherosclerosis in men and women. Associations between bone density and atherosclerosis were positive in those with dyslipidemia and inverse in those without dyslipidemia.

# I. INTRODUCTION

## Initial investigations

The recognition of bone-like tissues in atherosclerotic lesions dates to the time of Rudolph Virchow in the middle 19th century.(Mody, Parhami et al. 2001) The epidemiologic association between atherosclerosis and diminished bone integrity was first noted nearly 50 years ago by Elkes.(Elkeles 1957) After early speculation on a causal association between bone and arterial pathology, research programs studying the distribution and determinants of atherosclerosis and osteoporosis diverged.

## Divergence and convergence of research agendas

For some time atherosclerosis was considered to affect women very little but to inflict its greatest burden on men, largely as a result of aging, smoking, hypertension, diabetes and lipids. During this time, osteoporosis was largely considered a disease of women that resulted from estrogen deficiency. These sex-specific understandings of the diseases may have encouraged separate research programs for the two conditions. Since this period, these divergent lines of investigation have resulted in expanded understandings of atherosclerosis and osteoporosis, and their distribution and determinants have converged, to some extent. Atherosclerosis is now understood as a disease of women as well as men, and osteoporosis is known to affect large numbers of men, in addition to women. In both diseases, cells and hormones of the immune system have been implicated as have smoking, physical activity, type I diabetes mellitus, and lipid biology.

New technologies and methods: Raising the bar for epidemiologic studies

Interest in the bone-artery association has re-emerged with new radiologic methods for assessing bone integrity (from X-ray plain films, radiogrammetry, dual energy X-ray absorptiometry, ultrasound, and quantitative computed tomography) and with new methods for assessing atherosclerosis (lateral abdominal X-ray, ultrasound methods to asses the ankle-brachial index and carotid artery structure, computed tomography to score arterial calcium). In addition, laboratory experiments using whole animal models, as well as tissue and cell culture, have demonstrated the molecular bases for many individual risk factors and have raised the possibility of novel, shared determinants.(Demer 1995; Doherty, Asotra et al. 2003) These refinements in disease ascertainment and compelling findings from the bench have required that new epidemiologic investigations of a bone-artery association utilize more informative assessments of disease status and measure an array of potential confounding factors that may be responsible for spurious associations.

Shortcomings of previous investigations

The results of previous investigations(Elkeles 1957; Dent, Engelbrecht et al. 1968; Boukhris and Becker 1972; Mori, Oku et al. 1990; Reid, Evans et al. 1991; Frye, Melton et al. 1992; Jie, Bots et al. 1996; Uyama, Yoshimoto et al. 1997; Vogt, Cauley et al. 1997; Vogt, San Valentin et al. 1997; Barengolts, Berman et al. 1998; von der Recke, Hansen et al. 1999; Hak, Pols et al. 2000; Aoyagi, Ross et al. 2001; Kiel, Kauppila et al. 2001; Van Der Klift, Pols et al. 2002; Van Der Klift, Pols et al.

2002; Tanko, Bagger et al. 2003; Davutoglu, Yilmaz et al. 2004; Jorgensen, Joakimsen et al. 2004; Kammerer, Dualan et al. 2004; Montalcini, Emanuele et al. 2004; Pennisi, Signorelli et al. 2004; Samelson, Kiel et al. 2004; Schulz, Arfai et al. 2004; Aksoy, Yagmur et al. 2005; Bakhireva, Barrett-Connor et al. 2005; Magnus and Broussard 2005; Rajzbaum, Roger et al. 2005; Tanko, Christiansen et al. 2005; Wong, Kwok et al. 2005; Yamada, Inaba et al. 2005; Saverino, Del Sette et al. 2006) into the bone-artery association are summarized in Table 1. As a whole, these investigations have demonstrated some evidence of an epidemiologic link between atherosclerosis and poor bone integrity in some manifestation or another. These studies have typically relied on X-ray, radiogrammetry, ultrasound or DXA methods to assess bone density. Only volumetric bone density by computed tomography is unaffected by secular trends of height and age-, sex-, or ethnic-differences in bone size and shape.(Lang, Guglielmi et al. 2002) And no population-based investigation of the bone-artery association has measured volumetric bone density. Further, no investigation has assessed multiple measures of atherosclerosis or investigated large samples of men and women of multiple ethnic groups, among whom patterns of atherosclerosis and osteoporosis are known to vary. Finally, no previous epidemiologic investigations have specifically studied the effects of lipids, inflammatory makers and sex hormones on the bone-artery association. Investigation of these variables through serial modeling of epidemiologic data would test hypotheses about these factors as potential confounders, mediators or shared risk factors contributing to the association.

Summary of rationale and aims

Despite progress in understanding both atherosclerosis and bone loss, the epidemiology of an association between atherosclerosis and bone density remains unclear, its determinants unexplored. The association stands to benefit from a re-appraisal in a non-patient, multi-ethnic sample, using volumetric assessment of bone density and multiple measures of atherosclerosis in the context of extensive data on shared risk factors. These investigations attempted just that.

We utilized radiologic data from approximately 2000 participants in the Multi-Ethnic Study of Atherosclerosis – Abdominal Aortic Calcium Study (MESA-AACS), a prospective epidemiologic study investigating multiple subclinical cardiovascular disease (CVD) measures and CVD risk factors including both traditional risk factors and newer measures such as inflammatory markers. We examined the cross-sectional association of multiple measures of subclinical atherosclerosis with volumetric bone density (BMD) at the lumbar vertebra in the context of lipids, inflammatory markers and sex hormones, each of which may influence that association. The underlying hypotheses of the work are that 1) bone density is robustly, inversely associated with multiple measures of atherosclerosis 2) this association exists independent of covariates including potential confounders and 3) this association is independent of shared risk factors for atherosclerosis and osteoporosis. Because there is little previous data on the bone-artery in non-White ethnic groups, we also investigated ethnic-specific variations on the association.

Enumeration of chapters

The second chapter of this manuscript reports on tests of associations between bone density and the presence and extent of calcified atherosclerosis in the coronary arteries and abdominal aorta, controlling for multiple potential contributors to the association.

The third chapter reports on tests of associations between bone density and the ankle-brachial index, a functional measure of advanced atherosclerosis, the intima-media thickness of the common and internal carotid arteries, measures of earlier structural atherosclerosis, and carotid plaque presence and composition, controlling for multiple potential contributors to the association.

The fourth chapter of this manuscript describes a series of tests of the hypothesis that the bone-artery association varies according to patterns of dyslipidemia. Other investigations have indicated that lipids produce changes in the arteries and bones, and that some form of dyslipidemia may drive the bone-artery association.

The appendices to this manuscript provide additional information on the collection of bone density data (Appendix I) and a summary of feasibility studies of these investigations (Appendix II).

## Table 1. Previous epidemiologic studies of atherosclerosis and osteoporosis.

| First Author | Journal (Year) | Women Pre… Race N | Women Post… Race n | Men Race n | Atherosclerosis Site | Atherosclerosis Measure | Bone Site | Bone Measure | Finding *=Significant association **=Significant adjusted association |
|---|---|---|---|---|---|---|---|---|---|
| van der Klift M | Bone (2002) | | W 3374 | W 2445 | All Cause | Death | FN | DXA | Men: U-relationship with ACD: Women: Nothing |
| Tanko L B | JBMR (2005) | | W 2576 | | Cardiovascular | Morbidity | H L | fracture/DX | Women: OP RR=3.9, LBD RR=2.1 |
| von der Recke P | Am J Med (1999) | | W 1063 | | Cardiovascular | Mortality | M | | Women: HR 2.0 for Q1 and Q4 of BMD |
| Magnus JH | Osteoporos Int (2005) | | Mixed 2572 | Mixed 2478 | CHD | MI (self-report) | F | DXA | Men:**MI is assoc with "low BMD" OR=1.2-1.4 |
| Samelson E | AJE (2004) | | W 1236 | W 823 | CHD | Event | M | RG | Women:** M ARR=1.4 |
| Rajzbum G | J Int Med (2005) | | W 327 | | Aortic calcium | X-ray | FN | DXA | Women:** Aortic calcium prevalence and BMD |
| Schulz E | J Clin Endocriol Metab (2004) | | W 2348 | | Aortic calcium | CT | L | CT | Women:** Aortic calcium and L BMD |
| Schulz E | J Clin Endocriol Metab (2004) | | W 228 | | Aortic calcium | CT | L | CT | Women:** Changes in aortic calcium and L BMD |
| Tanko L B | Calcif Tissue Int (2003) | | W 963 | | Aortic calcium | X-ray | R, L, R | DXA | Women:** F BMD and aortic calcium |
| Kiel D P | Calcif Tissue Int (2002) | | W 364 | W 190 | Aortic calcium | X-ray | M | RG | Women:** Changes in aortic calcium and L BMD |
| Aoyagi K | Calcif Tissue Int (2001) | | A 524 | | Aortic calcium | X-ray | C, R | DXA | Women: AOR (1SD of BMD) for any calcium=1.1 |
| Hak E | ATVB (2000) | Peri | W 720 | | Aortic calcium | X-ray | M | RG | Women:** Aortic calcification and BMD |
| Hak E | ATVB (2000) | Peri | W 236 | | Aortic calcium | X-ray | M | RG | Women:** Change aortic calcification and BMD |
| Vogt M | J Am Geriatr Soc (1997) | | W 2051 | | Aortic calcium | X-ray | C, H, L, R | DXA | Women:** Aortic calcium and R BMD |
| Jie KS | Calcif Tissue Int (1996) | | W 113 | | Aortic calcium | X-ray | M | RG | Women:** Aortic calcium and low BMD |
| Frye M A | Bone and Mineral (1992) | | W 200 | | Aortic calcium | X-ray | FN, L | DXA | Women:* L BMD and aortic calcium |
| Reid I R | J Clin Endocriol Metab (1991) | | W 130 | | Aortic calcium | X-ray | L, F | X-ray | Women: Osteophytes affect BMD measurements |
| Boukhris R | JAMA (1972) | | W 196 B 94 | W 201 B 98 | Aortic calcium | X-ray | L | X-ray | Each:** Inverse Spearman correlation |
| Dent C E | BMJ (1968) | | W 100, B 200 | | Aortic calcium | X-ray | L | X-ray | Women: No relationship |
| Aksoy Y | Coron Art Dis (2005) | | W 55 | W 65 | Valve: Mitral | X-ray | FN, L1-L4 | DXA | Results not interpretable |
| Davutoglu V | Am Heart J (2004) | Peri | W 340 | | Valce: Mitral | Echo | H, L1-L4 | DXA | Women:** AOR (Osteo w. mitral calc) 2.7 |
| Mori H | J Cardio (1990) | | A (39) | | Valve:Mitral, Aortic | Echo | L | CT | Women:* BMD and Mitral calcium |
| Bakhireva L | Menopause (2005) | | W 186 | W 180 | Coronary calcium | EBCT | H, L | DXA | Men: Nothing; Women: (HT: Hip OR=0.6) |
| Barenglots E I | Calcif Tissue Int (1998) | | W 45 | | Coronary calcium | EBCT | FN, L1-L4 | DXA | Women:* L,FN BMD with coronary calcium |
| Wong SY | Osteoporos Int (2005) | | A 1963 | A 1994 | PAD | ABI | H, L1-L4 | DXA | Combined:** ABI and H BMD |
| van der Klift M | Calcif Tissue Int (2002) | | W 3053 | W 2215 | PAD | ABI<0.90 | H, L1-L4 | DXA | Women:** H AOR=1.5: L nothing |
| Vogt, M | JBMR (1997) | | W 1292 | | PAD | ABI linear | C, H, R | DXA | Women: ABI change predicts C, H change |
| Saverino A | Eur Neuro (2006) | | W33 | W16 | Carotid | US-plaque type | H | DXA | Combined:**Osteo and Echogenic plaque OR=6.6 |
| Yamada A | Atherosclerosis (2005) | J 46 | J 103 | | Carotid, Femoral | IMT | C, L | OSI, DXA | Women: OSI inverse with FaIMT |
| Pennisi P | Osteoporos Int (2004) | | W31 | W 35 | Carotid, Femoral | IMT | L, F | DXA | Combined:**Mean L,F BMD less in PAD "cases" |
| Jorgensen L | AJE (2004) | | W 2726 | W 2543 | Carotid | US-plaque type | R | DXA | Combined:** BMD lower for echogenic plaques |
| Kammerer CM | Calcif Tissue Int (2004) | H 306 | H 165 | | Carotid | US-IMT | H, L1-L4, R | DXA | Women: L,H:** BMD and IMT among old. |
| Montalcini T | Am J Cardiol (2004) | | W 157 | | Carotid | US-IMT | C | US | Women: No relationship. |
| Uyama O | Stroke (1997) | | W 50 | | Carotid | US-IMT | L2-L4, T | DXA | Women:** Total BMD and Carotid IMT |

Pre/Post-Menopausal; Peri=Perimenopausal; PAD=Peripheral Arterial Disease; A=Asian, W=Non-Hispanic White, H=Hispanic; C=Calcaneus; FN=Femoral neck; H=Hip; L=Lumbar spine; M=metacarpal; R=Radius;
US=Ultrasound, RG=Radiogrammetry, DXA=Dual energy X-ray Absorptiometry, CT=computer tomography; ARR=Adjusted Risk Ratio; AOR=Adjusted Odds Ratio

# II. CORONARY ARTERY AND AORTIC CALCIUM ARE STRONGLY ASSOCIATED WITH LUMBAR BONE DENSITY: THE MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS, ABDOMINAL AORTIC CALCIUM STUDY

## A. ABSTRACT

Background: Atherosclerosis and osteoporosis share some risk factors, but investigations of their association have been inconclusive. We investigated whether volumetric, trabecular lumbar bone mineral density (vBMD) is associated with coronary artery calcium (CAC) and abdominal aortic calcium (AAC) after adjustment for standard and novel cardiovascular as well as osteoporosis risk factors.

Methods and Results: Between 2002 and 2005, we used quantitative computed tomography to assess vBMD and the presence (Agatston score>0) and extent of CAC and AAC in participants in a sub-study of the Multi-Ethnic Study of Atherosclerosis (MESA). Sequential, sex-specific regression models adjusted associations for age, ethnicity, body mass index, hypertension, dyslipidemia, diabetes, smoking, alcohol consumption, physical activity, interleukin-6, C-reactive protein, homocysteine, and sex hormones. Among 946 women (65.5 years) and 963 men (64.1 years), the prevalence of CAC was 47% and 68%, respectively, and the prevalence of AAC was 70% and 73%, respectively. After adjustment, CAC in women was more prevalent in lower quartiles of vBMD ($p=0.05$ for trend across quartiles). In men, AAC was more prevalent in lower vBMD quartiles ($p$ for trend $<0.01$). Lower vBMD was associated with greater CAC score among women ($p=0.02$ for trend) and men ($p=0.02$ for trend)

with CAC and was associated with greater AAC score (p=0.01 for trend) among women with AAC. Partially and fully adjusted models showed similar results. Conclusions: In cross-sectional analyses, robust, significant associations between lower vBMD and calcified atherosclerosis in both men and women are independent of shared risk factors, suggesting that bone loss and atherosclerosis are related processes.

B. INTRODUCTION

Arterial calcium in the coronary arteries (CAC) and the abdominal aorta (AAC) are closely related to the volume of atherosclerosis in arterial sites(Sangiorgi, Rumberger et al. 1998) and share histological patterns with bone tissues in skeletal sites.(Demer and Abedin 2004) Both arterial calcium and bone density have demonstrated incremental ability to predict future death from cardiovascular disease (CVD),(von der Recke, Hansen et al. 1999; Greenland, LaBree et al. 2004; Samelson, Kiel et al. 2004; Magnus and Broussard 2005) and large, observational studies(Kiel, Kauppila et al. 2001; Van Der Klift, Pols et al. 2002; Jorgensen, Joakimsen et al. 2004; Samelson, Kiel et al. 2004; Bakhireva, Barrett-Connor et al. 2005; Wong, Kwok et al. 2005) have reported significant inverse associations between bone density and atherosclerosis. Both calcified atherosclerosis and bone density are strongly correlated with age, sex and ethnicity, but other determinants are less robust.(Bild, Detrano et al. 2005; Heaney and Recker 2005; Raisz 2005; Rosen 2005)

Laboratory investigations have demonstrated that compounds of oxidized low density lipoproteins (LDL) inhibit normal osteoblast development from bone marrow stromal cells and also promote the calcification of vascular smooth muscle cells,(Parhami,

Morrow et al. 1997) suggesting that the link between bone density and atherosclerosis may be stronger for calcified atherosclerosis.(Demer and Tintut 2003) However, explanations of a bone-artery association typically implicate lipids,(Parhami, Garfinkel et al. 2000) inflammation(Demer and Abedin 2004) or sex hormones(Tanko, Bagger et al. 2003; Bakhireva, Barrett-Connor et al. 2005) to explain the common disease patterns. To date, however, no epidemiologic investigations have been able to evaluate simultaneously the roles of each of these factors in contributing to an association, so the independence of the association is unclear. With few exceptions,(Dent, Engelbrecht et al. 1968; Boukhris and Becker 1972) previous studies of the association have not included a multi-ethnic sample, and patterns of atherosclerosis and bone density are known to vary by ethnicity.

We hypothesized that calcified atherosclerosis, specifically CAC and AAC, would be inversely associated with volumetric, trabecular lumbar bone density of the lumbar spine (vBMD) in an ethnically-diverse population free of symptomatic cardiovascular disease. We also hypothesized that the association between the two would attenuate upon adjustment for lipids, multiple inflammatory markers, sex hormones, and other shared risk factors, if these factors do contribute to the association.(Greenland and Morgenstern 2001)

## C. METHODS

### Study participants

The methods of the Multi-Ethnic Study of Atherosclerosis (MESA) have been described previously.(Bild, Bluemke et al. 2002) In brief, the MESA observational cohort was recruited between July 2000 and August 2002 from six field centers around the United States. The study population consists of 6814 men and women who were

between 45 and 84 years of age and identified themselves as Non-Hispanic White (NHW), Chinese American, African-American or Hispanic.

This report deals with a random sample of MESA participants who participated in the MESA Abdominal Aortic Calcium Study (MESA-AACS). MESA-AACS participants were recruited during one of two follow-up visits between August 2002 and September 2005 from five of the six MESA field centers: Northwestern University (Chicago, Illinois), Wake Forest University School of Medicine (Forsyth County, North Carolina), the University of California Los Angeles (Los Angeles County, California), Columbia University (New York, New York), and the University of Minnesota (St. Paul, Minnesota). Of 2202 MESA participants recruited, 2172 agreed to participate, and 1990 satisfied eligibility criteria including post-menopausal status (women) and no recent prior diagnostic abdominal computed tomography. In all, 1968 participants (974 women and 994 men) completed scanning. Subsequently, 28 women and 31 men were excluded because of vertebral abnormalities complicating bone density measurement. Concurrent coronary calcium scores were available for all but 28 women and 25 men. For these participants coronary calcium scores were replaced with scores from a visit two years prior. There were no missing aortic calcium data, leaving 946 female and 963 male participants for investigation. Signed informed consent was obtained for all participants, and institutional review board approval was obtained from all participating institutions.

Computed Tomography Scanning

Participants were randomly selected for computed tomography (CT) scanning of the chest at one of two clinical visits between August 2002 and September 2005. Scans were performed either with an ECG-triggered (at 80% of the RR interval) electron-beam computed tomography scanner (Chicago and Los Angeles; Imatron C-150)(Breen, Sheedy et al. 1992) or with prospectively ECG-triggered scan acquisition at 50% of the RR interval with a multidetector computed tomography system(Carr, Danitschek et al. 2001) that acquired 4 simultaneous 2.5-mm slices for each cardiac cycle in a sequential or axial scan mode (New York, Forsyth County, and St. Paul field centers; Sensation 64, GE Lightspeed, Siemens S4+ Volume Zoom and Siemens Sensation 16). For accuracy, two chest scans were performed for each individual.

Computed tomography of the abdomen was performed a single time for each individual. For electron-beam computed tomography, scanners were set as follows: scan collimation of 3mm; slice thickness of 6mm; reconstruction using 25 6mm slices with 35 cm field of view and normal kernel. For multi-detector computed tomography, images were reconstructed in a 35 cm field of view with 5mm slice thickness. Participants were scanned along with phantoms of known physical calcium concentration to convert CT numbers directly to equivalent vBMD in mg/cc.(Cann 1988)

Calcium Scoring

Scans were read centrally by the MESA CT Reading Center at Harbor-University of California, Los Angeles Research and Education Institute to identify and quantify calcium in the coronary arteries and in an 8cm segment of the distal

abdominal aorta ending at the aortic bifurcation. Calcium scores among scanning centers and between participants were adjusted with a standard calcium phantom scanned simultaneously for each participant. At least two adjacent pixels with an attenuation coefficient >130 Hounsfield units (modified to adjust for section thickness) defined a calcified lesion, and the average of two coronary calcium scores were calculated using the method of Agatston.(Agatston, Janowitz et al. 1990) Rescan agreement was found to be high with both electron beam tomography and multi-detector computed tomography scanners.(Detrano, Anderson et al. 2005) Inter-observer agreement and intra-observer agreement were very high ($\kappa$=0.93 and 0.90, respectively).(Carr, Nelson et al. 2005)

Bone density measurement

CT data were collected using the Image Analysis QCT 3D PLUS software program (Image Analysis, Columbia, Kentucky), and scans were read centrally at the MESA CT Reading Center at Harbor-University of California Medical Center (Los Angeles, California) by a reader blinded to the results of arterial calcium scoring. Measurements of vBMD in a virtual 10mm-thick slice of trabecular bone from each vertebra (L2 to L4) used soft-ware directed, automated placement of the region of interest (ROI) in the anterior one-half to one-third of the vertebral body where it (1) encompassed a large area exclusively of trabecular, or cancellous bone, (2) excluded cortical bone, and (3) excluded the basivertebral plexus. A trained reader examined each ROI and changed its placement to exclude vertebral abnormalities including bone islands and diffuse density variations or excluded a vertebra entirely only if the

following abnormalities were noted: fractures, metastatic lesions, osteophytes or benign focal lesions within the vertebra. These analyses use bone density from the third lumbar vertebra as it was most commonly available for all participants.

In a random sample of 25 scans re-read on three occasions by the blinded scan reader, there was 100 percent agreement in inclusion or exclusion for all vertebrae (L2-L4). Reader drift was tested using both a multivariate approach to repeated measures analysis of variance and serial t-tests, and neither analysis provided evidence of systematic differences between reads or a time effect in the data.

Clinical Measurements

Upon enrollment in MESA between 2000 and 2002, participants completed a clinical examination and detailed questionnaire. Covariate data from the first exam are used in the present analyses. Age, sex, ethnicity, height, weight, current medications from pill bottles, physical activity patterns (met x min/week), smoking history and alcohol consumption (never/former/current), and previous medical diagnoses were recorded. Hormone therapy among women was defined as recent if hormone use was reported in the previous two years. In addition, dietary intake was assessed with a self-administered food frequency questionnaire (FFQ) and dietary supplement form, and total dietary calcium, and phosphorus were calculated. Body mass index was calculated as mass in kilograms divided by height in meters squared from data concurrent with bone density assessment. Blood pressure was measured 3 times with a Dinamap model Pro 100 automated oscillometric sphygmomanometer (Critikon) with participants at rest in the seated position. The average of the last two

measurements was used to define hypertension as systolic pressure ≥140 mm Hg or diastolic pressure ≥90 mm Hg or current use of antihypertensive medication.(1997)

Laboratory measurements

C-reactive protein (CRP), interleukin-6 (IL-6) and homocysteine were measured. The CRP was measured using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, Illinois) at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, Vermont). Analytical intra-assay coefficient of variations ranged from 2.3% to 4.4%, and inter-assay coefficient of variation ranged from 2.1% to 5.7%. Interleukin-6 concentrations were measured by ultrasensitive enzyme-linked immunosorbent assay (Quantikine HS human interleukin-6 immunoassay; R&D Systems, Minneapolis, Minnesota). Analytical coefficients of variation for this assay were 6.3 percent. Plasma total homocysteine was measured by a fluorescence polarization immunoassay (IMx Homocysteine Assay, Axis Biochemicals ASA, Oslo, Norway) using the IMx Analyzer (Abbott Diagnostics, 100 Abbott Park Rd, Abbott Park, Illinois 60064) at the Biochemical Genetics Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN) with a laboratory CV of 5.1%.

Serum sex hormone concentrations were measured from stored samples at the University of Massachusetts Medical Center in Worcester, MA. Total testosterone and dehydroepiandrosterone (DHEA) were measured directly using radioimmunoassay kits, and SHBG was measured by chemiluminescent enzyme immunometric assay using Immulite kits obtained from Diagnostic Products

Corporation (Los Angeles, CA). For serum total T, the intra-assay CV and inter-assay CV were 10.0% and 13.2%; for SHBG, the inter-assay CV was 9.1%, and the intra-assay CV was 9.8%. Estradiol was measured by use of an ultra-sensitive radioimmunoassay kit from Diagnostic System Laboratories (Webster, TX); for estradiol, the inter-assay was CV=5.2% ,and the intra-assay was CV=2.6%.

Total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, and glucose levels were measured from blood samples obtained after a 12-hour fast. Analyses reported here use measurements ascertained at the initial visit to minimize missing values. Low density lipoprotein (LDL) cholesterol was calculated with the Friedewald equation.(Friedewald, Levy et al. 1972) Diabetes was categorized if either fasting plasma glucose was greater than 126 mg/dL or if the participant reported previous diagnosis of diabetes or the use of hypoglycemic medications.

Statistical Analyses

All analyses were stratified by sex given the significantly different distributions of calcified atherosclerosis(Allison, Criqui et al. 2003) and bone density in men and women.(Riggs, Khosla et al. 2002) Ethnic-specific associations are presented because of limited published data on a bone-atherosclerosis association in non-white ethnic groups. Chi-square tests of association and generalized linear models were used to compare distributions of categorical and continuous variables, respectively, across ethnic groups and bone density quartiles.

To determine the association between bone density and calcified atherosclerosis, two part analyses were used. The association between bone density

and presence versus absence of calcified atherosclerosis was directly estimated as the relative risk (or relative cumulative incidence, or relative prevalence) using regression models with robust (generalized estimating equations (GEE) or "sandwich") estimates of variance and assumed Gaussian error. This model form was selected because the odds ratio requires the rare disease assumption to estimate accurately the relative risk.(Greenland 2004) The second stage analyses included only those with detectable CAC or AAC and used multiple linear regression models to estimate the mean (ln)Agatston score for each bone density quartile.

We aimed to test the contributions of different suites of variables to the associations between bone density and atherosclerosis. The following terms were conceived in mutually exclusive theoretical concepts as follows: confounders are covariates that are associated with both bone density and atherosclerosis but are causally associated with only one of those outcomes; mediators are covariates that mediate, or are in a causal pathway between atherosclerosis and bone density; common risk factors are covariates that cause both atherosclerosis and low bone density, but through independent mechanisms. The empirical effect of adjusting for a confounder, mediator or common risk factor in serial models is an attenuation or augmentation of an association between atherosclerosis and bone density. No change in the association with adjustment indicates that the added covariates make no contribution to the association.(Greenland and Morgenstern 2001)

Associations were adjusted for suites of covariates in serial models. Model 1 adjusted for age in quartiles and ethnicity, given the strong, documented associations

of these covariates with both bone density and atherosclerosis. Model 2 adjusted additionally for total cholesterol, HDL-cholesterol, lipid medication, hypertension, diabetes, smoking (never/former/current), body mass index, physical activity, LN(dietary calcium), alcohol consumption (never/former/current), and hormone therapy (women only). The final model (Model 3) additionally adjusted the RR for the natural log (LN) of interleukin-6, LN(C-reactive protein), homocysteine, and sex-specific quartiles of total testosterone, estradiol, and serum hormone binding globulin (but not for recent hormone therapy). For women, quartiles of sex hormones were investigated when defined separately for those using and not using supplemental estrogens in an unordered eight-level variable and when groups were collapsed to form quartiles making a four-level variable. Models were inspected for attenuation and/or augmentation of an association, defined as a change in the relative risk of greater than 25% or, for relative risk estimates between 0.9 and 1.1, a change in the estimate of 0.10.

Tests of interaction by age, ethnicity, and hormone therapy (in women) were performed. Multiple variable forms for age including linear continuous, age by decades of life, and age quartiles were investigated, as well as categories of time since the menopause began (women only) with little difference in results. Final models use unordered age quartiles. Multi-collinearity was investigated, but not found, by screening for tolerance values of less than 0.15 for variables in fully adjusted models. Models of ethnic-specific results are reported after adjustment for ethnic differences in other covariates.

D. RESULTS

The characteristics of women and men in this study are shown in Table 2. The average age of women was 65 years, and the average age of men was 64 years. Non-white participants were 62% of women and 59% of men. In women, coronary artery calcium (CAC) and abdominal aortic calcium (AAC) prevalence were 47% and 70%. In men, CAC and AAC prevalence were 68% and 73%. Among women, 37% had taken estrogen within fours years of bone density assessment.

Age- and ethnicity-adjusted characteristics of women and men by sex-specific quartiles of bone density are shown in Table 3. Bone density was significantly and inversely associated with age in both women ($p<0.001$) and men ($p<0.001$). In both women ($p<0.001$) and men ($p<0.001$), ethnicity was significantly associated with bone density, with African Americans being more likely to be in the highest quartiles, and whites and Chinese being more likely to be in the lowest quartiles. After adjustment for age and ethnicity, body mass index was positively associated with bone density ($p=0.04$), and C-reactive protein was inversely associated with bone density ($p=0.004$) in women. Recent hormone therapy was more common in women in the highest quartiles of bone density ($p=0.002$). In women not taking estrogens (69% of women, n=649), mean estradiol, serum hormone binding globulin (SHBG) and total testosterone did not differ significantly by bone density quartile after adjustment. In men, greater estradiol ($p=0.01$) and lower SHBG ($p=0.045$) were significantly associated with greater vBMD quartile after adjustment. In all, few covariates other than age and ethnicity were strongly associated with BMD for either men or women.

Table 4 displays relative risks (RR) of any CAC and AAC derived from sex-specific sequential models. Among women, quartile of vBMD was significantly associated with CAC presence. Comparing the highest to the lowest quartile of bone density, this association differed little when adjusted for age and ethnicity (RR-1.17; 95%CI 0.95-1.43, p for trend across quartiles 0.05) or when fully adjusted (RR=1.15; 95%CI 0.96-1.39, p for trend across quartiles 0.05). In contrast, vBMD quartile was not significantly associated with AAC presence in any models among women.

In men, vBMD was not significantly associated with CAC presence, but was significantly associated with AAC presence. After adjustment for age and ethnicity, men in the lowest quartile of vBMD had 1.19 greater prevalence (95% CI 1.07-1.32, p for trend across quartiles <0.01) of AAC compared with men in the highest quartile of vBMD. The magnitude and significance of this association did not change after full adjustment.

Table 5 displays the adjusted quartile differences in mean values of LN(CAC) and LN(AAC) among participants with positive CAC or AAC scores, respectively. The adjusted mean values for those in the highest quartile of vBMD are compared with those in lower vBMD quartiles after sequential adjustments for multiple covariates as in Table 3. In women with CAC, lower vBMD was significantly associated with greater CAC score in all models ($p<0.02$ for trend for all models). Lower vBMD was also significantly associated with greater AAC score among women with AAC, regardless of adjustments ($p<0.01$ for trend for all models). In men with CAC, lower vBMD quartile was associated with greater CAC score, and this

association was significant after full adjustment (p for trend across quartiles 0.02). In men with AAC, lower vBMD quartile was associated with greater AAC, but differences across quartiles were not significant in any models.

We conducted multiple sensitivity analyses with the following variations for the analyses presented in Table 4 and Table 5: substitution of L3 vBMD with vBMD values from the fourth lumbar vertebra (Table 4 and 5), exclusion of participants without concurrent vBMD and CAC assessment (Table 4 and 5), alternative sex hormone adjustment using bio-available testosterone and dihydroepiandrosterone instead of total testosterone and SHBG (Table 4 and 5), alternative sex hormone adjustment using quartiles specific to women taking or not taking supplemental estrogen (Table 4 and 5), calcium presence defined as Agatston score of 10 or greater (Table 4), use of age quartiles and vBMD quartiles specific for those with CAC or AAC (Table 5). In these alternative models, patterns of associations were similar across models, and the results of final models did not differ materially from those shown.

Figure 1 displays the adjusted, sex-specific relative risks for CAC and AAC by ethnicity comparing those in the highest vBMD quartile with those in the lowest vBMD quartile. Although no overall interactions by ethnicity were significant, associations between vBMD and CAC prevalence in women appear strongest in white and African American women. In men, however, lower vBMD was significantly associated with lower CAC prevalence in Chinese participants (comparing highest to lowest vBMD quartile, RR=0.72; 95%CI 0.53-0.98). This positive association is in

contrast to the inverse associations between vBMD and arterial calcium in the general sample, in other ethnic groups, and for other measures of arterial calcium.

Figure 2 displays the adjusted, sex-specific mean values for CAC and AAC score by ethnicity for all quartiles of vBMD for those with either CAC or AAC. Although no overall interactions were significant, the significant associations between vBMD and CAC and AAC score were seen in white and Hispanic women. In men, there was a suggestion of a positive association between vBMD and CAC score in Chinese participants, but this association was not significant, and pair-wise tests of interaction with other ethnic groups for this outcome were not significant ($p>0.10$ for all). For all ethnic-specific estimates, additional analyses using ethnic-specific quartiles for bone density yielded similar patterns of associations.

E. DISCUSSION

This study demonstrated robust, inverse, and independent associations between volumetric, trabecular lumbar bone density and calcified atherosclerosis. These associations were with coronary artery calcium prevalence and extent in women, greater aortic calcium score in women, and aortic calcium prevalence in men. In sequential models, associations between bone density and atherosclerosis showed little evidence of attenuation or augmentation upon adjustment for lipids, inflammatory markers, and sex hormones, as well as other covariates including shared risk factors for atherosclerosis and bone density such as physical activity and smoking. Age alone appeared to be the only covariate for which adjustment attenuated the associations.(Greenland and Morgenstern 2001)

The present study was the first of its kind (Dent, Engelbrecht et al. 1968; Boukhris and Becker 1972) to include both white and non-white participants. Patterns were generally similar across ethnic groups, except for the anomalous finding of a positive association between bone density and coronary calcium prevalence among 136 Chinese men. This finding could be due to random error, although the pair-wise interaction terms comparing men in the other three ethnic groups were of borderline significance.

The significant, inverse associations between bone density and atherosclerosis in the present study are consistent with previous investigations in large samples of white women and extend previous findings to the coronary arteries, to men, and to non-white populations.(Vogt, San Valentin et al. 1997; Hak, Pols et al. 2000; Schulz, Arfai et al. 2004) The present study is, to our knowledge, the first population-based test of a bone-artery association to assess bone density volumetrically, which uniquely permits the specific measurement of trabecular bone, the exclusion of vertebrae with bony islands or osteophytes, and volumetric accounting of differences in vertebra size by age, sex and ethnicity.(Lang, Li et al. 1999; Lang, Guglielmi et al. 2002; Riggs, Melton III et al. 2004) In addition, this study attempted to examine the effects of adjustment for lipids, multiple inflammatory markers, and sex hormones.

Evidence indicating possible roles for lipids, inflammation, and sex hormones in the association between osteoporosis and atherosclerosis comes indirectly from epidemiologic studies, animal models and cell culture studies.(McFarlane, Muniyappa et al. 2004) Some,(Adami, Braga et al. 2003; Hsu, Venners et al. 2006) but not

all,(Wu, Yang et al. 2003; Solomon, Avorn et al. 2005) epidemiologic investigations have reported that lipids are associated with bone density, and that saturated fat intake is associated with bone density.(Corwin, Hartman et al. 2006) Atherosclerosis as well as osteoporosis-like disease can be induced by assignment to a high-fat diet in mice prone to atherosclerosis.(Parhami, Tintut et al. 2001) Cell-culture studies have demonstrated that oxidized LDL can inhibit the differentiation of osteoblasts in bone, as in osteoporosis, and promote the calcification of smooth muscle vascular cells, as in atherosclerosis.(Parhami, Morrow et al. 1997) In prospective, observational studies, interleukin-6 has been independently associated with CVD events(Ridker, Rifai et al. 2000) as well as bone loss,(Scheidt-Nave, Bismar et al. 2001) and homocysteine has been demonstrated as risk factor for CVD(Wald, Law et al. 2002) as well as osteoporotic fractures.(McLean, Jacques et al. 2004; van Meurs, Dhonukshe-Rutten et al. 2004) Finally, the enigmatic role of sex hormones in CVD is complemented by their clearer roles in bone loss in both men and women.(Riggs, Khosla et al. 2002)

Using serial models in this study, these factors did not attenuate the associations between bone density and atherosclerosis. Nor did multiple attempts to adjust for age fully attenuate the associations. Reports from this and other large, population-based, observational studies including the Framingham,(Kiel, Kauppila et al. 2001) Rancho Bernardo,(Bakhireva, Barrett-Connor et al. 2005) Tromso,(Jorgensen, Joakimsen et al. 2004) and Rotterdam studies,(Van Der Klift, Pols et al. 2002) suggest that previously published, significant bone-artery associations are not the result of publication bias alone.(Ioannidis 2005) In response, we offer three

explanations for the association here. First, lipids, inflammation and sex hormones may account for the association, but our cross-sectional study design did not allow for a comprehensive assessment of their effects. Alternatively, other factors, not measured here, such as genetic environments, may explain what is a non-causal association. Finally, and most parsimoniously, atherosclerosis and bone density loss may be related processes.

A cross-sectional study is limited in its ability to evaluate the effects of long-acting factors such as lipids, inflammation, and sex hormones. However, large cross-sectional studies often detect associations with lipids, inflammatory markers or sex hormones that are subsequently validated in prospective studies. Regarding other factors, many factors not measured in the MESA are known to affect disease development in both the bones and arteries. In recent studies the cannabinoid system has been implicated with atherosclerosis and osteoporosis in mice and in humans. (Karsak, Cohen-Solal et al. 2005; Steffens, Veillard et al. 2005; Ofek, Karsak et al. 2006) Cannabinoid receptors are found in atherosclerotic lesions but also exert effects on bone in part through the osteoprotegerin/RANKL system which affects both atherosclerotic(Erdogan, Aslan et al. 2004) and osteoporotic change(Raisz 2005).

Alternatively, trabecular bone loss may result, in part, from atherosclerotic disease in bone arteries.(Parhami and Demer 1997) Thus osteoporosis may represent, to some extent, bone vascular disease.(Jordan 2005) Recent magnetic resonance imaging studies have demonstrated that diminished bone perfusion is independently correlated with greater carotid atherosclerosis, lower bone density, and greater bone

marrow fat content, with the latter two being highly characteristic of osteoporosis.(Chen, Ting-Fang Shih et al. 2004; Shih, Liu et al. 2004; Griffith, Yeung et al. 2005) Lower bone density is common among type1 diabetics among whom micro-vascular disease is present,(Tuominen, Impivaara et al. 1999) and in a trial among rats, assignment to hormone therapy or nitroglycerin, a potent vasodilator, resulted in equal improvements in bone density after surgical menopause.(Wimalawansa 2000) The recent application of volumetric roentographic methods has demonstrated that trabecular bone loss may begin as early as the second and third decades of life in men, coinciding with the early development of atherosclerotic disease, typically in the aorta.(Allison, Criqui et al. 2003; Riggs, Melton III et al. 2004) The rapid loss of trabecular bone over the early menopause in women crudely mirrors the "catch-up" phase of increased cardiovascular risk in women during that time.(Riggs, Melton III et al. 2004) Trabecular bone density may prove useful for refined CVD risk stratification.

Despite the small RR estimates generated by the GEE models, bone density was strongly associated with CAC in women and AAC in men, especially compared to standard cardiovascular risk factors including those making up the Framingham risk equations. For example, the RR for CAC in women associated with a difference of two quartiles of vBMD, such as the difference between lowest (Q1) and next to highest (Q3) vBMD quartiles, is 1.16 (95%CI 1.03-1.31). This effect size was greater than and more significant than those effects associated with a two quartile decrease in HDL-cholesterol (RR=1.07; 95%CI 0.96-1.18) and is comparable to that associated

with use of lipid medication (RR=1.18, 95%CI 1.04-1.34) or hypertension (RR=1.29; 95%CI 1.13-1.46). Similarly for men, the RR for AAC associated with the difference between the lowest (Q1) and next to highest (Q3) vBMD quartiles was 1.09 (95%CI 1.03-1.16). This effect size is greater than that associated with a two quartile decrease in HDL-cholesterol (RR=1.03; 95%CI 0.98-1.08) or use of lipid medication (RR=1.07, 95%CI 1.00-1.14) or hypertension (RR=1.07; 95%CI 1.01-1.13).

Limitations of our study include a cross-sectional design, bone density measurements from a single site, one-time measures of sex hormones and inflammatory factors, and lack of data describing vitamin D status, parathyroid hormone levels, the use of selective estrogen receptor modulators, and anti-resorptive agents such as bisphosphonates. In light of these limitations, conclusions about causality from these data are speculative. Local effects of lipids, inflammation or sex hormones in the bone arteries, effects not quantifiable by assessment of systemic venous blood here, may yet explain the association. This is the first investigation of its kind with a multi-ethnic sample, but conclusions about any ethnic differences are further limited by the smaller sizes of sub-groups. Although the use of asymptomatic study samples and subclinical endpoints, as in this study, has clear advantages when attempting to study two disease processes,(Pearl 1929) any associations between bone density and atherosclerosis or covariance with other factors could be different in this sample than in another samples that include individuals with extensive disease. Use of an asymptomatic sample would likely bias age-adjusted associations to the null, however.

In summary, this study investigated a large, multi-ethnic, population-based sample with precise measures of atherosclerosis from multiple sites, accurate and precise measures of volumetric bone density, and extensive data on risk factors for atherosclerosis and low bone density including lipids, inflammatory markers, and sex hormones. We found that trabecular bone density was significantly associated with coronary calcium presence and amount in women, amount of aortic calcium in women, and aortic calcium prevalence in men. These associations did not change upon adjustment for multiple hypothesized correlates of an association, suggesting that bone loss and atherosclerosis are related processes and may be causally linked. Clinically, the utility of bone density for refined CVD risk stratification and/or assessment of biological age merits further investigation. Joint laboratory and epidemiologic investigations using repeated measures and assays sensitive to potential micro-vascular or local effects may elucidate the determinants and implications of a bone-artery association, including improved understanding of aging and potential dual-purpose therapies.

**Table 2. Characteristics of women and men in the MESA, AACS, 2000-2005.**

| | Women | Men |
|---|---|---|
| **Characteristic** | **All** | **All** |
| Sample size No. | 946 | 963 |
| Age, y, mean (SD) | 65.2 (9.2) | 64.1 (9.9) |
| Ethnicity % (N) | | |
| White | 38 (360) | 41 (404) |
| Chinese | 13 (119) | 14 (136) |
| Black | 23 (218) | 18 (172) |
| Hispanic | 26 (249) | 26 (251) |
| Bone density, mean (SD), mg/cc | 111.1 (40.5) | 120.9 (38.9) |
| Coronary Artery Calcium | | |
| Prevalence (%) | 47 (442) | 68 (655) |
| LN(Score+1), mean (SD) | 4.2 (1.6) | 4.7 (1.8) |
| Abdominal Aortic Calcium | | |
| Prevalence (%) | 70 (662) | 73 (703) |
| LN(Score+1), mean (SD) | 6.2 (1.8) | 6.4 (1.7) |
| Physical activity, met-min/week*100 | 120 (59) | 120 (73) |
| Body mass index, kg/m^2 | 28.4 (5.8) | 27.8 (4.4) |
| Total cholesterol, mg/dL | 200.9 (33.1) | 190.5 (33.9) |
| HDL cholesterol, mg/dL | 56.6 (15.6) | 45.2 (11.8) |
| Cholesterol medication (%) | 17.5 (165) | 14 (136) |
| Diabetes (%) | 12 (110) | 14 (136) |
| Hypertension (%) | 47 (448) | 44 (424) |
| Cigarette smoking (%) | | |
| Never | 60 | 42 |
| Former | 28 | 45 |
| Current | 12 | 13 |
| LN(dietary calcium) mg/day | 6.4 (0.6) | 6.5 (0.6) |
| LN(Interleukin-6) pg/mL | 0.85 (0.36) | 0.81 (0.37) |
| LN(C-reactive protein) mg/L | 1.35 (0.78) | 1.03 (0.64) |
| Homocysteine, µmol/L | 8.7 (4.6) | 10.0 (3.4) |
| Recent hormone therapy† (%) | 37 (346) | - - - |
| Estradiol, nmol/L | 0.07 (0.07) | 0.12 (0.04) |
| SHBG, nmol/L | 56.7 (33.7) | 43.2 (17.9) |
| Testosterone, nmol/L | 1.10 (0.88) | 15.0 (5.6) |

Percentage(N) and mean(SD) are displayed for discrete and continuous variables, respectively.

Means of calcium scores includes only participants with positive scores.

†Estrogen use in the previous two years; Values for estradiol, SHBG (serum hormone binding globulin) and testosterone in women include only 587 women not taking estrogens.

**Table 3. Age and ethnicity adjusted distribution of participants by sex-specific quartile of lumbar bone density in the MESA, AACS, 2000-2005.**

| | WOMEN | | | | | MEN | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Bone Density Quartiles | | | | | Bone Density Quartiles | | | | |
| **Characteristic** | **1 Low** | **2** | **3** | **4 High** | **p-value** | **1 Low** | **2** | **3** | **4 High** | **p-value** |
| Sample size No. | 242 | 235 | 235 | 234 | | 244 | 236 | 241 | 242 | |
| Bone density, mg/cc (range) | 11, 82 | 83, 107 | 108, 136 | 137, 271 | | 10, 88 | 89, 110 | 111, 138 | 139, 274 | |
| Age, years (SD) | 73 (8) | 66 (8) | 63 (8) | 59 (8) | <0.001 | 70 (9) | 66 (9) | 60 (9) | 59 (9) | <0.001 |
| Ethnicity (%) | | | | | <0.001 | | | | | <0.001 |
| White | 32 | 23 | 25 | 20 | | 31 | 27 | 25 | 17 | |
| Chinese | 31 | 32 | 18 | 19 | | 32 | 28 | 29 | 11 | |
| Black | 13 | 20 | 27 | 39 | | 10 | 16 | 21 | 53 | |
| Hispanic | 25 | 28 | 26 | 21 | | 23 | 24 | 26 | 27 | |
| Physical activity, met-min/week*100 | 107±4 | 116±4 | 115±4 | 128±4 | 0.007 | 117±5 | 120±5 | 122±5 | 117±5 | 0.802 |
| Body mass index, kg/m^2 | 27.4±0.4 | 27.4±0.4 | 28.2±0.4 | 28.8±0.4 | 0.040 | 27.1±0.3 | 27.3±0.3 | 27.5±0.3 | 27.6± | 0.723 |
| Total cholesterol, mg/dL | 202±2 | 200±2 | 202±2 | 197±2 | 0.376 | 189±2 | 190±2 | 190±2 | 192±2 | 0.753 |
| HDL cholesterol, mg/dL | 56±1 | 56±1 | 55±1 | 56±1 | 0.728 | 45±1 | 46±1 | 45±1 | 46±1 | 0.530 |
| Cholesterol medication (%) | 17±3 | 19±3 | 16±3 | 17±3 | 0.825 | 13±2 | 12±2 | 10±2 | 15±2 | 0.480 |
| Diabetes (%) | 12±2 | 12±2 | 11±2 | 14±2 | 0.729 | 16±2 | 14±2 | 15±2 | 15±2 | 0.885 |
| Hypertension (%) | 41±4 | 45±3 | 50±3 | 52±3 | 0.142 | 45±3 | 43±3 | 41±3 | 47±3 | 0.570 |
| Cigarette smoking (%) | | | | | 0.401 | | | | | 0.396 |
| Never | 68 | 65 | 66 | 64 | | 39 | 40 | 43 | 46 | |
| Former | 19 | 22 | 25 | 29 | | 43 | 45 | 41 | 45 | |
| Current | 13 | 13 | 9 | 7 | | 18 | 15 | 16 | 9 | |
| LN(dietary calcium) mg/day | 6.4±0.05 | 6.3±0.04 | 6.4±0.04 | 6.3±0.05 | 0.277 | 6.5±0.04 | 6.6±0.04 | 6.5±0.04 | 6.5±0.04 | 0.515 |
| LN(Interleukin-6) pg/mL | 0.78±0.03 | 0.81±0.02 | 0.84±0.02 | 0.87±0.03 | 0.142 | 0.82±0.03 | 0.79±0.02 | 0.83±0.02 | 0.79±0.02 | 0.510 |
| LN(C-reactive protein) mg/L | 1.16±0.06 | 1.22±0.05 | 1.36±0.05 | 1.43±0.05 | 0.004 | 1.00±0.04 | 0.92±0.04 | 1.06±0.04 | 1.03±0.04 | 0.102 |
| Homocysteine, µmol/L | 8.5±0.3 | 8.8±0.3 | 8.9±0.3 | 8.2±0.3 | 0.420 | 9.8±0.2 | 10.4±0.2 | 10.6±0.2 | 9.6±0.2 | 0.004 |
| Recent hormone therapy† (%) | 22±3 | 28±3 | 34±3 | 45±3 | 0.002 | - - - | - - - | - - - | - - - | - - - |
| Estradiol, nmol/L † | 0.066±0.006 | 0.073±0.005 | 0.076±0.006 | 0.091±0.007 | 0.091 | 0.110±0.003 | 0.114±0.003 | 0.118±0.003 | 0.125±0.003 | 0.010 |
| SHBG, nmol/L † | 57.5±3.0 | 57.7±2.7 | 52.3±2.9 | 52.1±3.5 | 0.431 | 46.4±1.2 | 43.8±1.2 | 42.3±1.1 | 41.9±1.2 | 0.048 |
| Testosterone, nmol/L † | 0.99±0.08 | 1.06±0.07 | 1.09±0.08 | 1.28±0.09 | 0.156 | 14.6±0.4 | 14.9±0.4 | 14.9±0.4 | 15.4±0.4 | 0.525 |

*Values other than age , ethnicity, and bone density are adjusted for age and ethnicity. LN denotes natural log. Mean±standard error and percentage±standard error are displayed for continuous and discrete variables, respectively. † Estrogen use in the previous two years; Values for estradiol, SHBG (serum hormone binding globulin) and testosterone in women include only 587 women not taking estrogens.

**Table 4. Relative risk (RR) for coronary artery calcium (CAC) and abdominal aortic calcium (AAC) associated with sex-specific quartiles of lumbar trabecular bone density (vBMD), the MESA, Abdominal Aortic Calcium Study 2000-2005.**

| | | WOMEN | | | | | | MEN | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Model 1* n=946 | | Model 2† n=865 | | Model 3‡ n=769 | | Model 1* n=963 | | Model 2† n=887 | | Model 3‡ n=848 | |
| | | RR | 95%CI | RR | 95%CI | RR | 95%CI | RR | 95%CI | RR | 95%CI | RR | 95%CI |
| **CAC** | | | | | | | | | | | | | |
| | | p=0.05 | | p<0.02 | | p=0.05 | | p=0.48 | | p=0.19 | | p=0.22 | |
| vBMD | Q1 | 1.17 | 0.95-1.43 | 1.16 | 0.96-1.41 | 1.15 | 0.96-1.39 | 1.02 | 0.91-1.14 | 1.06 | 0.94-1.19 | 1.05 | 0.93-1.19 |
| vBMD | Q2 | 1.09 | 0.88-1.35 | 1.06 | 0.87-1.29 | 1.05 | 0.86-1.29 | 0.98 | 0.87-1.10 | 1.01 | 0.89-1.14 | 1.01 | 0.90-1.15 |
| vBMD | Q3 | 0.97 | 0.77-1.22 | 0.92 | 0.74-1.16 | 0.96 | 0.77-1.20 | 0.95 | 0.83-1.08 | 0.98 | 0.86-1.12 | 0.97 | 0.85-1.11 |
| vBMD | Q4 | 1 | - - - - | 1 | - - - - | 1 | - - - - | 1 | - - - - | 1 | - - - - | 1 | - - - - |
| **AAC** | | | | | | | | | | | | | |
| | | p=0.17 | | p=0.14 | | p=0.15 | | p<0.01 | | p<0.01 | | p<0.01 | |
| vBMD | Q1 | 1.03 | 0.93-1.14 | 1.02 | 0.93-1.12 | 1.02 | 0.93-1.12 | 1.19* | 1.07-1.32 | 1.19* | 1.07-1.33 | 1.19 | 1.07-1.32 |
| vBMD | Q2 | 1.02 | 0.91-1.14 | 0.99 | 0.89-1.10 | 0.98 | 0.88-1.09 | 1.13* | 1.01-1.27 | 1.14* | 1.02-1.28 | 1.15 | 1.03-1.29 |
| vBMD | Q3 | 0.91 | 0.79-1.03 | 0.88 | 0.78-0.99 | 0.88 | 0.78-0.99 | 1.11 | 0.98-1.25 | 1.13 | 1.00-1.27 | 1.11 | 0.99-1.26 |
| vBMD | Q4 | 1 | - - - - | 1 | - - - - | 1 | - - - - | 1 | - - - - | 1 | - - - - | 1 | - - - - |

P-values are for trend

* Adjusted for age and ethnicity.

† Also adjusted for total cholesterol, HDL-cholesterol, lipid medication, hypertension, diabetes, smoking (never/former/current), body mass index, physical activity, LN(dietary calcium), alcohol consumption (never/former/current), and hormone therapy (women only).

‡Also adjusted for LN(IL-6), LN(C-reactive protein), homocysteine, and quartiles of total testosterone, estradiol, and serum hormone binding globulin.

**Table 5. Adjusted difference from reference for coronary artery calcium (CAC) and abdominal aortic calcium (AAC) for sex-specific quartiles of lumbar trabecular bone density (vBMD), the MESA, Abdominal Aortic Calcium Study 2000-2005.**

| | | WOMEN | | | MEN | | |
|---|---|---|---|---|---|---|---|
| | | **Model 1*** | **Model 2†** | **Model 3‡** | **Model 1*** | **Model 2†** | **Model 3‡** |
| | | Difference (SE) | Difference (SE) | Difference (SE) | Difference (SE) | Difference (SE) | Difference (SE) |
| **ln(CAC+1)** | | n=442 | n=405 | n=383 | n=655 | n=599 | n=573 |
| | | p=0.02 | p<0.01 | p<0.01 | p=0.14 | p=0.13 | p=0.02 |
| vBMD | Q1 | ^+0.51 (0.25) | ^+0.74 (0.26) | ^+0.80 (0.37) | +0.32 (0.22) | +0.39 (0.23) | ^+0.77 (0.31) |
| vBMD | Q2 | +0.40 (0.24) | ^+0.51 (0.25) | +0.11 (0.36) | +0.25 (0.22) | +0.28 (0.22) | +0.47 (0.30) |
| vBMD | Q3 | +0.06 (0.25) | +0.14 (0.26) | -0.51 (0.37) | +0.16 (0.22) | +0.31 (0.23) | +0.50 (0.31) |
| vBMD | Q4 | Reference | Reference | Reference | Reference | Reference | Reference |
| **ln(AAC+1)** | | n=662 | n=601 | n=561 | n=703 | n=652 | n=621 |
| | | p<0.01 | p<0.01 | p<0.01 | p=0.32 | p=0.17 | p=0.20 |
| vBMD | Q1 | ^+0.57 (0.22) | ^+0.64 (0.22) | ^+0.71 (0.22) | +0.15 (0.19) | +0.23 (0.19) | +0.23 (0.20) |
| vBMD | Q2 | +0.32 (0.21) | +0.26 (0.21) | +0.26 (0.22) | +0.01 (0.19) | +0.11 (0.19) | +0.14 (0.20) |
| vBMD | Q3 | +0.10 (0.21) | +0.04 (0.21) | +0.13 (0.22) | -0.06 (0.19) | +0.01 (0.19) | +0.03 (0.20) |
| vBMD | Q4 | Reference | Reference | Reference | Reference | Reference | Reference |

^ denotes significant difference from reference. P-values are for trend

* Adjusted for age and ethnicity.

† Also adjusted for total cholesterol, HDL-cholesterol, lipid medication, hypertension, diabetes, smoking (never/former/current), body mass index, physical activity, LN(dietary calcium), alcohol consumption (never/former/current), and hormone therapy (women only).

‡Also adjusted for LN(IL-6), LN(C-reactive protein), homocysteine, and quartiles of total testosterone, estradiol, and serum hormone binding globulin

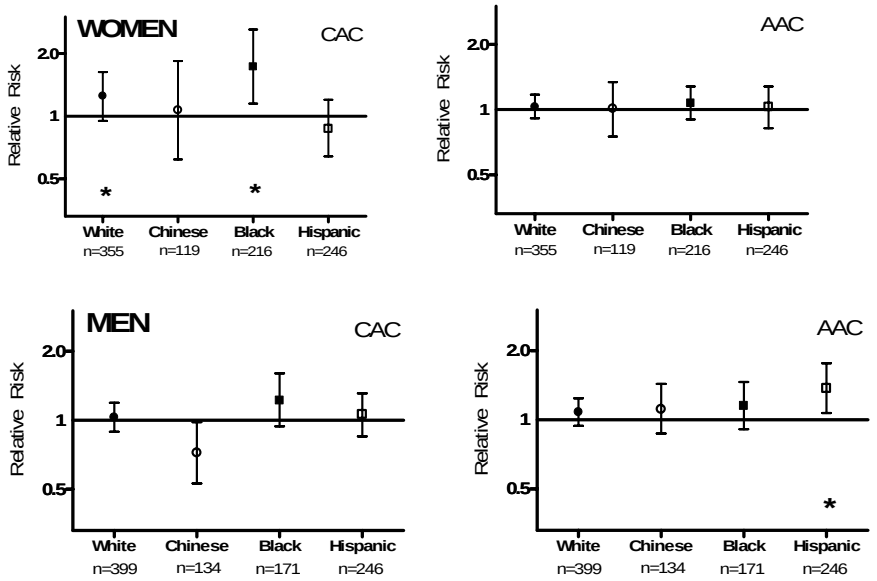


**Figure 1. Adjusted† relative risk and 95%CI for CAC and AAC for those in with the lowest bone density quartile compared to those with the highest bone density for women and men of different ethnic groups, MESA, Abdominal Aortic Calcium Study 2000-2005.**

* $p<0.05$ for overall bone density variable

† Adjusted for age, ethnicity, total cholesterol, HDL-cholesterol, lipid medication, hypertension, diabetes, smoking, body mass index, and physical activity and hormone therapy (women only)

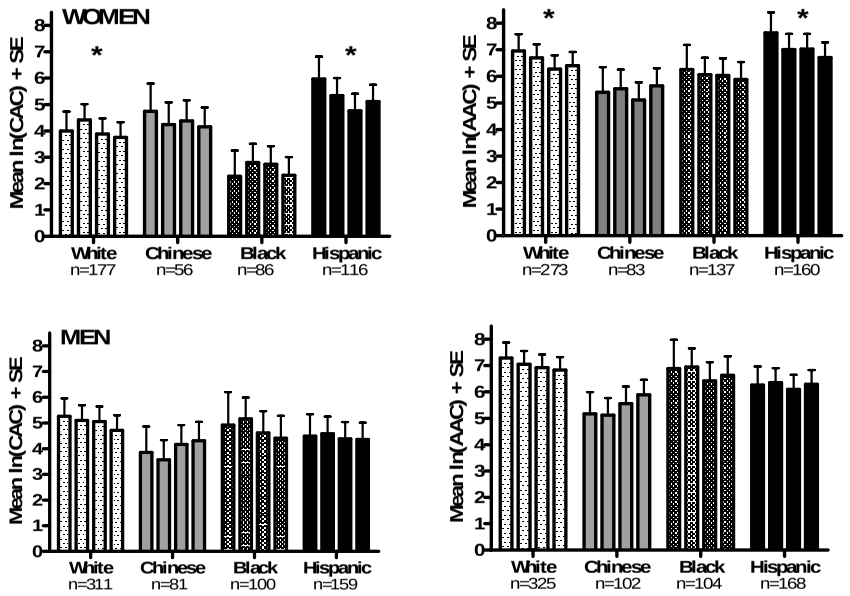


**Figure 2 Adjusted mean natural log coronary calcium score ln(CAC) and abdominal aortic calcium sore ln(AAC) with standard error by sex, ethnicity and bone density quartile (left to right, lowest to highest) for those with either coronary or aortic calcium, the Multi-Ethnic Study of Atherosclerosis, Abdominal Aortic Calcium Study 2000-2005.**

* $p<0.05$ for overall bone density variable

† Adjusted for age, ethnicity, total cholesterol, HDL-cholesterol, lipid medication, hypertension, diabetes, smoking, body mass index, and physical activity and hormone therapy (women only)

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# III. BONE DENSITY AND PERIPHERAL ATHEROSCLEROSIS: THE MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS, ABDOMINAL AORTIC CALCIUM STUDY

## A. ABSTRACT

Background: Molecular and cell biology studies have demonstrated an association between bone and arterial wall disease. However epidemiologic evidence of an independent bone-artery association has been inconclusive. We tested the association of volumetric trabecular lumbar bone mineral density (vBMD) with the ankle-brachial index (ABI), intima-media thickness of the common carotid (CCA-IMT) and internal carotid (ICA-IMT) arteries, and carotid plaque echogenicity.

Methods and Results: Between 2002 and 2005, we used computed tomography to assess vBMD in a random subset of 904 post-menopausal female (62.4 years; 62% non-white; 12% ABI<1; 17% CCA-IMT>1mm; 33% ICA-IMT>1mm) and 929 male (61.4 years; 58% non-white; 6% ABI<1; 25% CCA-IMT>1mm; 40% ICA-IMT>1mm) Multi-Ethnic Study of Atherosclerosis (MESA) participants. In serial, sex-specific regression models adjusting for age, ethnicity, body mass index, dyslipidemia, hypertension, smoking, alcohol consumption, diabetes, homocysteine, interleukin-6 and sex hormones, higher vBMD was associated with low ABI in men (difference of -0.03; 95%CI -0.05 to -0.01 between highest and lowest vBMD quartiles, overall $p<0.01$), and greater ICA-IMT in men (difference of +0.12mm 95%CI 0.05mm-0.19mm between highest and lowest vBMD quartile, $p<0.02$). CCA-

IMT was not associated with vBMD in men or women. Greater carotid plaque echogenicity on ultrasound was independently associated with lower vBMD in both men (trend p=0.01) and women (trend p<0.04). In all models, adjustment did not materially affect results.

Conclusions: Bone density is independently associated with structural and functional measures of atherosclerosis in men but only with more advanced carotid atherosclerotic plaques in women. These results suggest that bone loss and atherosclerosis are related processes.

B. INTRODUCTION

Evidence from cell-culture, animal models, and large epidemiologic studies have demonstrated significant associations between bone density and atherosclerosis, two of the most common diseases of aging.(Demer 1995; Vogt, San Valentin et al. 1997; Hak, Pols et al. 2000; Doherty, Asotra et al. 2003; Tanko, Bagger et al. 2003; Schulz, Arfai et al. 2004) Since the association was first noted,(Elkeles 1957) many bone-artery studies have emphasized calcified atherosclerosis rather than other measures, reflecting the practicality of investigating outcomes that can be assessed with similar technologies, the observation that calcified atherosclerotic lesions are histologically similar to skeletal bone,(Demer 1995) and, more recently, evidence that oxidized low density lipoprotein (oxLDL) both inhibits osteoblast development and stimulates the calcification of vascular smooth muscle cells.(Parhami and Demer 1997)

However, a few large observational studies have demonstrated associations between bone density and measures of atherosclerosis not specific to calcification.(Vogt, Cauley et al. 1997; Van Der Klift, Pols et al. 2002; Jorgensen, Joakimsen et al. 2004; Wong, Kwok et al. 2005) These studies have been frequently limited, however, by exclusively white populations, use of a single measure of atherosclerosis, inability to distinguish cortical from trabecular bone types, or inability to adjust for many proposed confounders, mediators, or shared risk factors of atherosclerosis and osteoporosis.(Vogt, Cauley et al. 1997; Van Der Klift, Pols et al. 2002; Jorgensen, Joakimsen et al. 2004; Wong, Kwok et al. 2005) Because osteoporosis and atherosclerosis differ by sex and ethnicity but share risk factors, an investigation of their association would benefit from an ethnically-diverse, well-characterized study sample.

In a previous report, we demonstrated significant, independent associations between volumetric, lumbar bone density (vBMD) and calcified atherosclerosis in the coronary arteries and abdominal aorta in a sub-sample of the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, an ethnically-diverse population free of symptomatic cardiovascular disease.(Hyder 2006) Here we test associations between vBMD and the ankle-brachial index (ABI), carotid artery intima-media thickness (IMT), functional and structural measures of peripheral atherosclerosis not specific to calcification. In addition we test the association between vBMD and carotid plaque composition assessed by ultrasound, to evaluate the extent to which any bone-artery association is stronger for calcified, or more advanced, atherosclerosis. These

analyses evaluate associations in the context of CVD and osteoporosis risk factors, including inflammatory markers and sex hormones.

C. METHODS

Study participants

The methods of the Multi-Ethnic Study of Atherosclerosis (MESA) have been described previously.(Bild, Bluemke et al. 2002) Briefly, volunteers for the MESA observational cohort were recruited between July 2000 and August 2002 from six field centers around the United States. The study population consists of 6814 men and women who were aged between 45 and 84 years of age and identified themselves as Non-Hispanic White (NHW), Chinese American, African-American or Hispanic.

This report describes a random sample of MESA participants who participated in the MESA Abdominal Aortic Calcium Study (MESA-AACS). MESA-AACS participants were recruited during one of two follow-up visits between August 2002 and September 2005 from five of the six MESA field centers: Northwestern University (Chicago, Illinois), Wake Forest University School of Medicine (Forsyth County, North Carolina), the University of California Los Angeles (Los Angeles County, California), Columbia University (New York, New York), and the University of Minnesota (St. Paul, Minnesota). Of 2202 MESA participants recruited, 2172 agreed to participate, and 1990 satisfied eligibility criteria including post-menopausal status (women) and no recent prior diagnostic abdominal computed tomography. In all, 1968 participants (974 women and 994 men) completed scanning. Subsequently, 28 women and 31 men were excluded because of vertebral pathology complicating

bone density measurement. An additional 41 women and 21 men were excluded due to missing measures of carotid atherosclerosis or ankle-brachial index (ABI). Subsequently, additional 1 woman and 13 men were excluded based on "stiff arteries" as characterized by an ABI greater than 1.4. (McDermott, Liu et al. 2005) Informed consent was provided by all participants, and institutional review board approval was obtained from each participating institution.

Bone mineral density

Participants were randomly selected to undergo computed tomography (CT) scanning during one of two clinical visits between August 2002 and September 2005. CT scanning of the abdomen was performed on each participant using electron-beam computed tomography scanner (Chicago, New York City and Los Angeles; Imatron C-150, General Electric Medical Systems)(Breen, Sheedy et al. 1992) or with a multi-detector computed tomography system that utilized helical scanning with reconstruction in 5 mm thick cuts and 350 mm field of view(New York, Forsyth County, and St. Paul field centers; Siemens Inc, GE Medical Systems). A previous study had demonstrated the comparability, accuracy and reproducibility of these scanners.(Carr, Nelson et al. 2005) Participants were scanned along with phantoms of known physical calcium concentration to convert CT numbers to directly to equivalent volumetric bone mineral density (vBMD) in mg/cc.(Cann 1988) CT data were collected using the Image Analysis QCT 3D PLUS software program (Image Analysis, Columbia, Kentucky), and scans were read centrally at Harbor-University of California

Medical Center (Los Angeles, California) by a reader blinded to the results of ABI and carotid measures.

Measurements of vBMD in a virtual 10mm-thick slice of trabecular bone from each vertebra (L2 to L4) used soft-ware directed, automated placement of the region of interest (ROI) in the anterior one-half to one-third of the vertebral body where it (1) encompassed a large area exclusively of trabecular, or cancellous bone, (2) excluded cortical bone, and (3) excluded the basivertebral plexus. A trained reader examined each ROI and changed its placement to exclude vertebral abnormalities such as bone islands and diffuse density variations or to exclude a vertebra entirely if the following abnormalities were noted: fractures, metastatic lesions, osteophytes or benign focal lesions within the vertebra. These analyses use bone density from the third lumbar vertebra because it was most commonly available for all participants.

In a random sample of 25 scans re-read on three occasions by the blinded scan reader, there was 100 percent agreement as to inclusion or exclusion of vertebrae (L2-L4). Reader drift was tested using both a multivariate approach to repeated measures analysis of variance and serial t-tests, and neither analysis provided evidence of systematic differences between reads or a time effect in the data.

Ankle-brachial Index

Measurements for calculation of the ankle-brachial index were made between July 2000 and August 2002. Using a hand-held Doppler instrument with a 5-mHz probe (Nicolet Vascular, Golden, Colorado), systolic blood pressure measurements were obtained from bilateral brachial, dorsalis pedis, and posterior tibial

arteries.(McDermott, Criqui et al. 2000) Brachial artery pressures were averaged to obtain the ABI denominator. When the two brachial artery pressures differed by 10 mmHg or more, the highest brachial artery pressure was used as the denominator.(Shadman, Criqui et al. 2004) For each lower extremity, the ABI numerator was the highest pressure (dorsalis pedis or posterior tibial) from that leg. Measurement variability of ABI values has been estimated to be approximately 12%.(Fowkes, Housley et al. 1988)

Carotid artery imaging

Carotid artery intima-media thickness was assessed between July 2000 and August 2002. Images of both far and near walls of the bilateral common carotid and internal carotid arteries were obtained by trained personnel using high-resolution B-mode ultrasonography.(O'Leary, Polak et al. 1996) A Logiq 700 ultrasound machine (GE Medical Systems, Waukesha, Wisconsin) was used at all centers.

Carotid artery intima-media thickness

Central reading of intima-media thickness (IMT) was done at the Tufts-New England Medical Center (Boston, Massachusetts).(O'Leary, Polak et al. 1996) Common carotid IMT (CCA-IMT) and internal carotid artery IMT (ICA-IMT) were calculated from pre-determined sets of long and short axis views of the respective arteries. CCA-IMT was determined from maximal values obtained from the near and far walls of the left and right carotid arteries. ICA-IMT was determined from maximal measures from internal carotid arteries obtained from near and far walls of the right and left anterior-oblique, lateral, and posterior-oblique views. On a random sample of

77 participants, Pearson correlations for between-reader values were 0.767 for common carotid IMT and 0.917 for internal carotid IMT values. Among 71 randomly selected participants, Pearson correlations for within-reader values were 0.965 for common carotid IMT and 0.990 for internal carotid IMT.

Carotid plaque echogenicity

Carotid plaque echogenicity was assessed by sonographic echogenicity relative to the surrounding arterial wall in the largest plaque imaged. Readers graded plaques based on grayness, noting if a plaque was darker than the surrounding tissue (echolucent, implying a lipid-rich lesion), had the same level of brightness as the surrounding tissue (isolucent), appeared brighter than the surrounding tissue and some of the tissue beneath the plaque was shadowed (echogenic, implying a lesion with dense fibrous tissue), or if the plaque appeared much brighter than the surrounding tissue and all of the tissue beneath the plaque was shadowed (implying a calcified lesion).(Polak, O'Leary et al. 1993; Joakimsen, Bonaa et al. 1997) Between-reader (n=77) kappa scores for plaque classification were 0.675 and 0.724 for right and left carotid plaque echogenicity, respectively. Within-reader (n=71) kappa scores for carotid plaque echogenicity were 0.970 and 0.914 for right and left carotid arteries, respectively.

Clinical Measurements

Participants completed a clinical examination and detailed questionnaire. Age, sex, ethnicity, height, weight, current medications from pill bottles, physical activity patterns (met x min/week), smoking history and alcohol consumption

(never/former/current), and previous medical diagnoses were recorded. Recent hormone therapy among women was defined (yes/no) as estrogen use within the past two years. Data from a self-administered food frequency questionnaire (FFQ) and dietary supplement form were used to calculate total dietary calcium and phosphorus were calculated. Body mass index was calculated as mass in kilograms divided by height in meters squared. Blood pressure was measured 3 times with a Dinamap model Pro 100 automated oscillometric sphygmomanometer (Critikon) with participants at rest in the seated position. The average of the last 2 measurements was used to define hypertension as systolic pressure ≥140 mm Hg or diastolic pressure ≥90 mm Hg or current use of antihypertensive medication.(1997)

Laboratory measurements

C-reactive protein (CRP), interleukin-6 (IL-6) and homocysteine were measured. The CRP was measured using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, Illinois) at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, Vermont) with analytical intra-assay and inter-assay coefficient of variations (CV) of 2.3%-4.4% and 2.1%-5.7%. Interleukin-6 concentrations were measured by ultrasensitive enzyme-linked immunosorbent assay (Quantikine HS human interleukin-6 immunoassay; R&D Systems, Minneapolis, Minnesota) with CV of 6.3%. Plasma total homocysteine was measured by a fluorescence polarization immunoassay (IMx Homocysteine Assay, Axis Biochemicals ASA, Oslo, Norway) using the IMx Analyzer (Abbott Diagnostics, 100 Abbott Park Rd, Abbott Park, Illinois 60064) at the Biochemical Genetics

Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN) with a laboratory CV of 5.1%.

Serum sex hormone concentrations were measured from stored samples at the University of Massachusetts Medical Center in Worcester, MA. Total testosterone and dehydroepiandrosterone (DHEA) were measured directly using radioimmunoassay kits, and SHBG was measured by chemiluminescent enzyme immunometric assay using Immulite kits obtained from Diagnostic Products Corporation (Los Angeles, CA). For serum total T, the intra-assay CV and inter-assay CV were 10.0% and 13.2%; for SHBG, the inter-assay CV was 9.1%, and the intra-assay CV was 9.8%. Estradiol was measured by use of an ultra-sensitive radioimmunoassay kit from Diagnostic System Laboratories (Webster, TX). For estradiol, the inter-assay was CV=5.2% ,and the intra-assay was CV=2.6%.

Total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, and glucose levels were measured from plasma samples obtained after a 12-hour fast. Analyses reported here used measurements ascertained at the initial visit to minimize the number of missing values. Low density lipoprotein (LDL) cholesterol was calculated in specimens that had a plasma triglyceride level less than 400 mg/dL, using the Friedewald equation.(Friedewald, Levy et al. 1972) Diabetes was categorized if either fasting plasma glucose was greater than 126 mg/dL or if the participant reported previous diagnosis of diabetes or the use of hypoglycemic medications.

Statistical analyses

Given documented differences between men and women in the distributions(Riggs, Khosla et al. 2002) of bone density and the relatively few reports in the literature on the association between bone and atherosclerosis in men, all analyses were stratified by sex. Age and bone density were categorized by sex-specific quartiles. Differences in characteristics by ethnicity and bone density quartile were tested with analysis of variance (linear variables) and chi-square tests (categorical variables.)

Mean difference in ABI, common carotid IMT and internal carotid IMT by bone density quartile were tested by analysis of covariance first after adjustment for age and race and then after full adjustment. Adjusted means for log-transformed ICA-IMT values are geometric means.(O'Leary, Polak et al. 1999)
Associations between bone density and carotid artery plaque echogenicity were also tested by analysis of covariance, however vBMD was the outcome, and carotid artery plaque echogenicity was the independent variable. For all outcomes, ethnic-specific results are reported after adjustment for ethnic differences in other covariates. For each outcome, formal tests of interactions of bone density by age, ethnicity, and recent hormone therapy (women only) were performed. None were significant (all $p>0.10$), so age, ethnic and hormone therapy groups were collapsed.

We aimed to test the contributions of different sets of variables to the associations between bone density and atherosclerosis. The following terms were conceived in mutually exclusive theoretical concepts as follows: confounders are covariates that are associated with both bone density and atherosclerosis but are

causally associated with only one of those outcomes; mediators are covariates that mediate, or are in a causal pathway between atherosclerosis and bone density; common risk factors are covariates that independently cause both atherosclerosis and low bone density. The empirical effect of adjusting for a confounder, mediator or common risk factor in the serial models is either attenuation or augmentation of the association between atherosclerosis and bone density. No change in the association with adjustment indicates that the added covariates made no contribution to the association.(Greenland and Morgenstern 2001)

Associations were adjusted for sets of covariates in serial models. Model 1 adjusted for age in quartiles and ethnicity, given the known associations of these covariates with both atherosclerosis and bone density. The second model, Model 2, adjusted additionally for total cholesterol, HDL-cholesterol, lipid medication, hypertension, diabetes, smoking (never/former/current), body mass index, physical activity, the natural log (ln) of dietary calcium, alcohol consumption (never/former/current), and recent hormone therapy (women only). Final model, Model 3, additionally adjusted the RR for ln(interleukin-6), ln(C-reactive protein), homocysteine, and sex-specific quartiles of total testosterone, estradiol, and serum hormone binding globulin (but not for recent hormone therapy). For women, quartiles of sex hormones were defined separately for those using and not using supplemental estrogens. This generated unordered, eight-level variables that accounted for the level of a sex hormone measure as well as the use of supplemental estrogen. Sensitivity analyses of sex hormones included models stratified by hormone use and models

where sex hormone measures were categorized for a combined sample of hormone-using and non-hormone-using groups. In addition, alternative variable forms for age, including linear age and n-tiles based on decades of life, were investigated for the potential effect of age as a confounder. Models were inspected for attenuation and/or augmentation of an association, defined as a change of more than 25% in the difference between the means of the lowest and highest bone density quartiles, a change corresponding to a 25% change in effect size. Multi-collinearity was not detected during screening for tolerance values of less than 0.15 for all variables in fully adjusted models. All analyses were executed using SAS version 8.1 (SAS Institute, Cary, NC, U.S.A.).

D. RESULTS

The sex-specific characteristics of 904 female and 929 male participants are shown in Table 6. The average age of women was 65 years, and the average age of men was 64 years. Non-Hispanic white participants were 62% of women and 58% of men. In women, mean (SD) values for ABI, CCA-IMT and ICA-IMT were: 1.09 (0.10), 0.85 (0.18) and 1.02 (0.58). In men, mean values for ABI, CCA-IMT and ICA-IMT were: 1.15 (0.11), 0.89 (0.20) and 1.11 (0.60). For both men and women, IMT mean values and variability were greater for the ICA-IMT than for the CCA-IMT.

Age- and ethnicity-adjusted characteristics of women and men by sex-specific quartiles of vBMD are shown in Table 7. Bone density was significantly, inversely associated with age in both women ($p<0.001$) and men ($p<0.001$). In both women

($p<0.001$) and men ($p<0.001$), ethnicity was significantly associated with bone density, with African Americans being more likely to be in the highest vBMD quartiles, and whites and Chinese being more likely to be in the lowest quartiles. After adjustment for age and ethnicity, C-reactive protein ($p=0.02$) and physical activity ($p=0.02$) were positively associated with vBMD ($p=0.02$) in women. Recent hormone therapy was significantly more common in women in the highest quartiles of bone density ($p=0.002$). In women not taking estrogens (n=618; 68%), mean estradiol, serum hormone binding globulin (SHBG) and total testosterone did not differ significantly by vBMD quartile after adjustment. In men, both homocysteine and the mean natural log of C-reactive protein varied significantly, but in a non-linear pattern, with vBMD ($p<0.01$ and $p=0.05$, respectively). In men, greater estradiol ($p=0.016$) and lower SHBG ($p=0.030$) were significantly associated with greater vBMD quartile after adjustment. There were few strong correlates of vBMD other than age and ethnicity in either men or women.

Table 8 displays mean ankle-brachial index (ABI), common carotid artery intima-media thickness (CCA-IMT), internal carotid artery IMT (ICA-IMT) values for men and women according to sex-specific quartiles of vBMD and multiple, sequential adjustments for covariates. In women, there were no significant associations between vBMD and ABI, CCA-IMT or ICA-IMT, in any model. In men, however, lower vBMD was significantly associated with lower ABI in all models with no evidence of attenuation or augmentation of associations across models. Although trends across quartiles of vBMD were significant (Model 3, p for trend=0.008), there was some evidence of a non-linear association wherein

ABI was much lower in the lowest vBMD quartile Q1 compared to other quartiles (Model 3: Q4 ABI=1.14; Q3 ABI=1.15; Q2 ABI=1.13; Q1 ABI=1.11; p for ANOVA=0.004).

Table 9 presents the sex-specific distributions of carotid plaque types by echolucency and mean vBMD for each plaque type after multiple adjustments. In women, the most common findings on carotid ultrasound were no carotid lesion (61%) and iso-echoic carotid lesions (23%). Similarly in men, the most common findings on carotid ultrasound were no carotid lesions (56%) and iso-echoic carotid lesions (26%). Greater plaque echogenicity, indicative of greater fibrous tissue content and calcium, was significantly associated with lower vBMD in all models, independent of adjustments, including adjustment for age, ethnicity, body mass index, total cholesterol, HDL, use of cholesterol medication, hypertension, diabetes, cigarette smoking, alcohol consumption, physical activity, homocysteine, C-reactive protein, interleukin-6, total testosterone, serum hormone binding globulin and estradiol for both men (p=0.011) and women (p=0.035). Significant associations persisted in both sexes when those with hyperechoic and calcified carotid lesions or no lesions were excluded, indicating that the trend was not driven exclusively by these individuals (results not shown).

In sensitivity analyses, results did not differ materially when the analyses were repeated using vBMD from the fourth lumbar vertebra (L4) instead of L3. Alternative adjustment for bio-available testosterone in place of total testosterone did not materially alter the results in either sex. In women we conducted additional analyses stratified by use of hormone therapy. Results of partially or fully adjusted models did not different from those shown.

Figure 3 displays mean values of CCA-IMT, ICA-IMT and ABI for men and women by ethnic group, adjusted for age, body mass index, total cholesterol, HDL, use of cholesterol medication, hypertension, diabetes, cigarette smoking, physical activity, homocysteine and interleukin-6 and hormone therapy (women only). Greater ICA-IMT was significantly associated with vBMD in white men (p for trend <0.05) and African American women (p for trend =0.05). Lower ABI was associated with lower vBMD in African American women, and the association was highly significant in African American men (p<0.001).

E. DISCUSSION

In the present study, we found that low trabecular lumbar bone density was independently associated with subclinical atherosclerosis assessed by internal carotid intima-media thickness (ICA-IMT) and ankle-brachial index (ABI) in men but not women. In contrast, more advanced carotid plaque morphology, evidenced by greater plaque echogenicity and calcification, was significantly associated with lower bone density in both men and women. In all cases, significant associations attenuated slightly after adjustment for age, but minimally for other covariates including classic cardiovascular disease risk factors, inflammatory measures, and sex hormones. In ethnic subgroups, associations between ABI and bone density were strongest in African American men, and associations between ICA-IMT and bone density were strongest in white men. ICA-IMT was also significantly associated with bone density in African American women. In general ethnic differences were small in comparison to sex differences.

Three previous studies of similar size have used areal measurements of bone density to demonstrate significant associations between ABI and hip bone density, but none detected associations with lumbar bone density(Vogt, Cauley et al. 1997; Rubin and Silverberg 2004; Wong, Kwok et al. 2005) and none found associations in men alone.(Rubin and Silverberg 2004; Wong, Kwok et al. 2005) The significant association between vBMD and the ABI in men in this study may have resulted from the use of volumetric bone densitometry and/or evaluation of the lumbar spine which may have differential susceptibility to confounding by low physical activity,(Laroche, Pouilles et al. 1994; Vogt, Cauley et al. 1997; Laroche, Moulinier et al. 2003) a known risk factor for bone loss and both a cause and consequence of low ABI. (Wang, Criqui et al. 2005)

Few studies of similar size have tested associations between bone density and carotid atherosclerosis. An investigation by Jorgenson and colleagues of over 5,000 white men and women found no association between areal BMD of the forearm and IMT summed across the carotid bifurcation, internal and common carotid arteries.(Jorgensen, Joakimsen et al. 2004) A study of 165 postmenopausal Hispanic women found an association between areal bone density (hip, spine, radius) and CCA-IMT that was strongest among women over 60 years old and stronger for the distal radius than for the less trabecular radius midpoint, suggesting that the bone-artery association is stronger for trabecular bone than for cortical bone.

The present study differs from previous investigations with its multi-ethnic, population-based sample, extensive covariate data including multiple inflammatory

markers, homocysteine and sex hormones, as well as its use of volumetric rather than areal bone density measurements. Areal bone density values summarize data from cortical and trabecular bone in a single measure and vary by bone size and type as well as density. The volumetric estimates here are independent of secular trends in height or age-, ethnic- or sex-differences in bone size and shape and are specific to the trabecular bone compartment.(Seeman 1997; Lang, Li et al. 1999; Bolotin 2001; Bolotin and Sievanen 2001; Lang, Guglielmi et al. 2002; Bolotin, Sievanen et al. 2003; Riggs, Melton III et al. 2004) Patterns of cortical and trabecular bone loss differ. Trabecular bone loss begins before cortical or net bone loss, as early as the second and third decades of life,(Riggs, Melton III et al. 2004) at the same time when early atherosclerotic lesions (fatty streaks) are thought to develop. Trabecular bone is more sensitive to sex hormones than cortical bone, so the rate of decline increases greatly around the time of the menopause in women before slowing later in life.(Riggs, Melton III et al. 2004) In this cross-sectional analysis, sex differences in the bone-artery association may have resulted from cross-sectional, trabecular-specific estimates of age-related bone loss in women that would bias associations with bone to the null.(Melton, Khosla et al. 2000) Differences between this report and previous reports may be driven in part by the precise trabecular measures here compared to integrated trabecular and cortical areal measures in other reports.

The present investigation found differences in bone-artery associations by anatomic site, sex, and ethnicity. Significant associations were found for vBMD with ABI and ICA-IMT in men but not women. No significant associations were found

between vBMD and CCA-IMT in men or women. Additional analyses pooling IMT measures in women yielded no evidence of an association (analyses not shown). Differences in the association by anatomic site, sex, and ethnicity in the present study may have resulted from differences in the processes of bone loss and/or atherosclerosis, but these differences in the present study may be explained more parsimoniously by the patterns rather than processes of atherosclerotic disease. In this study sample that was free of symptomatic CVD at enrollment, significant associations between vBMD and the IMT were found at the ICA, where atherosclerotic disease was greater than for the CCA. The internal carotid arteries typically have ultrasound evidence of atherosclerotic disease earlier, more frequently, and in greater amounts that the common carotid arteries.(Espeland, Evans et al. 2003) ICA-IMT baseline and change correlate more strongly with age and male sex than do baseline and change measure of CCA-IMT. In addition, the ICA-IMT was assessed in this study with a summary of 18 composite measures versus six measures for the CCA-IMT, which may have resulted in more stable values for ICA-IMT that were less susceptible to measurement error.

Regarding sex and ethnic differences, men have greater IMT values, particularly ICA-IMT values, than women at a given age.(Tell, Howard et al. 1989; Espeland, Tang et al. 1999) In this and other studies, whites have a greater range of ICA-IMT values than African Americans.(Tell, Howard et al. 1989) And African American men, in whom vBMD was significantly associated with ABI, have more peripheral arterial disease, evidenced by lower ABI values, than whites.(Criqui,

Vargas et al. 2005; McDermott, Liu et al. 2005) Thus, anatomic, sex, and ethnic differences in the magnitude of CVD may underlie the differences in the associations between vBMD and atherosclerosis seen here. Regardless, other factors may explain the sex and ethnic differences in the association between bone density and atherosclerosis.

Numerous factors affect disease development in both the bones and arteries. In recent studies the cannabinoid system has been implicated with both atherosclerosis and osteoporosis in mice and in humans.(Karsak, Cohen-Solal et al. 2005; Steffens, Veillard et al. 2005; Ofek, Karsak et al. 2006) Cannabinoid receptors are found in atherosclerotic lesions but also exert effects on bone in part through the osteoprotegerin/RANK/RANKL system which receives input from estrogen and, itself, affects both atherosclerotic(Erdogan, Aslan et al. 2004) and osteoporotic change(Raisz 2005).

Whether these factors are common risk factors or whether these factors mediate a causal association is not clear from this or other studies. The hypothesis of atherosclerosis begetting diminished bone perfusion and subsequent osteoporosis-like disease is consistent with other experimental data. Impaired bone perfusion has been associated with greater carotid IMT and lower bone density.(Chen, Ting-Fang Shih et al. 2004; Shih, Liu et al. 2004; Griffith, Yeung et al. 2005) And nitroglycerin, a potent vasodilator, resulted in equal improvements in bone density after surgical menopause when compared with hormone therapy in rats.(Wimalawansa 2000) Oxidized LDL, commonly found in atherosclerotic lesions, is known to promote the calcification of

vascular smooth muscle cells as well as inhibit the differentiation of bone-building osteoclastic cells which are typically located adjacent to the subendothelial matrix of bone vessels.(Parhami and Demer 1997)

Given that oxidized LDL may promote calcification of atherosclerosis, the association between bone and atherosclerosis may be stronger for calcified atherosclerosis than atherosclerosis in general. The present study examined a similar sample as a previous report from the MESA where vBMD was robustly, independently, and significantly associated with coronary and aortic calcium in women and with aortic calcium in men.(Hyder 2006) In the present study, associations between vBMD and the IMT and ABI, measures of atherosclerosis that do not specifically include calcium, were not as consistent across sex or measure of atherosclerosis. However associations with more advanced, or more fibrous and calcified carotid plaques were significant in both men and women. These findings are consistent with a study by Jorgensen and colleagues(Jorgensen, Joakimsen et al. 2004) of over 5000 white men and women, where bone density was not associated with carotid IMT or lipid-rich echolucent plaques but was significantly associated with fibrous and calcified echogenic plaques. Thus, the bone-artery association may be stronger for measures of calcified atherosclerosis than other measures of atherosclerosis in general, although the latter do show associations in higher risk groups. Regardless of causality or specificity for calcified atherosclerosis, trabecular bone density may prove useful in men for refined stratification of CVD.

Limitations of our study include a cross-sectional design, bone density measurements from a single site and bone type, single measures of sex hormones and inflammatory factors, and lack of data describing the use of selective estrogen receptor modulators, and anti-resorptive agents. Accurate estimation of the effects of long-acting factors such as sex hormones and inflammatory markers may require repeated measurements over time. The interpretation of cross-sectional associations for the ABI, the carotid IMT and bone density are further complicated because these outcomes may have different determinants for initial values than for change. For instance, carotid IMT is strongly associated with prevalent and incident CVD, but IMT increases, and thus a one-time high IMT value, may result from factors other than atherosclerosis such as adaptive response to shear and tensile stress associated with physical activity.(Glagov, Zarins et al. 1988; Bots, Hofman et al. 1997) In all, conclusions about causality are not feasible. Although the use of asymptomatic participants and subclinical endpoints has clear advantages when attempting to study two disease processes simultaneously,(Pearl 1929) any associations between bone density and atherosclerosis are applicable only in similarly healthy populations where age-adjusted associations are likely to be smaller than in more diseased populations. Finally, conclusions about any ethnic differences are further limited by the smaller sizes of sub-groups.

This strengths of this study include a multi-ethnic sample of men and women, detailed measurement of multiple shared risk factors for atherosclerosis and osteoporosis, including sex hormones, IL-6, C-reactive protein, and homocysteine.

The volumetric bone density measurements used in this study specifically assessed the more metabolically active trabecular bone and were not confounded by secular trends in height or age-, sex- and ethnic- differences in bone size.

In conclusion, this investigation demonstrated significant associations between lumbar bone density and atherosclerosis, as assessed by the ankle-brachial index and internal carotid intima-media thickness, in men but not in women. The significant, independent associations between bone density and more echogenic carotid plaques in both men and women may indicate that bone density is more strongly associated with more advanced or more calcified atherosclerosis.

**Table 6. Characteristics of participants from the MESA, Abdominal Aortic Calcium Study, 2000-2005.**

| Characteristic | WOMEN | MEN |
|---|---|---|
| N | 904 | 929 |
| Age y | 65 (9) | 64 (10) |
| Ethnicity (%) | | |
| White | 38 (344) | 42 (389) |
| Chinese | 13 (116) | 14 (133) |
| Black | 23 (206) | 18 (167) |
| Hispanic | 26 (238) | 26 (240) |
| ABI | 1.09 (0.10) | 1.15 (0.11) |
| Mean CCA-IMT† mm | 0.85 (0.18) | 0.89 (0.20) |
| Mean ICA-IMT ‡ mm | 1.02 (0.58) | 1.11 (0.60) |
| Bone density, mg/cc | 112 (40) | 121 (39) |
| Body mass index, kg/m^2 | 28.3 (5.8) | 27.8 (4.3) |
| Total cholesterol, mg/dL | 201 (33) | 190 (34) |
| HDL-cholesterol, mg/dL | 57 (16) | 45 (12) |
| Cholesterol medication (%) | 16.7 (151) | 14 (130) |
| Diabetes (%) | 10 (93) | 13 (127) |
| Hypertension (%) | 47 (422) | 44 (408) |
| Cigarette smoking (%) | | |
| Never | 60 | 42 |
| Former | 28 | 45 |
| Current | 12 | 14 |
| Alcohol consumption (%) | | |
| Never | 31 | 11 |
| Former | 19 | 25 |
| Current | 50 | 64 |
| LN(Interleukin-6) pg/mL | 0.84 (0.36) | 0.81 (0.37) |
| LN(C-reactive protein) mg/L | 1.35 (0.77) | 1.02 (0.65) |
| Homocysteine, µmol/L | 8.7 (4.7) | 10.0 (3.4) |
| Physical activity, met-min/week*100 | 121 (59) | 119 (73) |
| Hormone therapy§ (%) | 37 (332) | - - - |
| Estradiol, nmol/L | 0.07 (0.07) | 0.12 (0.04) |
| SHBG, nmol/L | 56.7 (33.7) | 43.2 (18.0) |
| Testosterone, nmol/L | 1.10 (0.89) | 15.0 (5.5) |

Mean (SD) and percentage (N) are shown for continuous and discrete variables
*ABI is ankle-brachial index
†CCA-IMT is common carotid artery intima-media thickness
‡ICA-IMT is internal carotid artery intimal-media thickness, not transformed
§Estrogen use in the previous two years; Values for estradiol, SHBG (serum hormone binding globulin) and testosterone in women include only 559 women not taking estrogens

**Table 7. Distribution of participant characteristic by sex-specific quartile of lumbar bone density (mg/cc) in the MESA, AACS, 2000-2005.**

| | WOMEN | | | | | MEN | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Bone Density Quartiles | | | | | Bone Density Quartiles | | | | |
| **Characteristic** | **1** | **2** | **3** | **4** | **p-value** | **1** | **2** | **3** | **4** | **p-value** |
| N | 225 | 225 | 229 | 225 | | 231 | 228 | 236 | 234 | |
| Bone density, mg/cc (range) | 10, 82 | 83, 107 | 108, 136 | 137, 274 | | 11,93 | 94, 117 | 118, 144 | 145, 271 | |
| Age, years (SD) | 73 (8) | 66 (8) | 63 (8) | 59 (8) | <0.001 | 70 (9) | 66 (9) | 60 (9) | 59 (9) | <0.001 |
| Ethnicity (%) | | | | | <0.001 | | | | | <0.001 |
| White | 31 | 23 | 26 | 20 | | 31 | 28 | 25 | 16 | |
| Chinese | 30 | 32 | 18 | 20 | | 30 | 29 | 30 | 11 | |
| Black | 13 | 19 | 28 | 40 | | 10 | 15 | 22 | 54 | |
| Hispanic | 25 | 29 | 26 | 21 | | 23 | 24 | 26 | 27 | |
| Body mass index, kg/m^2 | 27.2±0.4 | 27.4±0.4 | 28.1±0.4 | 28.8±0.4 | 0.035 | 27.0±0.3 | 27.4±0.3 | 27.5±0.3 | 27.6±0.3 | 0.493 |
| Total cholesterol, mg/dL | 203±3 | 200±2 | 202±2 | 196±2 | 0.214 | 188±2 | 190±2 | 190±2 | 192±2 | 0.740 |
| HDL cholesterol, mg/dL | 57±1 | 56±1 | 55±1 | 56±1 | 0.662 | 45±1 | 46±1 | 45±1 | 46±1 | 0.447 |
| Cholesterol medication (%) | 14±3 | 18±3 | 16±3 | 18±3 | 0.672 | 12±3 | 12±2 | 11±2 | 16±2 | 0.460 |
| Diabetes (%) | 10±2 | 11±2 | 11±2 | 13±2 | 0.873 | 15±2 | 13±2 | 15±2 | 15±2 | 0.859 |
| Hypertension (%) | 41±4 | 45±3 | 50±3 | 51±3 | 0.163 | 46±4 | 43±3 | 41±3 | 47±3 | 0.603 |
| Cigarette smoking (%) | | | | | 0.353 | | | | | 0.389 |
| Never | 67 | 66 | 66 | 65 | | 38 | 41 | 43 | 45 | |
| Former | 20 | 21 | 25 | 28 | | 44 | 44 | 42 | 45 | |
| Current | 13 | 13 | 9 | 6 | | 18 | 15 | 15 | 10 | |
| Alcohol consumption (%) | | | | | 0.770 | | | | | 0.765 |
| Never | 40 | 37 | 38 | 37 | | 16 | 13 | 13 | 15 | |
| Former | 16 | 16 | 21 | 21 | | 24 | 26 | 30 | 27 | |
| Current | 44 | 48 | 41 | 42 | | 60 | 61 | 58 | 58 | |
| LN (Interleukin-6) pg/mL | 0.77±0.03 | 0.80±0.02 | 0.83±0.02 | 0.86±0.03 | 0.174 | 0.81±0.03 | 0.78±0.03 | 0.83±0.02 | 0.80±0.03 | 0.530 |
| LN(C-reactive protein) mg/L | 1.15±0.06 | 1.23±0.05 | 1.36±0.05 | 1.39±0.06 | 0.023 | 1.00±0.05 | 0.90±0.04 | 1.07±0.04 | 1.04±0.04 | 0.048 |
| Homocysteine, µmol/L | 8.5±0.4 | 8.9±0.3 | 9.0±0.3 | 8.3±0.3 | 0.371 | 9.8±0.2 | 10.4±0.2 | 10.5±0.2 | 9.6±0.2 | 0.006 |
| Physical activity, met-min/week*100 | 108±4 | 116±4 | 116±4 | 128±4 | 0.019 | 117±5 | 119±5 | 121±5 | 117±5 | 0.900 |
| Hormone therapy† (%) | 24±3 | 29±3 | 34±3 | 43±3 | 0.002 | - - - | - - - | - - - | - - - | - - - |
| Estradiol, nmol/L | 0.066±0.006 | 0.073±0.006 | 0.076±0.006 | 0.092±0.007 | 0.102 | 0.110±0.003 | 0.114±0.003 | 0.117±0.003 | 0.125±0.003 | 0.016 |
| SHBG, nmol/L | 58.7±3.1 | 57.4±2.8 | 52.0±2.9 | 51.7±3.6 | 0.338 | 46.7±1.2 | 43.9±1.2 | 42.0±1.1 | 42.0±1.2 | 0.030 |
| Testosterone, nmol/L | 0.98±0.08 | 1.05±0.08 | 1.10±0.08 | 1.28±0.10 | 0.196 | 14.8±0.4 | 15.0±0.4 | 14.9±0.4 | 15.4±0.4 | 0.691 |

*Values other than age and race are adjusted for age and ethnicity.
Mean±standard error and percentage±standard error are displayed for continuous and discrete variables, respectively. P-values are for ANCOVA, not for trend.
†Estrogen use in the previous two years; Values for estradiol, SHBG (serum hormone binding globulin) and testosterone in women include only 559 women not taking estrogens

**Table 8. Mean ankle-brachial index (ABI), common carotid artery intima-media thickness (CCA-IMT), and internal carotid artery IMT (ICA-IMT) by sex-specific quartile of trabecular lumbar bone density (vBMD), MESA, AACS, 2000-2005.**

| | WOMEN | | | MEN | | |
|---|---|---|---|---|---|---|
| | Model 1* n=904 Mean±SE | Model 2† n=868 Mean±SE | Model 3‡ n=807 Mean±SE | Model 1* n=929 Mean±SE | Model 2† n=898 Mean±SE | Model 3‡ n=884 Mean±SE |
| **Mean ABI** | | | | | | |
| vBMD Q1 | 1.09±0.007 | 1.08±0.008 | 1.08±0.009 | 1.12±0.008 | 1.11±0.008 | 1.11±0.008 |
| vBMD Q2 | 1.10±0.007 | 1.09±0.007 | 1.09±0.008 | 1.14±0.007 | 1.13±0.008 | 1.13±0.008 |
| vBMD Q3 | 1.08±0.007 | 1.08±0.007 | 1.08±0.008 | 1.15±0.007 | 1.15±0.008 | 1.15±0.008 |
| vBMD Q4 | 1.10±0.007 | 1.09±0.008 | 1.09±0.009 | 1.14±0.007 | 1.13±0.008 | 1.14±0.008 |
| p for ANCOVA | 0.119 | 0.202 | 0.215 | 0.017 | 0.007 | 0.004 |
| p for trend | 0.828 | 0.928 | 0.976 | 0.041 | 0.017 | 0.009 |
| **Mean CCA-IMT (mm)** | | | | | | |
| vBMD Q1 | 0.84±0.01 | 0.85±0.01 | 0.85±0.01 | 0.87±0.01 | 0.90±0.01 | 0.89±0.01 |
| vBMD Q2 | 0.83±0.01 | 0.83±0.01 | 0.83±0.01 | 0.88±0.01 | 0.90±0.01 | 0.90±0.01 |
| vBMD Q3 | 0.86±0.01 | 0.85±0.01 | 0.85±0.01 | 0.88±0.01 | 0.89±0.01 | 0.89±0.01 |
| vBMD Q4 | 0.85±0.01 | 0.85±0.01 | 0.86±0.01 | 0.89±0.01 | 0.90±0.01 | 0.90±0.01 |
| p for ANCOVA | 0.217 | 0.461 | 0.523 | 0.856 | 0.867 | 0.761 |
| p for trend | 0.199 | 0.536 | 0.445 | 0.419 | 0.793 | 0.619 |
| **Mean ICA-IMT§ (mm)** | | | | | | |
| vBMD Q1 | 0.90±0.03 | 0.97±0.04 | 0.98±0.04 | 1.01±0.03 | 1.04±0.03 | 1.05±0.03 |
| vBMD Q2 | 0.86±0.03 | 0.89±0.03 | 0.91±0.03 | 0.95±0.03 | 0.96±0.03 | 0.97±0.03 |
| vBMD Q3 | 0.87±0.03 | 0.89±0.03 | 0.91±0.03 | 0.96±0.03 | 0.99±0.03 | 0.98±0.03 |
| vBMD Q4 | 0.86±0.03 | 0.89±0.03 | 0.89±0.03 | 0.91±0.03 | 0.93±0.03 | 0.93±0.03 |
| p for ANCOVA | 0.703 | 0.167 | 0.163 | 0.106 | 0.061 | 0.053 |
| p for trend | 0.462 | 0.106 | 0.071 | 0.033 | 0.023 | 0.015 |

§ Mean values are geometric means for ICA-IMT
* Adjusted for age and ethnicity.
† Adjusted for age, ethnicity, body mass index, total cholesterol, HDL, use of cholesterol medication, hypertension, diabetes, cigarette smoking, alcohol consumption, physical activity, homocysteine, C-reactive protein, interleukin-6 and hormone therapy (women only)
‡Additionally adjusted for unordered quartiles of total testosterone, serum hormone binding globulin and estradiol.

**Table 9. Mean lumbar bone density (vBMD) by sex and carotid artery plaque characteristic, MESA, AACS, 2000-2005.**

| | WOMEN | | | | MEN | | | |
|---|---|---|---|---|---|---|---|---|
| | % (N) | Model 1* n=904 vBMD Mean±SE | Model 2† n=868 vBMD Mean±SE | Model 3‡ n=807 vBMD Mean±SE | % (N) | Model 1* n=929 vBMD Mean±SE | Model 2† n=898 vBMD Mean±SE | Model 3‡ n=884 vBMD Mean±SE |
| **Carotid plaque** | | | | | | | | |
| No plaque | 61 (548) | 113±1 | 113±2 | 113±2 | 56 (516) | 126±1 | 125±2 | 126±2 |
| Hypoechoic | 7 (66) | 108±4 | 108±4 | 108±4 | 9 (87) | 125±4 | 123±4 | 122±4 |
| Iso-echoic | 23 (207) | 109±2 | 109±3 | 109±3 | 26 (244) | 121±2 | 120±3 | 120±2 |
| Hyperechoic | 5 (41) | 106±5 | 105±5 | 106±5 | 3 (30) | 121±6 | 119±6 | 119±6 |
| Calcified | 5 (42) | 103±5 | 104±5 | 106±5 | 6 (52) | 117±5 | 116±5 | 118±5 |
| p for ANCOVA | | 0.316 | 0.231 | 0.295 | | 0.230 | 0.175 | 0.129 |
| p for trend | | 0.043 | 0.014 | 0.035 | | 0.025 | 0.013 | 0.010 |

* Adjusted for age and ethnicity.

† Adjusted for age, ethnicity, body mass index, total cholesterol, HDL, use of cholesterol medication, hypertension, diabetes, cigarette smoking, alcohol consumption, physical activity, homocysteine, C-reactive protein, interleukin-6 and hormone therapy (women only)

‡Adjusted for age, ethnicity, body mass index, total cholesterol, HDL, use of cholesterol medication, hypertension, diabetes, cigarette smoking, alcohol consumption, physical activity, homocysteine, C-reactive protein, interleukin-6 and unordered quartiles of total testosterone, serum hormone binding globulin and estradiol. In women, these quartiles are specific for women taking and not taking supplemental estrogens.

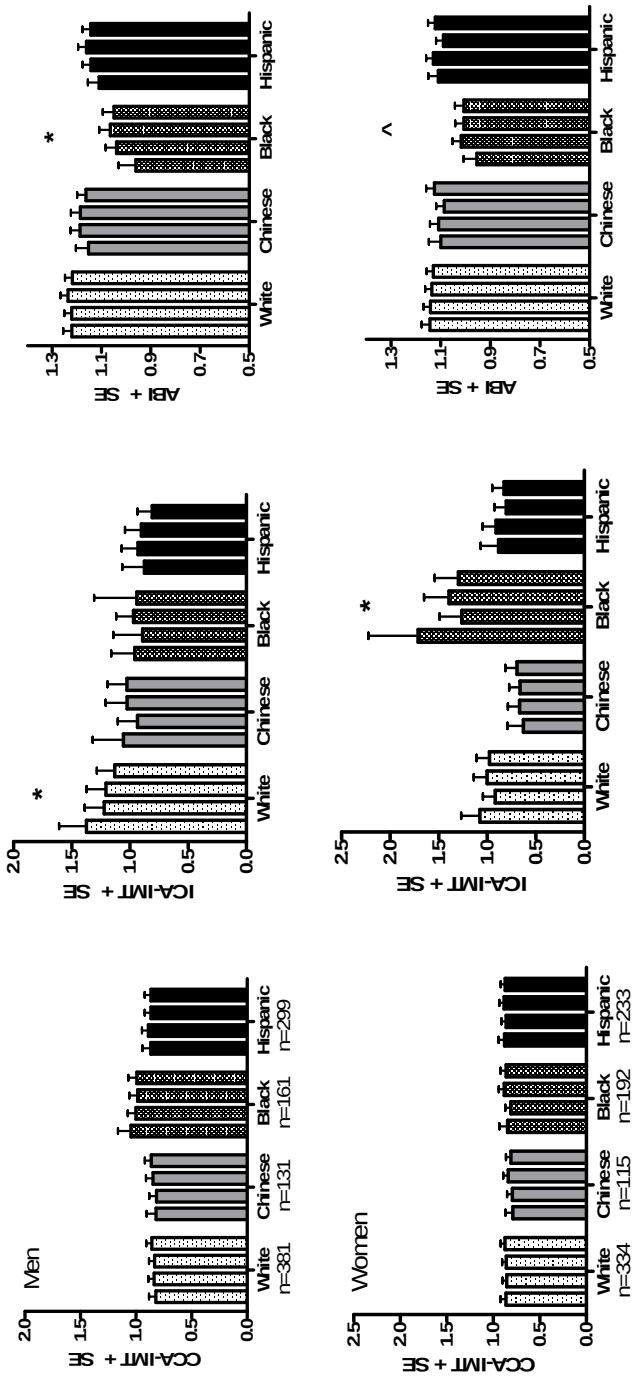


**Figure 3. Mean ankle-brachial index (ABI), common carotid intima-media thickness (CCA-IMT) and internal carotid IMT (ICA-IMT) plus standard error (SE) by ethnicity in men and women. Bone density quartiles are displayed from lowest to highest (left to right) for each ethnic group. Multi-Ethnic Study of Atherosclerosis, Abdominal Aortic Calcium Study, 2000-2005.**
Values are adjusted for age, body mass index, total cholesterol, HDL, use of cholesterol medication, hypertension, diabetes, cigarette smoking, physical activity, homocysteine and interleukin-6 and hormone therapy (women only). ^ $p<0.10$ for ANOVA *$p<0.05$ for ANOVA

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# IV. DYSLIPIDEMIA MODIFIES THE ASSOCIATION BETWEEN BONE DENSITY AND CALCIFIED ATHEROSCLEROSIS

## A. ABSTRACT

Objective: In light of data implicating lipids in both osteoporosis and atherosclerosis, we tested the hypothesis that the association between bone density and atherosclerosis varies according to dyslipidemia.

Methods and Results: Between 2002 and 2005, 423 women (mean age 64.4 years) and 670 men (63.4 years) naïve to lipid-modifying medications were assessed for coronary artery calcium (CAC), abdominal aortic calcium (AAC), volumetric trabecular bone density (vBMD), and total cholesterol, (TC), HDL- and LDL-cholesterol. Prevalence of CAC, AAC, TC:HDL≥5.0 were (59% , 63%, 15%) for women and (64%, 70%, 27%) for men. In relative risk (RR) regression models adjusting for age, ethnicity, body mass index, hypertension, diabetes, alcohol consumption, cigarette smoking, and physical activity, greater vBMD was associated with greater prevalence of CAC and AAC in those with TC:HDL≥5.0 but with lower prevalence of CAC and AAC in those with TC:HDL<5.0 (p for interaction for CAC and AAC were <0.01 and <0.02 in women and 0.36 and <0.01 in men). Similarly significant interactions, were noted in men and women for dyslipidemias defined by LDL or HDL, but not by TC or triglycerides.

Conclusion: Dyslipidemia modifies the associations between bone density and calcified atherosclerosis in both sexes. Lipid factors may link bone loss and atherosclerosis.

B. INTRODUCTION

Previous epidemiologic reports have demonstrated inverse associations between systemic atherosclerosis and bone density independent of their shared risk factors including as age, sex, ethnicity, cigarette smoking and physical activity.(Kiel, Kauppila et al. 2001; Jorgensen, Joakimsen et al. 2004; Schulz, Arfai et al. 2004; Hyder 2006; Hyder 2006) In addition, correlates of aging such as sex hormones, interleukin-6, and parathyroid hormone have not accounted for the association between atherosclerosis and bone density.(Kiel, Kauppila et al. 2001; Arlt and Hewison 2004; Jorgensen, Joakimsen et al. 2004; Schulz, Arfai et al. 2004; Hyder 2006; Hyder 2006)

A leading explanation of the association between osteoporosis and atherosclerosis implicates dyslipidemia and oxidized low density lipoprotein (oxLDL).(Demer 1995; Parhami and Demer 1997; Parhami, Garfinkel et al. 2000; Parhami 2003; McFarlane, Muniyappa et al. 2004; Rubin and Silverberg 2004; Koshiyama, Ogawa et al. 2006) Support for this thesis comes from experimental studies showing the common monocytic origin of multi-nucleated macrophages and osteoclasts and their dependence, in vivo, on exogenous LDL cholesterol.(Luegmayr, Glantschnig et al. 2004) Further experiments have shown that oxLDL inhibits osteoblastic differentiation of bone marrow stromal cells,(Parhami, Jackson et al.

1999) and induces the calcification of vascular cells.(Parhami, Morrow et al. 1997; Nuttall, Patton et al. 1998) In a test of the hypothesis that high lipids contribute to the association between atherosclerosis and osteoporosis, atherosclerosis-susceptible mice that were fed high-fat diets experienced greater atherosclerosis, failed osteoblastic differentiation, and reduced bone mineralization when compared to control mice.(Parhami, Tintut et al. 2001)

The related hypothesis that an inverse association between bone density and atherosclerosis is more exaggerated among individuals with dyslipidemia has not been tested. We used data on volumetric lumbar trabecular bone density (vBMD) and coronary artery (CAC) and abdominal aortic calcium (AAC) each measured by computed tomography to test this hypothesis in a large, ethnically diverse sample of men and women who were free of clinical cardiovascular disease and not taking lipid-modifying medications or estrogen therapy.

## C. METHODS

### Study Participants

The methods of the Multi-Ethnic Study of Atherosclerosis (MESA) have been published.(Bild, Bluemke et al. 2002) In brief, the MESA is an observational cohort of volunteers recruited between July 2000 and August 2002 from six field centers around the United States. The study population consists of 6814 men and women who were aged 45 to 84 years and identified themselves as Non-Hispanic White (NHW), Chinese American, African-American or Hispanic.

This report is based on a random sample of MESA participants who were participants in the MESA Abdominal Aortic Calcium Study (MESA-AACS). MESA-AACS participants were recruited during one of two follow-up visits between August 2002 and September 2005 from five of the six MESA field centers: Northwestern University (Chicago, Illinois), Wake Forest University School of Medicine (Forsyth County, North Carolina), the University of California Los Angeles (Los Angeles County, California), Columbia University (New York, New York), and the University of Minnesota (St. Paul, Minnesota). Of 2202 MESA participants recruited, 2172 agreed to participate, and 1990 satisfied eligibility criteria including post-menopausal status (women) and no recent prior diagnostic abdominal computed tomography. In all, 1968 participants (974 women and 994 men) completed scanning. To avoid pharmacologic effects, 292 women and 285 men were excluded based on reported use of lipid-lowering medications (statins, niacin, fibrates, and/or cholestyramines) in the past two years, and an additional 246 women were excluded based on reported use of postmenopausal estrogen therapy (hormone therapy) within two years. Another 2 women and 20 men were excluded for missing cholesterol data. Coronary calcium was not available for 14 women and 19 men; for these participants coronary calcium scores measured two years earlier were used for the present analyses. Aortic calcium data were available for all 423 women and 670 men. Written informed consent was given by each participant, and institutional review board approval was obtained from participating academic centers.

Computed Tomography Scanning

Participants were randomly selected for computed tomography (CT) scanning of the chest at one of two clinical visits between August 2002 and September 2005. Scans were performed either with an ECG-triggered (at 80% of the RR interval) electron-beam computed tomography scanner (Chicago and Los Angeles; Imatron C-150, Imatron)(Breen, Sheedy et al. 1992) or with prospectively ECG-triggered scan acquisition at 50% of the RR interval with a multidetector computed tomography system(Carr, Danitschek et al. 2001) that acquired 4 simultaneous 2.5-mm slices for each cardiac cycle in a sequential or axial scan mode (New York, Forsyth County, and St. Paul field centers; Imatron C-150 and Sensation 64, GE Lightspeed, Siemens S4+ Volume Zoom and Siemens Sensation 16). For accuracy, two chest scans were performed for each individual.

Computed tomography of the abdominal aorta was performed a single time for each individual. For electron-beam computed tomography, scanners were set as follows: scan collimation of 3mm; slice thickness of 6mm; reconstruction using 25 6mm slices with 35 cm field of view and normal kernel. For multi-detector computed tomography, images were reconstructed in a 35 cm field of view with 5mm slice thickness. Participants were scanned along with phantoms of known physical calcium concentration to convert CT numbers directly to equivalent vBMD in mg/cc.(Cann 1988)

Calcium Scoring

Scans were read centrally by the MESA CT Reading Center at Harbor-University of California, Los Angeles Research and Education Institute to identify and

quantify calcium in the coronary arteries and in an 8cm segment of the distal abdominal aorta ending at the aortic bifurcation. Calcium scores among scanning centers and between participants were adjusted with a standard calcium phantom scanned simultaneously for each participant. At least two adjacent pixels with an attenuation coefficient >130 Hounsfield units (modified to adjust for section thickness) defined a calcified lesion, and the average coronary calcium scores were calculated using the method of Agatston.(Agatston, Janowitz et al. 1990) Calcium was considered present given an Agatston score greater than 0. Rescan agreement was found to be high with both electron beam tomography and multi-detector computed tomography scanners.(Detrano, Anderson et al. 2005) Interobserver agreement and intraobserver agreement were very high ($r_s$=0.93 and 0.90, respectively).(Carr, Nelson et al. 2005)

Bone density measurement

CT data were collected using the Image Analysis QCT 3D PLUS software program (Image Analysis, Columbia, Kentucky). Measurements of vBMD in a virtual 10mm-thick slice of trabecular bone from each vertebra (L2 to L4) used soft-ware directed, automated placement of the region of interest (ROI) in the anterior one-half to one-third of the vertebral body where it (1) encompassed a large area exclusively of trabecular, or cancellous bone, (2) excluded cortical bone, and (3) excluded the basivertebral plexus. Scans were read centrally at the MESA CT Reading Center at Harbor-University of California Medical Center (Los Angeles, California) by a trained reader blinded to the results of arterial calcium scoring. The reader examined each

ROI and changed its placement to exclude vertebral abnormalities such as bone islands and diffuse density variations or to exclude an entire vertebra from measurement if the following abnormalities were noted: fractures, metastatic lesions, osteophytes, benign focal lesions within the vertebra, any other pathologically involved vertebrae. These present analyses use bone density from the third lumbar vertebra as it was most commonly available for all participants.

In a random sample of 25 scans re-read on three occasions by the blinded scan reader, there was 100 percent agreement in inclusion or exclusion for each vertebra (L2-L4). Reader drift was tested using both a multivariate approach to repeated measures analysis of variance and serial t-tests, and neither analysis provided evidence of systematic differences between reads or a time effect in the data.

Clinical measurements

Participants completed a clinical examination and detailed questionnaire. Age, sex, ethnicity, height, weight, current medications (based on self report and examination of pill bottles), physical activity patterns (mets x min/week), cigarette smoking history (ever/never/former), and previous medical diagnoses were recorded. In addition, total dietary calcium, phosphorus, fat, saturated fat, polyunsaturated fat and alcohol consumption (never/former/current and average drinks per week) were calculated from a self-administered food frequency questionnaire (FFQ) and dietary supplement form. Body mass index was calculated as mass in kilograms divided by height in meters squared. Blood pressure was measured three times with a Dinamap model Pro 100 automated oscillometric sphygmomanometer (Critikon) with

participants at rest in the seated position. The average of the last two measurements was used to define hypertension as systolic pressure ≥140 mm Hg or diastolic pressure ≥90 mm Hg or current use of antihypertensive medication.(1997)

Laboratory measurements

C-reactive protein (CRP), interleukin-6 (IL-6), homocysteine and fasting plasma glucose were measured using standard laboratory methods previously described.(Bild, Bluemke et al. 2002) Diabetes was defined by a baseline fasting plasma glucose greater than 126 mg/dL or reported previous diagnosis of diabetes or the use of hypoglycemic medications. Oxidized LDL (oxLDL) was measured in a random sample of 69 women and 103 men from the full MESA sample using competitive ELISA (Mercodia Oxidized LDL ELISA, Mercodia AB, Uppsala, Sweden) with a specific murine monoclonal antibody to oxLDL and copper-oxidized LDL as a competitive ligand.(Holvoet, Vanhaecke et al. 1998) This assay was performed (University of Leuven; Leuven, Belgium) with a total CV from 7.4-8.3%.

Total cholesterol, high density lipoprotein cholesterol(HDL-C), triglycerides were measured from morning blood samples obtained after a 12-hour fast (Roche Diagnostics, Indianapolis, IN 46250). Analyses reported here use measurements ascertained between 2002 and 2004. Total cholesterol was measured in EDTA plasma using a cholesterol oxidase method on a Roche COBAS FARA centrifugal analyzer with laboratory CV of 1.6%. HDL-cholesterol was measured in EDTA plasma using the cholesterol oxidase cholesterol method after precipitation of non-HDL-cholesterol with magnesium/dextran with laboratory CV of 2.9%. Triglycerides were measured in

EDTA plasma using Triglyceride GB reagent on the Roche COBAS FARA centrifugal analyzer with a laboratory CV of 4.0%. Low density lipoprotein (LDL) cholesterol was calculated for all participants with the Friedewald equation.(Friedewald, Levy et al. 1972)

Dyslipidemias were categorized based on lipid measures made between August 2002 and July 2004 during the second MESA examination because this visit had the least missing data, and these were the most recent lipid data confirmed to be in participants not using lipid-lowering medications or estrogen. The total cholesterol:HDL cholesterol (TC:HDL) ratio was chosen as the primary hypothesis because of its robust associations with CVD, because no other lipid measure has been shown to add predictive power in epidemiologic studies after first considering TC:HDL, and similarly, because no other lipid measure explains additional CVD outcome variance in randomized clinical trials after TC:HDL has been considered.(Natarajan, Glick et al. 2003; Ridker, Rifai et al. 2005) For convenience and comparison to other measures, a cut point of less than 5 was chosen for TC:HDL. For secondary dyslipidemia measures, the cut point were as follows: total cholesterol greater than 240 mg/dL; LDL cholesterol greater than 160 mg/dL; HDL cholesterol less than 40 mg/dL; triglycerides greater than 200 mg/dL.(2001)

Statistical Analyses

All analyses were stratified by sex given the well-known sex differences in distributions of calcified atherosclerosis(Allison, Criqui et al. 2003) and bone mineral density in men and women.(Riggs, Khosla et al. 2002) Chi-square tests of association

and generalized linear models were used to compare distributions of categorical and continuous variables, respectively. Because the odds ratio requires the rare disease assumption to accurately estimate the relative risk,(Greenland 2004) the association between bone density and presence of calcified atherosclerosis was directly estimated as the relative risk (or relative cumulative incidence, or relative prevalence). We used relative risk regression models with robust (generalized estimating equations (GEE) or "sandwich") estimates of variance and assumed Gaussian error. Bone density was initially investigated in quartiles to inspect and confirm the suitability of a linear continuous variable form in these analyses. Tests of interaction of bone density by dyslipidemia were performed first after adjustment for ethnicity and age, and then after adjustment for other covariates including potential CVD and osteoporosis risk factors as well as other covariates determined a priori from extensive literature review. Covariates were added individually to models to inspect their potential effects on the interaction. Changes in direction of effect for either lipid stratum or changes from significant ($p \leq 0.05$) to non-significant ($p > 0.05$) interactions were considered material changes in the results. Estimates of relative risk associated with a one standard deviation greater bone density were calculated from the models used to test the significance of interactions. These estimates are subsequently referred to as "adjusted stratum-specific" risks. We conducted sensitivity analyses with alternative definitions of calcium presence (Agatston score >10), lipid measurements from other visits, and additional adjustments for potential confounders as reported below.

D. RESULTS

The characteristics of study participants by sex and TC:HDL ratio are shown in Table 10. The average age of women was 64 years, and the average age of men was 63 years. Non-Hispanic white participants were 73% of women and 60% of men. Prevalence of CAC, AAC and TC:HDL-defined dyslipidemia was 59%, 63% and 15% in women and 64%, 70% and 27% in men. Prevalence of dyslipidemia defined by TC, LDL, HDL and triglycerides was 12%, 9%, 9%, and 13% in women and 7%, 8%, 31% and 16% in men. Each of these four dyslipidemias was more common among women and men whose TC:HDL ratio was greater than or equal to 5 ($p<0.05$ for each). A TC:HDL ratio greater than or equal to 5 was significantly associated with AAC in women ($p<0.01$) and men ($p<0.05$) but not with CAC in either sex.

Table 11 presents the sex-specific age- and ethnicity- adjusted characteristics according to TC:HDL ratio. After adjustment for age and ethnicity, the TC:HDL-defined dyslipidemia was associated with greater CAC and AAC in both sexes, but associations were significant only for AAC in both groups. Current alcohol consumption was more common in both women (46% vs 26%; $p<0.01$) and men (62% vs 54%; $p<0.10$) with a TC:HDL ratio less than 5.

Figure 4 displays age- and ethnicity- adjusted associations between bone density and CAC and AAC. The relative risk for CAC or AAC associated with a one standard deviation increase in vBMD is presented for men (1SD=39 mg/cc vBMD) and women (1SD=42 mg/cc vBMD), according to each defined category of dyslipidemia. In general, greater vBMD was associated with greater CAC and AAC prevalence in women with dyslipidemias but with lower CAC and AAC prevalence in

women without dyslipidemias. For men, similar patterns of inverse associations between bone and AAC were seen in those without dyslipidemias while positive associations were seen in those with dyslipidemias. These patterns were similar, but not significant, for CAC in men. This qualitative interaction of the bone-CAC association by dyslipidemia was significant ($p<0.05$) in women for all dyslipidemia definitions except TC, but not in men. For the bone-AAC association, in women the interactions were significant for TC:HDL, LDL, and HDL, and in men for TC:HDL and HDL.

Table 12 displays standardized associations between bone density and CAC and AAC by dyslipidemia, after adjustment for age, ethnicity, body mass index, hypertension, diabetes, alcohol consumption, cigarette smoking, and physical activity. Patterns were similar to those in Figure 4, and were not attenuated upon adjustment. In sensitivity analyses, results across models were comparable after the following modifications: adjustment for quantity of alcohol consumption, given its potential effects on lipid profiles,(Criqui 1998) defining CAC and AAC prevalence as Agatston score >10, use of lipid measurements made two to four years prior, use of the mean of all lipid measurements, additional adjustment for interleukin-6, C-reactive protein and dietary calcium intake.

To evaluate the hypothesis that oxidized LDL (oxLDL) contributes to an association between atherosclerosis and bone density, we examined measures of oxLDL that were available in a random sample of 103 men and 69 women. This random sample differed minimally from the study sample described in Table 1 in

terms of CAC prevalence, AAC prevalence, prevalence of dyslipidemia defined by TC:HDL ratio less than 5, or mean vBMD. In this sample, the mean of natural log-transformed oxLDL levels were significantly greater in both men ($p<0.01$) and women ($p<0.01$) with dyslipidemia defined by the TC:HDL ratio, after adjustment for age and ethnicity and even after adjustment for LDL-C. Mean LN(oxLDL) also differed significantly ($p<0.05$) in men and women when dyslipidemia was examined according to LDL and HDL cut-points. However, vBMD was not significantly associated with LN(oxLDL) in men or women in models adjusting for age and ethnicity.

E. DISCUSSION

We tested the hypothesis that lipids modify the association between atherosclerosis and bone density by investigating the associations between bone density (vBMD) and coronary artery calcium (CAC) and abdominal aortic calcium (AAC) in a sample of MESA-AAC study participants without clinical cardiovascular disease at enrollment and naïve to lipid-modifying medications. The study tested for differences in the bone-atherosclerosis association between those with dyslipidemia, defined by total cholesterol to HDL-cholesterol ratio (TC:HDL) of greater than or equal to 5, and those without dyslipidemia (TC:HDL<5.0). We found generally inverse associations between bone density and calcified atherosclerosis in those without dyslipidemia and generally positive associations between bone density and calcified atherosclerosis in those with dyslipidemia. These qualitative interactions, where effects were opposite in direction by strata of dyslipidemia, were highly significant for CAC and AAC in women and for AAC in men.

These findings were robust and consistent in multiple sensitivity analyses. Patterns of qualitative interaction between bone density and atherosclerosis by TC:HDL ratio were comparable for both coronary and aortic sites, both sexes, and for different measurements of lipids made over two to five years. These patterns were largely consistent in confirmatory analyses where dyslipidemia was defined as TC:HDL ratio>4.5 (not shown), LDL >160 or HDL <40 or HDL<50 (women only, not shown). We found no significant interactions by total cholesterol, and interactions by triglycerides were significant only for CAC in women. All significant interactions persisted after adjustment for multiple risk factors for atherosclerosis and osteoporosis as well as additional adjustment for other lipid measures (results not shown). These data suggest that the strongest and most consistent interactions were with the TC:HDL ratio.

The magnitude of the relative risk may differ between samples as a result of risk-stratification (rather than differences in absolute risk)(Rothman and Poole 1988) or they may vary due to truncation of a variable distribution because of reduced power to detect an association. To investigate the possibility that our findings resulted from these known these statistical artifacts, we performed additional tests of interaction between dyslipidemia and two known risk factors for calcified atherosclerosis, hypertension and diabetes. None of these tests for interaction were significant ($p>0.14$ for all), and, importantly, none indicated qualitative interaction as was found consistently with multiple dyslipidemia definitions. Finally, although those with and without a favorable TC:HDL ratio varied in their distributions of many covariates,

mean and range values for bone density were not different between lipid-defined groups. Thus the qualitative bone-lipid interactions demonstrated were unlikely to have resulted from known statistical artifacts.

The present investigation built on previous reports from the MESA-AACS that demonstrated significant, inverse associations between trabecular bone density and coronary artery calcium in women and with aortic calcium prevalence, ankle-brachial index (ABI) and internal carotid artery intima-media thickness (ICA-IMT) in men.(Hyder 2006; Hyder 2006) In light of these observations, we investigated effect modification by dyslipidemia on the associations between bone density and ABI, ICA-IMT and common carotid IMT (CCA-IMT) in the same sample. Similar patterns of significant, qualitative interaction were found for ICA-IMT in women but not for other measures of atherosclerosis. In men, these patterns of interaction varied considerably and did not inform the findings in the present study. The atherosclerosis-lipid-bone interactions for CAC and AAC reported here could be specific for calcified atherosclerosis. However, arterial calcium in the coronaries and aorta are considered to be markers for total atherosclerotic burden rather than specific measures of fractional calcification of atherosclerosis.

An atherosclerosis-lipid-bone model has been proposed to explain laboratory and epidemiologic associations between low bone density and atherosclerosis.(Parhami and Demer 1997; McFarlane, Muniyappa et al. 2004) In this model, LDL and oxidative stress result in oxidized LDL, a strongly atherogenic class of compounds that promote osteoblastic differentiation of vascular calcifying

cells.(Parhami, Morrow et al. 1997) Plaque formation takes place in arterial sites within the highly-vascularized bone tissue.(Parhami, Garfinkel et al. 2000) Here oxidized LDL stimulates bone marrow stromal cells, which are located adjacent to the subendothelial matrix of bone vessels, to favor adipogenic rather than osteoblastic differentiation.(Nuttall, Patton et al. 1998) The result is simultaneous arterial calcification and skeletal bone loss.

The present study was the first epidemiologic test of this atherosclerosis-lipid-bone model. In the present study, as expected, dyslipidemia defined by TC:HDL was associated with CAC and AAC, and with greater levels of oxidized LDL and fractional oxidized LDL in a sub-sample of participants. In the present study, however, the associations between bone density and atherosclerosis were positive in those with dyslipidemia, not inverse as would be predicted from the atherosclerosis-lipid-bone model. Also, bone density was not significantly associated with oxidized LDL levels.

Another class of compound associated with oxidized LDL and atherosclerosis may explain these inconsistencies. Oxysterols, active components of oxidized LDL,(Colles, Maxson et al. 2001) include numerous oxygenated derivatives of cholesterol and can be found in the circulation and body tissues as a result of dietary intake, auto-oxidation of cholesterol, and the action of mono-oxygenases.(Russell 2000) In a recent series of experiments, various oxysterols demonstrated strong osteogenic effects including inhibition of the adipogenic effects of oxidized LDL on marrow stromal cells and promotion of osteoblastic differentiation of these cells

through multiple effects.(Kha, Basseri et al. 2004; Liu, Yuan et al. 2005; Shouhed, Kha et al. 2005) In related experiments, oxysterols stimulated the calcification of osteoblast-like vascular smooth muscle cells.(Watson, Bostrom et al. 1994; Liu, Yuan et al. 2004) The actions of oxysterols vary according to their combinations and concentrations, which also vary widely in human tissues.(Colles, Maxson et al. 2001) The present findings, including null or limited interactions with total cholesterol and triglycerides, point to integrated effects of HDL-C, LDL-C, oxidized LDL, and various oxysterol compounds. These findings also raise the testable hypothesis that osteoblastic combinations of oxysterols predominate in dyslipidemic states but not normal lipid states.

Other components may explain the present findings. Moderate alcohol consumption is known to reduce the risk of cardiovascular disease, largely by elevating HDL-cholesterol,(Criqui 1998) but very high levels of alcohol intake are associated with lower bone density.(Kalbfleisch, Lindeman et al. 1963; Krawitt 1975; Rosen 2005) Multiple adjustments for alcohol consumption variables did not alter the patterns between lipids, bone, and atherosclerosis in this study (results not shown). Leptin,(Takeda, Elefteriou et al. 2002) the cannabinoid system,(Ofek, Karsak et al. 2006) the osteoprotegerin/RANKL system,(Tintut and Demer 2001) and saturated fat intake(Corwin, Hartman et al. 2006) all have been associated with bone density and atherosclerosis. In this study, however, adjustment for dietary intake of total fat, saturated fat, and poly-unsaturated fat did not alter the results (results not shown). The

present findings may reflect the effects of unknown factors such as certain genetic environments on both basal cholesterol levels and peak bone density.

The present study has several limitations including a cross-sectional design with one-time measures of bone density and atherosclerosis. Another limitation is the small sample to test associations with oxLDL. For some participants, the ascertainment of bone, atherosclerosis, and lipid measures was not concurrent, although this did not affect the results. In addition, some participants may have been using selective estrogen receptor modifiers, vitamin D supplements or anti-bone-resorptive agents, which (other than estrogen) were not assessed. The effects of these medications on the outcome depend, in part, on their differential distributions in dyslipidemic groups, but would likely bias observed effects to the null.

Despite these limitations, the study benefited from a multi-ethnic, population-based sample of both men and women, and state-of-the-art measurements of atherosclerosis and volumetric BMD, which is more accurate and precise than areal measurements.(Lang, Guglielmi et al. 2002) Participants taking the most common pharmacologic therapies for dyslipidemia and bone loss were excluded, thus minimizing confounding by a pharmacologic effect. Conclusions from the study are bolstered by the consistency of highly significant interactions between vBMD and atherosclerosis across two arterial sites, multiple established definitions of dyslipidemia, and multiple measurements of lipids at different times. In conclusion, this study supports the hypothesis that dyslipidemia modifies the association between bone density and atherosclerosis. In men and women with atherogenic total

cholesterol to high density lipoprotein cholesterol ratios, greater bone density was associated with greater prevalence of coronary or abdominal aortic calcium. In men and women with normal lipid levels, greater bone density was associated with lower prevalence of coronary or abdominal aortic calcium. Further laboratory and population-based investigations of regulation of bone and artery aging will benefit from the integrated study of bone and vascular biology.

**Table 10. Distribution of clinical characteristics according to sex and total cholesterol to high density cholesterol ratio (TC:HDL), the Multi-Ethnic Study of Atherosclerosis, Abdominal Aortic Calcium Study, 2000-2005.**

| | WOMEN | | | | MEN | | | |
|---|---|---|---|---|---|---|---|---|
| | | TC:HDL ≥ 5.0 | | | | TC:HDL ≥ 5.0 | | |
| | ALL | No | Yes | p | ALL | No | Yes | p |
| § | | | | | | | | |
| Sample % (n) | 423 | 85 (360) | 15 (63) | | 670 | 73 (490) | 27 (180) | |
| Age, years | 64±0.5 | 65±0.5 | 64±1 | | 63±0.4 | 64±0.5 | 62±0.7 | |
| Ethnicity | | | | | | | | ‡ |
| Non-Hispanic White | 27 (114) | 27 | 25 | | 40 (265) | 40 | 38 | |
| Chinese | 16 (68) | 16 | 14 | | 15 (99) | 16 | 12 | |
| African American | 27 (114) | 28 | 19 | | 19 (130) | 22 | 12 | |
| Hispanic | 30 (127) | 28 | 41 | | 26 (176) | 22 | 39 | |
| Coronary artery calcium | 59 (249) | 39 | 51 | | 64 (427) | 63 | 67 | |
| Abdominal aortic calcium | 63 (268) | 61 | 78 | † | 70 (468) | 67 | 77 | * |
| Total cholesterol > 240 mg/dL | 12 (49) | 7 | 37 | ‡ | 7.0 (47) | 3 | 18 | ‡ |
| LDL-C >160 md/dL | 9 (37) | 5 | 32 | ‡ | 8 (55) | 3 | 22 | ‡ |
| HDL-C <40 mg/dL | 9 (36) | 3 | 40 | ‡ | 31 (207) | 16 | 73 | ‡ |
| Triglycerides > 200 mg/dL | 13 (54) | 8 | 41 | ‡ | 16 (109) | 7 | 41 | ‡ |
| Hypertension | 42 (178) | 41 | 48 | | 38 (252) | 38 | 37 | |
| Diabetes | 10 (41) | 9 | 14 | | 11 (73) | 10 | 13 | |
| Ever smoked | 63 (268) | 63 | 68 | | 42 (279) | 42 | 41 | |
| Current alcohol consumption | 45 (190) | 48 | 27 | | 63 (422) | 65 | 59 | |
| ∞ | | | | | | | | |
| Bone density, mg/cc | 110±2 | 110±2 | 110±5 | | 122±1.5 | 122±1.7 | 124±2.9 | |
| TC:HDL ratio | 3.81±0.06 | 3.46±0.04 | 5.82±0.10 | ‡ | 4.31±0.05 | 3.76±0.03 | 5.82±0.06 | ‡ |
| BMI, kg/m^2 | 28.5±0.3 | 28.3±0.3 | 29.7±0.7 | | 27.6±0.2 | 27.3±0.2 | 28.5±0.3 | ‡ |
| Physical activity, met-min/week*100 | 122±3 | 122±4 | 123±8 | | 121±3 | 119±3 | 124±5 | |
| (LN)Interleukin-6, pg/mL | 0.86±0.02 | 0.85±0.02 | 0.89±0.05 | | 0.82±0.01 | 0.81±0.02 | 0.84±0.03 | |
| (LN )C-reactive protein, mg/L | 1.28±0.04 | 1.26±0.04 | 1.42±0.10 | | 1.02±0.02 | 0.99± 0.3 | 1.11±0.05 | * |
| Homocysteine, µmol/L | 9.0±0.3 | 9.0±0.3 | 8.9± 0.8 | | 10.0±0.1 | 10.0 | 10.2 | |
| Dietary calcium, mg/day | 730±26 | 735±28 | 704±66 | | 796±24 | 801±28 | 789±47 | |
| Dietary phosphorus, mg/day | 991±27 | 994±29 | 973±69 | | 1169±26 | 1157±31 | 1201±51 | |

§ Displays percentage (n) for discrete variables.
∞ Displays mean±standard error of the mean for continuous variables.
^TC is total cholesterol; LDL-C is low density lipoprotein cholesterol; HDL-C is high density lipoprotein cholesterol. LN is natural log.
*p<0.05 †p<0.01 ‡p<0.001

**Table 11. Age- and ethnicity-adjusted distribution of clinical characteristics according to sex and total cholesterol to high density cholesterol ratio (TC:HDL), the Multi-Ethnic Study of Atherosclerosis, Abdominal Aortic Calcium Study, 2000-2005.**

| | | WOMEN | | | MEN | | |
|---|---|---|---|---|---|---|---|
| | | TC:HDL ≥ 5.0 | | | TC:HDL ≥ 5.0 | | |
| | | No | Yes | * | No | Yes | * |
| Sample % (n) | | 85 (360) | 15 (63) | | 73 (490) | 27 (180) | |
| Coronary artery calcium | § | 39±2 | 51±6 | # | 59±2 | 65±3 | # |
| Abdominal aortic calcium | | 61±2 | 80±5 | * | 64±2 | 77±3 | † |
| Hypertension | | 40±2 | 49±6 | | 37±2 | 40±4 | |
| Diabetes | | 9±2 | 14±4 | | 11±1 | 13±2 | |
| Ever smoked | | 34±2 | 29±6 | | 43±2 | 43±4 | |
| Current alcohol consumption | | 46±3 | 26±6 | † | 62±2 | 54±4 | # |
| Bone density, mg/cc | ∞ | 109±2 | 109±4 | | 125±2 | 126±3 | |
| BMI, kg/m^2 | | 28.9±0.3 | 28.9±0.7 | | 26.9±0.2 | 27.9±0.3 | † |
| Physical activity, met-min/week*100 | | 120±3 | 121±8 | | 120±3 | 119±5 | |
| (LN)Interleukin-6, pg/mL | | 0.83±0.02 | 0.85±0.04 | | 0.80±0.02 | 0.83±0.03 | |
| (LN)C-reactive protein, mg/L | | 1.22±0.04 | 1.37±0.09 | | 0.97±0.03 | 1.06±0.05 | |
| Homocysteine, µmol/L | | 8.9±0.3 | 8.9±0.8 | | 9.8±0.2 | 10.4±0.3 | |
| Dietary calcium, mg/day | | 722±28 | 665±66 | | 778±29 | 713±48 | |
| Dietary phosphorus, mg/day | | 979±29 | 931±69 | | 1134±32 | 1126±52 | |

§ Displays percentage±standard error for discrete variables.
∞ Displays mean±standard error of the mean for continuous variables.
# p<0.10 *p<0.05 †p<0.01 ‡p<0.001

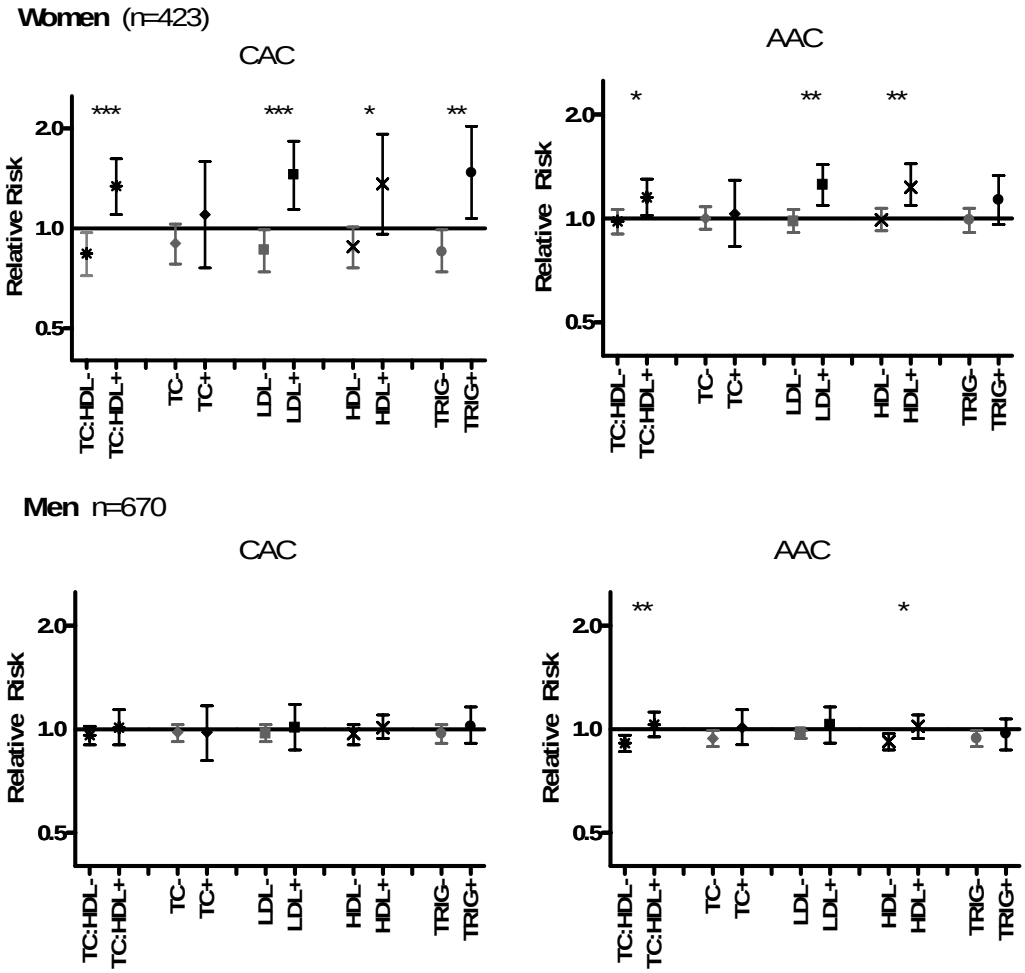


**Figure 4. Age- and ethnicity-adjusted relative risk (95%CI) for coronary artery calcium (CAC) and abdominal aortic calcium (AAC) associated with a standardized increase in trabecular, lumbar bone density for women and men with and without five dyslipidemias, the Multi-Ethnic Study of Atherosclerosis, Abdominal Aortic Calcium Study, 2000-2005.**

* $p<0.05$ for bone density by dyslipidemia interaction.

** $p<0.01$ for bone density by dyslipidemia interaction.

*** $p<0.001$ for bone density by dyslipidemia interaction.

"-" or "+" indicates absence or presence of dyslipidemia based on the following criteria: total cholesterol to HDL ratio (TC:HDL) ≥ 5.0; total cholesterol (TC) ≥ 240 mg/dL; LDL-cholesterol ≥ 160 mg/dL ; HDL-cholesterol<40 mg/dL; triglycerides (TRIG) ≥ 200.

**Table 12. Multiply-adjusted relative risk (RR) of coronary artery calcium (CAC) or abdominal aortic calcium (AAC) associated with a standardized increase in lumbar bone density for different lipid profiles, the Multi-Ethnic Study of Atherosclerosis, Abdominal Aortic Calcium Study, 2000-2005.**

| | WOMEN | | | | | MEN | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | CAC | | AAC | | | CAC | | AAC | |
| | N | RR | 95% CI | RR | 95% CI | N | RR | 95% CI | RR | 95% CI |
| | 421 | | | | | 668 | | | | |
| TC:HDL ratio ^ | | | p=0.002 | | p=0.038 | | | p=0.27 | | p=0.035 |
| < 5.0 | | 0.86 | 0.75-0.99 | 0.98 | 0.90-1.05 | | 0.96 | 0.91-1.02 | 0.93 | 0.88-0.98 |
| ≥ 5.0 | | 1.26 | 1.03-1.53 | 1.11 | 1.00-1.24 | | 1.03 | 0.92-1.14 | 1.02 | 0.94-1.11 |
| Total cholesterol | | | p=0.37 | | p=0.83 | | | p=0.86 | | p=0.35 |
| < 240 mg/dL | | 0.92 | 0.81-1.04 | 1.00 | 0.93-1.07 | | 0.98 | 0.93-1.03 | 0.94 | 0.90-0.99 |
| ≥ 240 mg/dL | | 1.09 | 0.75-1.59 | 1.02 | 0.81-1.27 | | 0.99 | 0.84-1.18 | 1.00 | 0.88-1.14 |
| LDL cholesterol | | | p=0.003 | | p=0.031 | | | p=0.49 | | p=0.19 |
| < 160 mg/dL | | 0.87 | 0.76-0.99 | 0.98 | 0.91-1.05 | | 0.97 | 0.92-1.03 | 0.94 | 0.90-0.99 |
| ≥160 mg/dL | | 1.33 | 1.04-1.71 | 1.17 | 1.01-1.37 | | 1.02 | 0.89-1.17 | 1.03 | 0.91-1.16 |
| HDL cholesterol | | | p=0.03 | | p=0.010 | | | p=0.22 | | p=0.044 |
| > 40 mg/dL | | 0.90 | 0.79-1.03 | 0.98 | 0.91-1.05 | | 0.96 | 0.91-1.03 | 0.92 | 0.88-0.98 |
| ≤ 40 mg/dL | | 1.32 | 0.94-1.85 | 1.17 | 1.03-1.33 | | 1.02 | 0.95-1.10 | 1.01 | 0.94-1.08 |
| Triglycerides | | | p=0.002 | | p=0.17 | | | p=0.39 | | p=0.60 |
| < 200 mg/dL | | 0.87 | 0.76-0.99 | 0.98 | 0.91-1.06 | | 0.97 | 0.92-1.02 | 0.94 | 0.90-0.99 |
| ≥ 200 mg/dL | | 1.44 | 1.06-1.95 | 1.11 | 0.94-1.33 | | 1.03 | 0.91-1.16 | 0.97 | 0.88-1.07 |

p-values are for interaction.
^TC:HDL ratio= total cholesterol to HDL-cholesterol ratio.
*Models are adjusted for age, ethnicity, body mass index, hypertension, diabetes, alcohol consumption, cigarette smoking, physical activity.

The text of Chapter Four, in part, will be submitted for publication as:

Hyder, J.A., Criqui, M. H., Barrett-Connor, E., Allison, M. A., Detrano, R., Wong, N. D. "Dyslipidemia modifies the association between bone density and calcified atherosclerosis," 2006.

# V. CONCLUSION

## Intention of the research program

At the time this study was conceived, conclusions about an association between atherosclerosis and osteoporosis from 50 years of literature were limited by the methods of bone and atherosclerosis measurement, study sample compositions and characterizations, and/or analytic approaches.(Elkeles 1957) To extend the findings of previous investigations, this study added precise measurements of bone density to detailed information on the distribution and determinants of atherosclerosis in a well-characterized, ethnically-diverse sample of men and women from the Multi-Ethnic Study of Atherosclerosis, Abdominal Aortic Calcium Study (MESA-AACS).

## Summary of findings

This series of investigations demonstrated associations between trabecular lumbar bone density (vBMD) and multiple measures of atherosclerosis in men and women (Chapters 2 and 3). Specifically, trabecular bone density was significantly, inversely associated with the presence of abdominal aortic calcium (AAC), the ankle-brachial index (ABI), the internal carotid intima-media thickness (ICA-IMT), and greater carotid artery plaque echogenicity in men. In women, trabecular bone density was associated with the presence and extent coronary artery calcium (CAC) and the extent of AAC as well as greater carotid artery plaque echogenicity.

Previous literature had implicated lipids, inflammation, and sex hormones as contributors to the bone-artery association. In serial analyses, adjustment for these

factors did not attenuate or augment associations between atherosclerosis and vBMD. We concluded that the bone-artery is not affected by these factors, whether these factors are confounders, mediators or common risk factors for bone loss and atherosclerosis. Thus bone density and atherosclerosis may be related through some other, unmeasured non-causal factors, or they may be causally related.

Because of limited published data describing associations between bone and atherosclerosis in non-white samples, the present work explored ethnic differences in the association between bone density and atherosclerosis. In these investigations, the associations between atherosclerosis and bone density varied little by ethnicity (Chapters 2 and 3). The major interaction by ethnicity was for CAC in men where greater vBMD was significantly associated with greater CAC presence in Chinese men, but with non-significantly lower CAC presence in other ethnic groups. This finding, discussed in Chapter 2, may have resulted from sampling differences between the Chinese men in MESA and the sub-sample in MESA-AACS. Overall, multiple interactions by ethnicity were tested and the finding of only one marginally statistically-significant difference is surprising given that patterns of bone density and atherosclerosis are known to vary by ethnicity.

In reality, previously reported ethnic differences in bone density were based on areal assessments that were confounded by differences in bone size and shape associated with ethnicity. In some studies, adjustment for body habitus resolved what appeared to be ethnic differences in bone density.(Russell-Aulet, Wang et al. 1993; Wang, Aguirre et al. 1997; Morton, Barrett-Connor et al. 2003) However, the present

study demonstrated significant differences in vBMD across ethnic groups in patterns similar to those reported in studies using areal measurements. We also demonstrated ethnic differences in atherosclerosis as would be expected from previous reports.(Bild, Detrano et al. 2005; McDermott, Liu et al. 2005) Thus, the lack of significant interaction by ethnicity here raises the possibility that the current study had limited power to detect ethnic-specific differences for some or all outcomes. Directionality of interaction by ethnic group was not hypothesized because explanations for ethnic differences are not clear. In that context, speculation about non-significant interactions is limited.

In further investigations (Chapter 4), we examined MESA-AACS participants naïve to lipid-modifying medications and demonstrated that the association between bone density and the presence of CAC and AAC was positive when dyslipidemia, defined by total cholesterol to high density cholesterol ratio (TC:HDL>5), was present but inverse in the absence of dyslipidemia. These population-based findings informed the increasingly-popular hypothesis that dyslipidemia and oxidized low density lipoproteins (oxLDL) were driving the inverse association between low bone density and atherosclerosis. As a result, this work raised the testable hypothesis that oxysterols, which are oxidized forms of cholesterol that associate with oxLDL and promote both atherosclerosis and osteoblastic differentiation, vary in concentration and/or combination in those with versus those without dyslipidemia.

Previous chapters have explained the ways that the present investigations have furthered the certainty regarding an association between bone density and

atherosclerosis as well as the scope of inquiry into this association. Briefly, the significant, inverse associations between bone density and atherosclerosis in the present study extended previous findings to the coronary arteries, to men, and to non-white populations. This work suggested that shared risk factors such as smoking and physical activity, as well as inflammatory markers and sex hormones do not contribute to the association. Future research may focus then on other factors as well as interactions with lipids such as oxidized LDL and oxidized cholesterol forms.

Limitations

Testing for an association between two diseases has challenged classical epidemiologic methods and resulted in spurious that were findings later rebutted.(Pearl 1929) However, in the present investigations, the use of non-patient participants who were free of symptomatic cardiovascular disease at intake, and the assessments of "subclinical" rather than "clinical" osteoporosis and atherosclerosis minimized the chances of spurious associations. This approach likely biased any associations to the null. The evaluation of subclinical endpoints in asymptomatic participants as part of a two-disease study does not preclude the possibility of previous diagnosis and treatment of these conditions. This principle was demonstrated in the case of many study participants taking lipid-modifying prescription drugs for what were, presumably, previously-diagnosed dyslipidemia. Another illustration of this point is made in dietary calcium intake data. Dietary calcium intake, including dietary supplements, was not inversely associated with bone density quartile in women in this sample. In fact women in the lowest quartile for bone density had a non-significantly

greater mean dietary calcium intake than women in the highest bone density quartile (Chapter 2).

Some limitations of this work, such as the classical limitations of cross-sectional studies and bone density measurement from a single site, have been discussed previously (Chapters 2, 3, 4). Others not previously discussed deserve attention. These present investigation includes only those MESA participants who attended a second or third clinical visit up to 4 years after initial enrollment. Of all participants attending the first exam, approximately 87% participated in both the second and third study visits. Those with greater disease, be it atherosclerosis or osteoporosis, would have been less likely to participate, but their absence is likely to diminish any associations found here.

Carotid IMT measurement by ultrasound, particularly of the anatomically-buried ICA, may suffer from limited reproducibility, largely as a result of differences in technique among practitioners who place the ultrasound probes on subjects' necks.(Riley 2002; Bots, Evans et al. 2003) The IMT measurements in the MESA were made based on established protocols for vessel interrogation and digital measurement of ultrasound data, in the form of videotape. The reproducibility of IMT measurements in the MESA appear to be calculated based on multiple readings of the videotapes produced during a single ultrasound assessment and not based on multiple repetitions of the ultrasound assessment. Thus, the largest component of variability in this measurement was not quantified, and measurement error for IMT was likely

greater than reported by the MESA Ultrasound Committee. Regardless, this error would likely bias results to the null.

Finally, data describing use of selective estrogen-receptor modulators (such as raloxifene), bisphosphonates (such as aledronate), and other agents(Rosen 2005) affecting bone density (such as parathyroid hormone, vitamin D or K status and calcitonin) were not available from MESA, and their measurement or imputation was not feasible. Unreported use of these medications, all of which increase bone density, would be likely to bias inverse associations to be more positive. Because positive associations between bone density and atherosclerosis were found only in a sub-set of individuals with dyslipidemia also not taking lipid-modifying medications, a spurious association is unlikely in the present study.

Future directions of research

The current investigations raise hypotheses and promise both for population-based and laboratory-based research into the bone-artery association. This discussion is concerned primarily with future population-based investigations. Future work may involve more detailed treatment of the MESA-AACS study sample or investigations of other study samples. The present series of investigations in the MESA-AACS could be enriched in two main ways as follows: 1) additional cross-sectional measurements of new variables and 2) additional prospective measurements of the measurements used here as well as measurement of new variables.

For continued cross-sectional analyses of the MESA-AACS, the most promising new variables are cannabinoid receptor genotypes and measurements evaluating the osteoprotegerin system. Cannabinoid receptors are found in

atherosclerotic lesions and exert effects on bone in part through the osteoprotegerin/RANK/RANKL system which, itself, affects both atherosclerotic(Erdogan, Aslan et al. 2004) and osteoporotic change(Raisz 2005). In recent studies the cannabinoid system has been implicated with atherosclerosis and osteoporosis in mice and in humans.(Karsak, Cohen-Solal et al. 2005; Steffens, Veillard et al. 2005; Ofek, Karsak et al. 2006) In mouse models, mice lacking the peripheral cannabinod receptor have a markedly accelerated age-related trabecular bone loss and cortical expansion, although cortical thickness remains unaltered. These changes are very similar to those found in human osteoporosis.(Seeman 2003) In another series of mice experiments, a diet rich in tetrahydrocannabinol, an agonist of the cannabinoid receptor, inhibited atherosclerosis progression in apolipoprotein E knockout mice that are highly susceptible to atherosclerosis.(Steffens, Veillard et al. 2005) In humans, a population-based investigation demonstrated a highly significant associations with single polymorphisms and haplotypes encompassing the peripheral cannabinoid receptor gene, but not the central cannabinoid receptor gene. These results demonstrate a role for the peripherally expressed receptor in the etiology of osteoporosis and provide a novel therapeutic target.(Karsak, Cohen-Solal et al. 2005) Assessment of cannabinoid genotypes in the present study may delineate populations susceptible to inverse and/or positive associations between bone density and atherosclerosis.

In Chapter 4, oxidized LDL (oxLDL) and oxysterols were discussed as potential actors in a bone-artery association. The utility of oxLDL measurement in the

full MESA-AACS sample, as well as oxysterol combinations and concentrations, is unknown at this time. In the case of oxysterols, these compounds are produced in bone tissue as well as in the circulation, so additional research is needed before the value of venipuncture samples is known. Similarly, the assessment of multiple parameters related to bone metabolism and/or atherosclerosis is of questionable value in any future investigation. For instance, vitamin D and its metabolites, markers of bone formation and resorption, parathyroid hormone, detailed information on ultraviolet light exposure (from weather records) and many other measures would be of some, if limited, use in a study of this association. This is likely the case for cross-sectional as well as repeated measurements of these factors.(Watson, Abrolat et al. 1997; Kamycheva, Sundsfjord et al. 2004; Shea, Wells et al. 2004)

Prospective data collection of ABI, AAC, CAC and CVD risk factors is underway in the MESA-AACS, as is the collection of events data including death and CVD-related events. The repeated measurement of vBMD from on-going AAC scans is imminently feasible. Serial measurements of vBMD, atherosclerosis, and CVD risk factors would permit testing of hypotheses more closely related to causality in the association as well as more clinically relevant evaluation of the bone-artery association. For instance, a prospective study could evaluate the incremental predictive value of vBMD for CVD risk assessment over and above other major CVD risk factors.

From other study samples, an investigation of bone density and small artery function, such as coronary function, would inform the hypothesis that atherosclerosis

causes bone loss. Such an investigation of bone density and coronary artery function, assessed by echocardiography, is underway in a convenience sample of men and women who underwent bone density assessment and echocardiography at the Mayo Clinic in Rochester, Minnesota. Additional, related work using magnetic resonance imaging to specifically assess bone perfusion as well as bone density and atherosclerosis would inform the understanding of the bone-artery association.(Chen, Ting-Fang Shih et al. 2004; Shih, Liu et al. 2004; Griffith, Yeung et al. 2005; Yeung, Griffith et al. 2005)

Finally, it should be mentioned that there are few, if any, reports describing ethnic differences in vBMD, and a more detailed investigation of vBMD and ethnicity would be a worthy contribution to the bone literature. In addition, data describing prospective changes in vBMD have not been published as of this writing. The current MESA-AACS data provide a unique opportunity to study these patterns and processes.

Closing

In closing, the nature of the association between age-related bone and artery pathology will likely be elucidated by the integration of findings from laboratory investigations and multiple, large, prospective studies. Until then, these reports from the MESA-AACS provide the most extensive cross-sectional investigation of the patterns of this association to date.

APPENDIX ONE

**1) Research and training steps prior to writing bone density collection protocol**
**2) Bone density collection protocol with example image**
**3) Training of JAH on Bone density readings**
**4) Procedures of data quality control and assurance**
- Initial note on blinding
- Scan Re-reads
- Tests of data quality over time

**5) CT model and bone density**
**6) Bone data and exclusions**

**1) Research and training steps prior to writing bone density collection protocol**
Prior to writing the protocol for bone density acquisition, the candidate (JAH) did the following trained in anatomy and spinal pathology with Agnes Papa, MD, a radiologist and primary reader for the MESA Reading Center responsible for arterial calcium scoring and bone density measurements for MESA and MESA-AACS, respectively. JAH also trained on the QCT 3D PLUS (Image Analysis, Inc, Columbia, Kentucky) automated bone mineral density analysis software at the MESA CT Reading Center at Harbor UCLA. This software and reading center were used for all bone density measurements. In this software environment, lateral as well as anterior-posterior projections of the spine are acquired to identify the patient's vertebrae and to align the scan plane along both axes. This lateral view also permits identification of spinal fractures, compressions or abnormalities that may cause inaccurate density measurement. Axial images are acquired for each vertebral body selected.

**2) Bone density collection protocol with example image**
The protocol is designed by JAH to maximize the automated selection of regions of interest by the QCT 3D PLUS bone density analysis software for high repeatability of measurements.

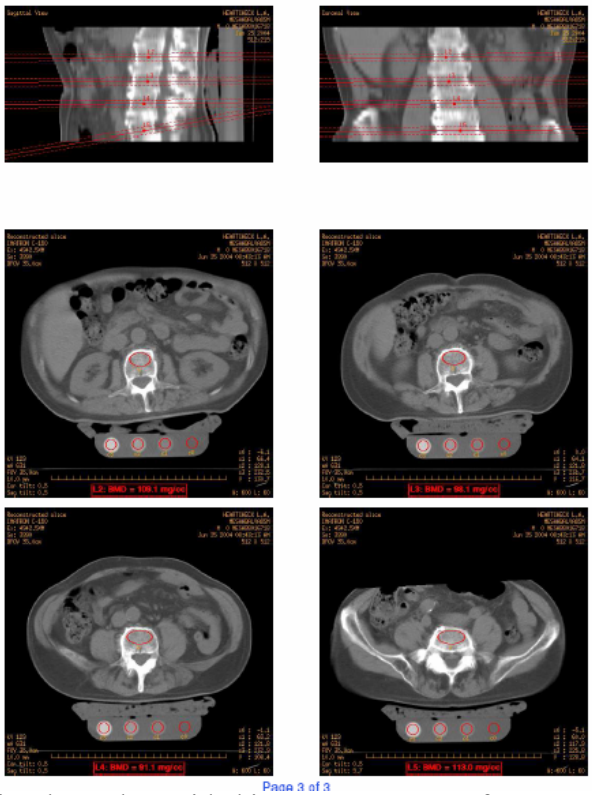

Figure 1. Example visual panel provided in QCT 3D PLUS for measurement of trabecular bone mineral density. In the four bottom panels, the largest red circles are automatically-placed regions of interest for measurement. The smaller red circles are places over hydoxyapatite calibration phantoms. The upper right and upper left panels are lateral and anterior-posterior images, respectively.

Each subject's abdominal scan will be loaded into the QCT 3D Plus (Image Analysis, Columbia, KY) for viewing and measurement.
The location of L4 will be identified by the presence of the superior border of the iliac crest. The L4/L5 border or distal area of L4 will be confirmed by counting backwards from the sacrum and by its proximity to the bifurcation of the aorta. If necessary, L2 may be confirmed by the emergence of the superior mesenteric artery (SMA).
To begin measurements, the reader will select any single vertebra between L2 and L5. Using the software, the reader will adjust the planes of measurement such that the virtual 10mm slice for reading passes through the center of the vertebra perpendicular to the posterior plane of the vertebra and not including cortical bone found at the superior or inferior margins of the vertebra.
Using the interactive software, regions of interest (ROI) will be assigned to the calibration phantom rods if such assignments are not properly made automatically by the software. C0 is the darkest phantom rod; C3 is the lightest phantom rod.
Measurements will be made by indicating the region of interest (ROI) where the ROI is placed in the anterior one-half to one-third of the vertebral body where it (1) encompasses a large area exclusively of trabecular bone, (2) excludes cortical bone,

(3) excludes the basivertebral plexus and (4) excludes abnormalities (fractures, metastatic lesions, osteophytes, bone islands, diffuse density variations, benign focal lesions within the vertebra, any other pathologically involved vertebrae).
A virtual 10mm-thick slice of each vertebra (L2 to L5) will be measured and its location will be recorded using a prompting pop-up window.
Measurements of all vertebrae L2 through L5 will be attempted.
If a vertebra shows evidence of fracture, measurements will not be attempted on that vertebra. If a 10mm slice or ROI not meeting the above criteria is not available, measurement will not be attempted. All measurement attempts and failures will be recorded in the data log where all data reads will be recorded. The below system was used to record readings:

0 (no markings) =read fine
1= not read - not available on screen
1*=availablle on screen but blurred
2=not read - fracture/compression fracture
3= not read - metastatic lesion
4= not read - osteophytes
5= not read - bone islands
6= not read - density variations
7= not read - other pathology
8=image cut off
88=read-cut off phantom
89=not read -phantom not visible
98=missing c2 from phantom not read
12 (xxx) phantom cut off and missing c1
### (13) with negative values probably due to artifact or an obese patient.
14 (M)-metal inside the vertebral bodies (secondary to an operation) not read
N=not read ( with negative value)

A pdf file, titled by anonymous subject ID, will be created to store measurement results for each subject. These files will be stored in the MESA computer under the file tree qCT's Home; bmd; pdf/ Files are saved by subject name. sent (in electronic copy) to Joe Hyder at jhyder@ucsd.edu with an electronic copy remaining on the MESA BMD computer at the Harbor reading center. If such copies cannot be sent electronically, arrangements will be made for data exchange.
Data describing if and why a vertebra was included/excluded were recorded for the second and third reads only using a coding system that identified the following possible reasons for excluding reads:

Reference: QCTPro Poster on BMD Readings
Other landmark – superior mesenteric artery generally occurs at L2
Bifrucation at L4 in 75% of subjects

**3) Training of JAH on Bone density readings**

Agnes and Joe will perform ~20 reads in duplicate to standardize the reading approach (selection of ROI, etc).

To confirm standardization, Agnes and Joe will each read ~20 new scans, blinded to the other's result. A measure of agreement will be performed. If variability exceeds 5-10% (?) then the reading approach will be re-standardized.

A protocol to repeat blinded, quarterly measurement of these 15 scans will be established before May 5, 2004 (Deborah will confirm BASH coding.)

These repeat scans will be used to examine reader migration over time.

May 20, 2004

In repeat, blinded reading of 30 scans (MESA Aortic CDs 19 and 20), 26 scans were acceptable for inclusion in the analysis of consistency (inclusion versus exclusion for subsequent read). Of those excluded for this analysis, one was a chest scan accidentally loaded. Two scans were not read by one of the readers (JH). Another scan included one vertebra that was read by one reader (AP) and not by another (JH). Of 26 scans which included the measurement of 103 vertebrae (of 108 possible; five vertebrae were not considered valid for reading by each of the readers) the mean variability between readers was 4.97%.

Of the scans read, the BMD values procured by Hyder were greater than the BMD values procured by Papa for 30 vertebrae, was less than the values procured by Papa for 73 vertebrae. Per subject, the estimates by Hyder were, on average, greater than those by Papa for 5 of 26 subjects. In no case were differences significant.

Together these results suggest that there is excellent reader agreement on these 26 scans between Hyder and Papa. The results also suggest the estimates by Hyder tended to be lower than those of Papa. Agnes Papa will perform additional data reads used for the present analyses. AP and JH discussed ROI technique and AP suggested the use of automated ROI unless recordable vertebral pathology or error were noted.

June 12, 2004

JH re-reads MESA Aortic CDs 19 and 20 and 10% sub-sample of MESA Aortic data. Of the scans re-read against AP initial scans from May 20, 2004, agreement on inclusion versus exclusion was 100% agreement between readers; variability was <5%, and the BMD values procured by Hyder varied by less than 5% for combined vBMD values over all vertebrae.

**4) Procedures of data quality control and assurance**

Initial note on blinding

The QCT 3D PLUS provides a lateral and anterior-posterior projections of the abdomen, including the spine. Such projections may allow the reader of bone density to see arterial calcification in the aorta, which raises the issue of reader bias. For data collection, the reader loaded and viewed each CT image two times – first to read aortic calcium and then to read BMD. The reads were never performed consecutively, given that different software environments were necessary for Agatston score and bone density measurements. Rather, days or weeks separated the viewing and reading of scans from each other as sets of data were read in batches first for aortic calcium and, when one or multiple incoming data batches were completed, then images were reloaded and read for BMD. Although some evidence of aortic calcium is apparent during BMD reads, the reader was not aware of a subjects' specific aortic calcium score or the hypothesis or directionality of association under investigation. Further, the reader for BMD and aortic calcium was not responsible for coronary calcium reads or measures of ABI or carotid atherosclerosis.

Scan Re-reads

Beginning 06/2004, 25 scans, or about 100 vertebrae attempted reads, were randomly selected to be read a second (10/2004) and third time (07/2005). Scans were loaded by someone (ER) other than the scan reader (AP). The scan reader was not aware of this serial repeat of subjects.

Tests of Data Quality over Time

The data from repeat scans were investigated to learn the following:
1) Were reader decisions about if and why a vertebra was or was not acceptable for measurement were consistent?
2) Was there evidence of "data drift" in bone density values over time.

Inspection of 100 paired repeated reads revealed that two vertebrae in the 10/2004 read were considered unreadable because of inadequate image size and two other vertebrae were excluded because inadequate image data resulted in negative values for BMD. In the 07/2005 read, the same exclusions were noted for the same vertebrae. This level of agreement makes formal tests of agreement unnecessary. Although only four of 100 vertebrae were excluded, these results provide considerable evidence for consistency of exclusion.

To investigate reader drift in bone density measurements over time, two approaches were employed. The more traditional approach used repeated measures ANOVA to test for within-subject effects for each of the vertebra (L2 ... L5) measures. Repeated measures ANOVA carries the standard set of assumptions associated with an ordinary analysis of variance, extended to the matrix case: multivariate normality, homogeneity of covariance matrices, and independence. Within-subject effect can be tested with a univariate or multivariate approach with the univariate approach favored because of greater power. The univariate approach to tests of the within-subject effects requires the assumption of sphericity – a common spherical covariance matrix. Testing of this assumption with Mauchley's test on the orthogonal components of the transformed variable (three levels of time) resulted in the rejection of the assumption of sphericity

(Chi-square 9.1; 2 degrees of freedom, $p<0.02$). Consequently the multivariate approach was employed. The Wilks' test of time effect yielded an F-equivalent of 0.18 with an equivalent 18 degrees of freedom for a p-value of 0.839 indicating no time effect in the data.

| Spinal Site | Univariate p-value | Multivariate p-value |
|---|---|---|
| L2 | NA | 0.839 |
| L3 | NA | 0.526 |
| L4 | 0.481 | (0.405) |
| L5 | NA | 0.395 |

A more sensitive approach to investigating reader drift used 12 paired t-tests for difference in BMD between reads. The 12 tests represented three comparisons (T1 vs T2, T1 vs T3, T2 vs T3) for each of four vertebrae (L2, L3, L4, L5). For the 12 tests, the greatest mean difference was 1.42 and the least mean difference was -2.4. Six mean differences were positive and six were negative. None of the differences were significantly different (least p-value=0.09).

In conclusion, there is no evidence of systematic reader error over time in bone density measurements. This is likely the result of the standardized protocol, an experienced reader, and the automated placement of the region of interest in the software package.

**5) CT model and bone density**

From the below table, presenting age, race and weight adjusted mean bone density values asccording to CT model, CT model is significantly associated with bone density in both men and women. Comparing rank of bone density values, we see that the patterns of high and low bone density are different for each sex, suggesting that CT model is unlikely to introduce serious bias in the bone-atherosclerosis relationships under investigation.

This suspicion was confirmed when adjustment for CT model did NOT alter any relationships between bone and atherosclerosis under investigation.

| | MEN | | | WOMEN | | | All | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | BMD* | Rank | N | BMD* | Rank | N | BMD | Rank |
| CT MODEL | p=0.01 | | | p=0.02 | | | | | |
| Cardiac Prevention | 1 | 105 | 7 | 1 | 74 | 7 | 2 | 91 | 7 |
| Imatron C-150 | 524 | 121 | 5 | 538 | 114 | 3 | 1062 | 117 | 3 |
| LightSpeed Plus | 70 | 112 | 5 | 70 | 116 | 2 | 140 | 116 | 4 |
| LightSpeed Pro 1 | 121 | 132 | 2 | 137 | 118 | 1 | 158 | 125 | 1 |
| Sensation 16 | 86 | 122 | 3 | 80 | 104 | 5 | 166 | 112 | 6 |
| Sensation Cardiac | 29 | 140 | 1 | 19 | 102 | 6 | 48 | 123 | 2 |
| Volume Zoom | 131 | 122 | 3 | 100 | 109 | 4 | 231 | 116 | 4 |
| Missing | 3 | x | x | 3 | x | x | 6 | x | x |

*Adjusted for age, race, weight, smoking (cig1c) and SEX

In separate analyses, CT model was also associated with ankle-brachial index after multiple adjustment. In sensitivity analyses, though, adjustment for site or CT model did not change the associations between bone density and atheroclerosis in Chapters 2 and 3. In conclusion, CT model is not likely responsible for spurious associations between bone density and atherosclerosis in these MESA-AACS reports.

```
proc freq;title'models and counts';
where bdl3 ne .;
tables gender1*modela/missing;run;quit;
proc glm;title'is site assoc with bmd - men';
where gender1=0;
```

```
class age5cat race1c cig1c modela;
model bdl3=age5cat race1c wtlb1  cig1c  modela;lsmeans
modela/pdiff;
run;quit;
proc glm;title'is site assoc with bmd - women';
class age5cat race1c cig1c modela;
model bdl3=age5cat race1c wtlb1  cig1c  gender1
modela;lsmeans modela/pdiff;
run;quit;
proc glm;title'is site assoc with bmd - ALL';
class age5cat race1c cig1c modela;
model bdl3=agexc race1c wtlb1  cig1c  gender1
modela;lsmeans modela/pdiff;
run;quit;
```

**6) Bone data and exclusions**

Bone density data as of April 17, 2006

Includes all bone density reads made as part of the MESA. Some missing bone data result from duplicate ID numbers.

| | Total | Men | Women |
|---|---|---|---|
| MESA Study | 6934 | 3213 | 3601 |
| MESA Aortic | 1968 | 994 | 974 |

| | **L2** | **L2** | **L2** | L3 | L3 | L3 | **L4** | **L4** | **L4** | L5 | L5 | L5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Total** | **Women** | **Men** | Total | Women | Men | **Total** | **Women** | **Men** | Total | Women | Men |
| Reason | **Not read** | **Not read** | **Not read** | Not read | Not read | Not read | **Not read** | **Not read** | **Not read** | Not read | Not read | Not read |
| Missing | 18 | 11 | 7 | 18 | 11 | 7 | 18 | 11 | 7 | 18 | 11 | 7 |
| NR- Not on screen | 7 | 0 | 7 | 2 | 1 | 1 | 0 | 0 | 0 | 15 | 6 | 9 |
| NR- Fracture/Compression fracture | 11 | 7 | 4 | 8 | 3 | 5 | 12 | 4 | 8 | 19 | 6 | 13 |
| NR- Metastatic lesion | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| NR- Osteophytes | 4 | 2 | 2 | 5 | 3 | 2 | 5 | 3 | 2 | 9 | 5 | 4 |
| NR- Bone islands | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NR- Density variations | 5 | 3 | 2 | 5 | 1 | 4 | 6 | 4 | 2 | 9 | 5 | 4 |
| NR- Other Pathology | 7 | 3 | 4 | 6 | 3 | 3 | 13 | 7 | 6 | 28 | 14 | 14 |
| NR - Metal in vertebral bodies | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 3 | 3 | 0 |
| NR - Phantom not visible | 7 | 4 | 3 | 11 | 6 | 5 | 9 | 6 | 3 | 6 | 3 | 3 |
| Read - None errors | 5 | 2 | 3 | 2 | 0 | 2 | 3 | 3 | 0 | 10 | 7 | 3 |
| Read - Image cut off | 142 | 19 | 123 | 1 | 0 | 1 | 1 | 0 | 1 | 59 | 0 | 59 |
| Read - Cut off phantom | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total Not Read | **207** | **52** | **155** | 59 | 28 | 31 | **70** | **41** | **29** | 176 | 60 | 116 |
| Total Read | **1761** | **922** | **839** | 1909 | 946 | 963 | **1898** | **933** | **965** | 1792 | 914 | 878 |

APPENDIX TWO

Title: **Low lumbar bone density is strongly associated with systemic calcified atherosclerosis**

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**ABSTRACT**

Background: Limited information exists on the extent of any association between calcified atherosclerosis and bone mineral density (BMD). We tested associations between lumbar BMD and the extent of arterial calcification in multiple vascular beds.

Methods: Electron beam computed tomography was performed on 233 female and 123 male consecutive asymptomatic subjects to measure the prevalence of atherosclerotic calcification in the carotids, coronaries, and iliac arteries, and the aorta as well as BMD in the third lumbar vertebra.

Results: The mean age of the cohort was 55.9 years. The prevalence of any arterial calcium was lower in women than men for the carotids (21%, 33%), coronaries (38%, 75%), aorta (56%, 60%) and iliac (45%, 61%) arteries, respectively. In sex-specific logistic regression models adjusting for age, smoking, hypertension, cholesterol, diabetes and hormone therapy (women only), men with a bone density below the median had significantly higher odds of any carotid (odds ratio [OR], 2.9; 95% confidence interval [CI] 1.02-8.0) or aortic calcium (OR, 5.9; CI 1.8-19.6). After the same adjustment, women with bone density below median had significantly greater odds of aortic (OR, 2.6; CI 1.2-5.3) or iliac (OR, 2.2; CI 1.1-4.3) artery calcium. In men, bone density remained significantly associated with aortic calcium after adjustment for coronary and carotid calcium (OR=5.9; 1.7-20.9).

Conclusions: This study offers evidence for a robust, independent association between bone density and peripheral, but not coronary artery calcium. Low bone density may be a marker for advanced, peripheral atherosclerosis in men and women.

**Introduction**

Both atherosclerosis and osteoporosis are responsible for significant morbidity and mortality,(Stafford, Drieling et al. 2004) are independent predictors of cardiovascular disease (CVD) events(Kado, Browner et al. 2000; Jorgensen, Engstad et al. 2001), and may share common regulatory mechanisms(Mody, Parhami et al. 2001; Tintut and Demer 2001; Adami, Braga et al. 2003) as well as histopathology(Demer and Tintut 2003). Multiple reports of weak or null relationships(Taylor, Feuerstein et al. 2001; Allison, Wright et al. 2003) between traditional CVD risk factors and calcified atherosclerosis has heightened interest in novel predictors of arterial calcium. One such hypothesis is for an inverse relationship between bone mineral density (BMD) and calcified coronary atherosclerosis.(Doherty, Asotra et al. 2003) Although contrary findings have been reported(Drinka, Bauwens et al. 1992; Aoyagi, Ross et al. 2001), the majority of cross-sectional(Jie, Bots et al. 1996; Barengolts, Berman et al. 1998; Hak, Pols et al. 2000; Kiel, Kauppila et al. 2001; Jorgensen, Joakimsen et al. 2004; Schulz, Arfai et al. 2004) and all prospective studies(Hak, Pols et al. 2000; Kiel, Kauppila et al. 2001; Schulz, Arfai et al. 2004) of this association have demonstrated a significant inverse association between arterial calcium deposits and bone mineral density. The few studies that include men are equivocal, and, to date, no study has investigated the relationship between BMD and systemic arterial calcification.

The aim of this study was to test the hypothesis that lumbar BMD would be significantly associated with the extent of arterial atherosclerotic calcification in the carotid, coronary, and iliac vascular beds as well as the aorta.

**Methods**

Participants

From February 2001 to July 2002, 365 consecutive patients who were free of clinical CVD (myocardial infarction, stroke, transient ischemic attack [TIA], coronary revascularization [CABG, PTCA or stent] or carotid artery surgery) and presented for preventive medicine services at a university affiliated disease prevention center in San Diego, California, were evaluated for the extent of calcified atherosclerosis in four different vascular beds: the carotid, coronary, and iliac vessels and the aorta (thoracic and abdominal). Most subjects were self-referred or referred on the advice of their primary care provider.

Participants completed a detailed health history questionnaire that collected information on history of hypertension, diabetes, high cholesterol, smoking, medications, family history of coronary heart disease, diet, exercise and prior surgeries. The study protocol was approved by the Human Research Protection Program at the University of California at San Diego, which granted a waiver of informed consent. Hormone therapy was limited to those taking medications containing estrogen.

Imaging

All patients underwent computed tomography (CT) imaging with an Imatron C-150 scanner. Images were obtained from each subject during a single session using a 100-millisecond scan time and proceeding caudally from the base of the skull to the symphysis pubis. Each bed was interrogated with a distinct scan of the segment in question using the following scan thicknesses: 3 mm for the coronary bed, 6 mm for the neck, abdomen and pelvis, and 5 mm for the thorax. Cardiac tomographic imaging was electrocardiographically triggered at 40% or 65% of the R-R interval, depending on the subject's heart rate. Imaging of the heart, thorax, and abdomen was conducted during separate breath-holds at half-maximal inspiration.

Arterial beds were grouped in the follow way: "Carotid" refers to the sum of left and right carotid artery values; "Coronary" refers to the sum of left main, left anterior descending, left circumflex and right coronary artery values and "Aortic" refers to the sum of values from the root of the aorta to the iliac bifurcation. "Iliac" refers to the sum of values from the left and right iliac arteries.

Atherosclerotic calcification was defined as a plaque of $\geq$ 1 mm with a density of $\geq$130 Hounsfield units. Quantitative calcium scores were determined according to the method described by Agatston and colleagues.(Agatston, Janowitz et al. 1990) Calcium scores for vascular beds other than the coronaries were adjusted for slice thickness using the following formula: Adjusted score=original score X slice thickness /3.0. Volume averaging was avoided by scoring each homogeneous slice-thickness segment separately. Calcium scoring of all beds was performed by one of us (M.A.A.) who had specific training for the methodology described.

Bone mineral density was assessed as mg/cm^2 using qCTPro (Mindways Software, Inc: San Francisco, CA.) The third lumbar vertebra (L3) was selected for measurement if it showed no evidence of previous fracture or compression fracture, metastatic lesion, osteophytes, bone islands, severe density variations or other pathology that would distort bone density measurement. The bone density reader was blinded from the results of arterial calcium scoring.

Laboratory

All subjects had blood pressure measured while in the seated position and after resting for 5 minutes. Casual serum lipid and glucose measurements were obtained by fingerstick using the Cholestec LDX® system. Weight was assessed with the patient lightly clothed and without shoes. Body mass index was calculated as the weight (kg) divided by the height ($m^2$).

Statistical Analyses

Smoking status was defined as current, former or never. Hypertension was defined as systolic or diastolic blood pressure > 140 or > 90 mmHg respectively, or current use of anti-hypertensive medication combined with a physician diagnosis of this condition. Diabetes was defined by current use of anti-glycemic medications or a casual serum glucose > 200 mg/dL. Individuals with a total cholesterol to HDL ratio greater than 5 or who reported using a medication for this condition were classified as dyslipidemic. Bone density was categorized into low and high for each sex based on median values obtained from the third lumbar vertebra. Arterial calcium was noted as present or absent. The analyses were stratified by sex given the significantly different

distributions of calcified atherosclerosis(Allison, Criqui et al. 2003) and patterns and determinants bone loss in men and women.(Riggs, Khosla et al. 2002) Generalized linear models were used to compare differences in means and to estimate age-adjusted prevalence of arterial calcium for each sex. Relative odds of any arterial calcium were estimated using multiple logistic regression models for each sex. Multiple variable forms for age including continuous linear age, age decade and age quartiles were explored in bivariate models to find forms most appropriate to control for confounding. Sex-specific age quartiles were used for final models. Analyses included investigation of model assumptions, regression diagnostics and interactions with arterial calcium. Odds ratios with 95% confidence intervals are reported. Associated p-values were not adjusted for multiple comparisons. All analyses were performed using SAS Version 8.3e (Cary, North Carolina.)

**Results**

The clinical characteristics of the 123 male and 233 female participants are presented in Table 1. Men and women in the study sample were middle-aged (mean age of 55.9 for both men and women) and predominantly non-Hispanic White. In the sample, 25.2% of men and 17.6% of women had been previously diagnosed with hypertension. Only 2.4% of men and 2.6% of women had been diagnosed with diabetes. Previous or current estrogen therapy was reported among 15% of women. Among men, the prevalence of calcified atherosclerosis was greatest in the coronary arteries (75%), iliacs (61%) , aorta (60%), and carotid (33%). In women, calcified atherosclerosis was most common in the aorta (56%), iliacs (45%), coronary (38%),

and carotid (21%). Bone density (mg/cm^2) did not differ between men and women in this sample (p=0.90).

Age-adjusted distributions of clinical characteristics by sex and bone density are presented in Table 2. In both sexes, those with lower bone density values were older (mean difference of 10 years, p<0.01 for both). Men with lower bone density did not differ significantly from men with higher bone density in terms of BMI, total cholesterol:HDL cholesterol ratio, hypertension, previous smoking or diabetes. Among women, those with lower bone density had significantly greater mean total cholesterol:HDL ratio (3.8 versus 3.3, p=0.02) and lower prevalence (n (%)) of hypertension (12.5% versus 22.5%, p=0.07). The prevalence of arterial calcium was greater for men with lower BMD in all arterial beds. These differences were significant for the aorta, marginally significant for the carotid arteries and iliacs (p=0.06 for both), and not significant for the coronary arteries (p=0.33). Similarly, arterial calcium was more common among women with lower BMD for all beds and significant at the p<0.05 level for the aorta and iliacs but of borderline significance for carotid (25% vs 17%, p=0.12) and coronary (43% vs 33%, p=0.13) beds.

In sex-specific logistic regression models adjusting for age (Table 3), lower bone density was significantly associated with increased odds of arterial calcium in the aorta in men and with increased odds of arterial calcium in the aorta or iliacs in women. In men, after further adjustment for hypertension, diabetes, smoking and total cholesterol: HDL cholesterol ratio, the odds of any arterial calcium was significant for the coronary arteries (OR=2.85; 1.02-7.96) and aorta (OR=5.90; 1.78-19.6). Except

for age, lower bone density was the only significant predictor of arterial calcium in models of carotid calcium in men. Among women, adjustment for hormone therapy, hypertension, diabetes, smoking and total cholesterol: HDL cholesterol ratio had little effect on the magnitude or significant associations between low bone density and calcified atherosclerosis. Further adjustment for aspirin use in men and women did not affect the results (not shown). Additional adjustment for BMI did not alter the results in either sex.

To test the robustness of associations between a lower bone density and presence of an arterial calcium, separate logistic regression models for any carotid, coronary, and aortic calcium were adjusted for age, hypertension, total cholesterol:HDL cholesterol ratio, diabetes as well as presence of calcium in other arterial beds. Iliac calcium was not included because of high co-linearity with aortic calcium. In men, lower bone density remained significantly associated with aortic calcium (OR=5.9; 1.7-20.9) after full adjustment for including adjustment for presence of calcium in the carotid and coronary beds. In all other models for men and women, associations between bone density and presence of arterial calcium were uniformly positive but not statistically significant with adjustment for presence of calcium in other arterial beds.

**Comment**

This investigation identified consistent, significant inverse associations between lumbar BMD and atherosclerotic arterial calcium. The magnitude of these associations varied by vascular bed with the strongest associations found for the aorta.

On multivariable adjustment, lower bone density was significantly associated with calcified atherosclerosis in some cases where traditional risk factors for CVD, such as hypertension and total cholesterol:HDL cholesterol ratio, were not. Specifically, this was the case for aortic calcium in men and coronary calcium in women. Among men, the inverse association remained significant for aortic calcium even after adjustment for arterial calcium at other sites.

With few exceptions,(Doherty, Fitzpatrick et al. 2004) findings of arterial calcium on CT are the consequence of atherosclerosis.(Agatston, Janowitz et al. 1990) Evidence indicates that calcified atherosclerosis, including coronary calcium, is modestly associated with traditional CVD risk factors other than age and sex,(Taylor, Feuerstein et al. 2001; Allison, Wright et al. 2003; Allison and Wright 2005) but is incrementally predictive of CVD mortality after standard adjustment for traditional CVD risk factors.(Kondos, Hoff et al. 2003; Greenland, LaBree et al. 2004; Pletcher, Tice et al. 2004) Thus arterial calcium may be a marker for atherosclerotic processes that are not explained by traditional risk factors but nonetheless contribute to CVD morbidity and mortality.(Witteman, Kok et al. 1986; Wilson, Kauppila et al. 2001; McClelland, Chung et al. 2006)

The findings presented here suggest that low bone density is a marker for similar processes and expand on a growing body of evidence supporting an age-independent relationship between low bone density and calcified atherosclerosis. As in the present study, previous cross-sectional investigations in men have detected associations between bone density and calcified carotid atherosclerosis,(Jorgensen,

Joakimsen et al. 2004) but not coronary atherosclerosis.(Bakhireva, Barrett-Connor et al. 2005) In contrast to the findings presented here, a prospective study of 190 men found no association between BMD of the spine or metacarpals and aortic calcium, but used plain X-rays to assess the latter.(Kiel, Kauppila et al. 2001)

The majority of previous studies have used methods less sensitive than CT to measure arterial calcium and/or bone density, and none have simultaneously investigated multiple arterial sites. Three reports have demonstrated that bone density is associated with carotid atherosclerosis as assessed by intima-medial thickness or echogenicity of plaques by ultrasound.(Uyama, Yoshimoto et al. 1997; Jorgensen, Joakimsen et al. 2004; Kammerer, Dualan et al. 2004) To our knowledge, three previous studies, all including women only, have used CT methods to investigate the relationship between bone and arterial calcium, and their results are generally consistent with the findings presented here. Specifically, Schulz and colleagues(Schulz, Arfai et al. 2004) found that lumbar bone density was associated with aortic calcium in both cross-sectional and prospective data. Two reports with a combined sample of 231 women demonstrated significant inverse relationships between coronary artery calcium and bone density.(Barengolts, Berman et al. 1998; Bakhireva, Barrett-Connor et al. 2005) Although bone density was not significantly associated with coronary calcium in the present analyses, analytic methods to detect risk factors for coronary calcium vary because of the skewed distribution of scores.(Reilly, Wolfe et al. 2004) The approach used in the present analysis may not

be the most sensitive but is more robust than methods used in the previous two investigations with significant findings.

In this study, the strongest relationships between lumbar bone density and atherosclerosis were found at arterial sites that give rise to the lumbar arterial supply the lumbar spine. Although speculation on this finding is limited by the magnitude of difference across arterial beds, experiments in animal models and tissue culture have demonstrated that calcium in skeletal sites and atherosclerotic lesions share regulatory factors.(Demer and Tintut 2003) One of these factors, oxidized low density lipoprotein cholesterol (LDL), is known to promote arterial calcification while inhibiting calcification of bone.(Parhami, Morrow et al. 1997) Thus, metabolites from extensive, calcified atherosclerotic lesions may pass downstream and affect bone density locally more so than systemically.

Other aspects of the epidemiology of calcified atherosclerosis may explain the link between bone and arteries. Specifically, the aorta and iliac arteries generally develop calcium earlier than the coronary and carotid arteries, and do so in greater amounts.(Allison, Criqui et al. 2003) Specific reasons for earlier calcified disease in the larger vessels are not clear, although the independent effect of greater surface area, and thus greater opportunity for any calcification, in these larger vessels is currently being investigated in the Multi-Ethnic Study of Atherosclerosis.(Bild, Bluemke et al. 2002) Regardless, the higher prevalence of calcified disease and greater range of calcium scores in the aorta compared to coronary arteries in general provides greater statistical power for detecting significant associations.

The present study benefits from the use of an asymptomatic-for-CVD sample of men and women, as well as whole-body CT for quantification of calcium from multiple arterial beds as well as bone mineral density. Limitations of our study include the cross-sectional design and relatively small sample size, which limit speculation on local effects or sex-differences in the relationship between bone and calcified atherosclerosis, as well as firm conclusions about hypothesized role of lipids(Parhami, Basseri et al. 2002) or hypertension,(Cappuccio, Meilahn et al. 1999) In conclusion, we show that bone density, a common clinical screening procedure for osteoporosis, is a strong marker for systemic calcified atherosclerosis.

**Acknowledgements**

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**Table 1. Characteristics of participants**

| | MEN | WOMEN |
|---|---|---|
| N | 123 | 233 |
| Age, mean (SD) y | 55.9 (12.2) | 55.9 (10.4) |
| Body mass index, mean (SD) kg/m^2 | 26.5 (4.9) | 25.9 (5.0) |
| Total cholesterol/HDL cholesterol, mean (SD) | 4.8 (1.5) | 3.6 (1.4) |
| Hypertension, No. (%) | 31 (25) | 41(18) |
| Ever Smoker, No. (%) | 45 (37) | 95 (41) |
| Diabetes, No. (%) | 3 (2.4) | 6 (2.6) |
| Estrogen Use, No. (%) | - - - | 35 (15) |
| Bone density, mean (SD), mg/cm^2 | 136 (37) | 137 (38) |
| Arterial calcium prevalence, N (%) | | |
| Carotid arteries | 41 (33) | 49 (21) |
| Coronary arteries | 92 (75) | 88 (38) |
| Aorta | 74 (60) | 130 (56) |
| Iliac arteries | 75 (61) | 104 (45) |

*Body mass index values are from 121 male and 228 female participants.

**Table 2. Age-adjusted characteristics of study participants by sex and bone density category.**

| | MEN | | | WOMEN | | |
|---|---|---|---|---|---|---|
| **Variable** | **Greater BMD** | **Lower BMD** | **p-value** | **Greater BMD** | **Lower BMD** | **p-value** |
| Age, mean (SE) y | 50.6±1.4 | 61±1.4 | <0.001 | 51.2±0.9 | 60.7±0.86 | <0.001 |
| Body mass index, mean (SE) kg/m^2 | 25.8 ±0.7 | 27.2±0.7 | 0.16 | 26.0±0.5 | 25.8±0.5 | 0.81 |
| Total cholesterol:HDL cholesterol, mean (SE) | 4.7 ±0.2 | 4.8±0.2 | 0.78 | 3.3±0.1 | 3.8±0.1 | 0.02 |
| Hypertension , %±SE | 30±6 | 20±6 | 0.26 | 23±4 | 12±4 | 0.07 |
| Ever Smoker, %±SE | 32±6 | 41±6 | 0.28 | 40±5 | 42±5 | 0.78 |
| Diabetes, %±SE | 2.6±2 | 2.2±2 | 0.90 | 1.9±1.5 | 3.3±1.6 | 0.56 |
| Estrogen Use, %±SE | - - - | - - - | - - - | 16±3 | 14±4 | 0.63 |
| Bone density, mean (SE), mg/cm^2 | 163.8±2.9 | 109.0±3.0 | <0.001 | 162±2.2 | 110±2.3 | <0.001 |
| Arterial calcium prevalence, %±SE | | | | | | |
| Carotid arteries | 25±5 | 40±5 | 0.06 | 17±4 | 25±4 | 0.12 |
| Coronary arteries | 71±5 | 79±6 | 0.33 | 33±4 | 43±4 | 0.13 |
| Aorta | 48±5 | 73±5 | 0.001 | 45±4 | 68±4 | <0.001 |
| Iliac arteries | 53±6 | 69±6 | 0.06 | 35±4 | 55±4 | 0.003 |

*Values other than age are adjusted for age. Before adjustment for age, prevalence of arterial calcium for each site was greater among those with lower BMD (Chi-square test, all p-values less than 0.01).
Greater BMD = BMD above the median, Lower BMD = BMD below the median

**Table 3 Sex-specific odds ratios (OR; 95% CI) of calcium in a given arterial bed associated with BMD lower than median. Results are adjusted for 1) age and 2) for age, total cholesterol:HDL-cholesterol ratio, hypertension, smoking history, diabetes status and hormone therapy (women only).**

| | MEN | | WOMEN | |
|---|---|---|---|---|
| | **Age-adjusted** | **Fully adjusted*** | **Age-adjusted** | **Fully adjusted*** |
| **Site** | **OR(95%CI)** | **OR(95%CI)** | **OR(95%CI)** | **OR(95%CI)** |
| Carotid arteries | 2.42 (0.93-6.27) | 2.85 (1.02-7.96) | 1.84 (0.84-4.04) | 1.97 (0.86-4.50) |
| Coronary arteries | 1.62 (0.61-4.30) | 1.72 (0.62-4.79) | 1.63 (0.86-3.09) | 1.51 (0.77-2.95) |
| Aorta | 4.71 (1.74-12.72) | 5.90 (1.78-19.6) | 3.13 (1.63-6.03) | 3.14 (1.55-6.38) |
| Iliac arteries | 2.35 (0.95-5.76) | 2.31 (0.86-5.93) | 2.42 (1.30-4.49) | 2.20 (1.13-4.29) |

**Adjusted for age, total cholesterol:HDL-cholesterol ratio, hypertension, smoking history, diabetes status and hormone therapy (women only).**

The text of Appendix II will be submitted in part as,

Hyder, J. A., Allison, M. A., Criqui, M. H., Wright C. M. “Low lumbar bone density is strongly associated with systemic calcified atherosclerosis,” 2006.

# APPENDIX TWO REFERENCES

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