

**THE ASSOCIATION OF BONE MINERAL DENSITY WITH
CARDIOVASCULAR DISEASE**

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Cardiovascular disease (CVD) and osteoporosis are common age-related conditions with major public health impact. Mounting evidence suggests a link between the two diseases.

The purpose of this research was to investigate the association of bone mineral density (BMD) measures (areal and volumetric) with prevalent CVD, incident CVD, and subclinical measures of atherosclerosis.

We utilized data from two prospective epidemiological studies: a) the Health, Aging, and Body Composition (Health ABC) study that enrolled a population of older men and women (age 68-80 years, 51% women, 42% black), and b) the Study of Women's Health Across the Nation (SWAN) that followed a cohort of women through the menopause transition (age 45-58 years, 61% white, 64% peri-menopausal).

In the cross-sectional Health ABC analysis, lower volumetric BMD (vBMD) measures of the spine were associated with higher CVD prevalence in men and women, and areal BMD (aBMD) of the trochanter was related to CVD in women. Additionally, aBMD of the total hip was related to subclinical peripheral arterial disease in men.

In the SWAN analysis, we observed an inverse cross-sectional association between trabecular vBMD of the spine and aortic calcification. Meanwhile, no associations with coronary artery calcification were noted after adjusting for age.

In the longitudinal Health ABC analysis, lower vBMD measures of the spine were associated with higher CVD incidence in white men, but not in blacks. In women, aBMD of the femoral neck was associated with incident CVD in the full cohort. In race-specific analyses, aBMD measures of the total hip, femoral neck, and trochanter exhibited significant relationships with incident CVD in black women, but not in whites.

These relationships were independent of age and shared risk factors between osteoporosis and CVD. The inflammatory cytokines interleukin-6 and tumor necrosis factor-alpha, oxidized LDL, and endogenous estradiol did not explain these associations.

Overall, our findings provide epidemiological evidence for the presence of an inverse association between BMD and CVD. An understanding of the common mechanisms underlying bone loss and atherogenesis has significant public health implications as it may set the stage for dual-purpose preventive and therapeutic interventions that target both osteoporosis and CVD.

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1.0 DISSERTATION OVERVIEW AND OBJECTIVE

Cardiovascular disease (CVD) and osteoporosis are common age-related conditions. Mounting biological [1-6] and epidemiological evidence supports a link between the two diseases. Low bone mineral density (BMD) was related to increased cardiovascular mortality, [7-12] cardiovascular morbidity, [13-19] and subclinical measures of atherosclerosis [20-40] in cross-sectional as well as longitudinal epidemiologic studies.

The objective of this dissertation was to examine the association of BMD measures with prevalent CVD, incident CVD, and subclinical measures of atherosclerosis; and to investigate potential explanations for the link between low BMD and cardiovascular disease. Specifically, the following research questions were addressed in a series of three research articles:

1. Are BMD measures associated with prevalent CVD, incident CVD, and subclinical measures of atherosclerosis?
2. Are these associations:
 - a) Independent of age?
 - b) Independent of shared risk factors between osteoporosis and CVD?
 - c) Explained by common pathophysiological factors such as inflammatory cytokines, oxidized lipids, or endogenous estradiol?

These research questions were investigated in two prospective epidemiological studies: the Health, Aging, and Body Composition (Health ABC) study that enrolled a population of

older men and women, and the Study of Women's Health Across the Nation (SWAN) that followed a cohort of women through the menopause transition.

2.0 BACKGROUND

2.1 OSTEOPOROSIS

2.1.1 Burden of Osteoporosis

Osteoporosis is defined as “a systemic skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures”. [41]

Osteoporosis is the most common metabolic bone disease and constitutes a major public health problem. In the United States today, it is estimated that 10 million individuals aged 50 years and older have osteoporosis while an additional 34 million have osteopenia or low bone mass, and are therefore at increased risk for the disease. [42] Estimates from the third National Health and Nutrition Examination Survey (NHANES III, 1988–1994) indicate that the prevalence of osteoporosis at different femoral regions (total femur, trochanter, intertrochanter, and femoral neck) in women aged 50 years and older is 13–18% and the prevalence of osteopenia is 37–50%. Men of the same age group have lower, albeit considerable, rates for osteoporosis (3–6%) and osteopenia (28–47%). These rates appear to vary by race. White women have an age-adjusted prevalence of osteoporosis that is 1.5-2.8 times higher than that of blacks. Similarly, white men have a greater prevalence of osteoporosis and osteopenia than black men. [43]

Fractures are the clinical manifestation of osteoporosis. Each year, 1.5 million fractures are attributed to osteoporosis. Of these fractures, approximately 700,000 are vertebral; 300,000

are at the hip; 250,000 are at the wrist; and 300,000 occur at other sites. [42] It is estimated that around 40% of U.S. white women and 13% of U.S. white men 50 years of age will experience at least one fragility fracture in their lifetime [44]. These fractures constitute a significant cause of disability and mortality, and result in substantial medical costs. About 7% of people who suffer from fragility fractures will have some degree of permanent disability, and another 8% will require long-term nursing home care. About 8% of men and 3% of women aged 50 years and older will die during hospitalization for a hip fracture event. Within the first year following a hip fracture, mortality rates are 36% for men and 21% for women. The direct cost attributed to fractures is estimated at \$20 billion per year in the US. [45, 46] Over the next few decades, the overall burden of osteoporosis and osteoporotic fractures is expected to increase dramatically due to population aging.

2.1.2 Pathogenesis of Osteoporosis

The basic pathogenetic mechanisms underlying osteoporosis include: a) failure to achieve optimal peak bone mass during growth and development, b) uncoupling in the bone remodeling process with excessive resorption and decreased formation resulting in net bone loss and architectural deterioration of the skeletal tissue. [47]

Peak bone mass, the maximal bone mass attained in life, is achieved by the age of 20-30 years and is predominantly determined by genetic factors. Heritability accounts for 50-85% of the variability in peak bone mass. [48] Environmental factors also modulate bone growth during childhood and adolescence. Nutrition (calcium and protein intake), physical activity, and gonadal steroids have positive effects on bone mass accrual. Building adequate bone mass and strength during growth can protect against developing osteoporosis later in life. [49]

The adult skeleton undergoes continuous remodeling; a tightly coupled cyclical process whereby osteoclasts (bone resorbing cells) and osteoblasts (bone forming cells) remove and replace small packets of bone at discrete and distinct points throughout the bone tissue. Remodeling begins with the differentiation of hematopoietic stem cells to osteoclasts. Osteoclasts initiate a bone resorption phase by: a) secreting protons that acidify the surrounding extracellular compartment and dissolve the hydroxyapatite crystals, and b) secreting lysosomal enzymes and metalloproteases (collagenase and gelatinase) which digest collagen fibers and the other matrix components. A brief reversal phase follows in which a layer of protein (called cement line) is deposited to facilitate the attachment of the old bone to the newly formed bone. Subsequently, bone formation is achieved by osteoblasts which produce successive layers of the collagenous matrix (osteoid). Mineralization of the osteoid tissue ensues and marks the final step in bone formation. Osteoblasts later on become embedded in the matrix as osteocytes, undergo apoptosis, or become flat lining cells. The complete remodeling cycle takes 3-6 months. [50]

This process of bone remodeling is tightly regulated by a set of complex interactions between systemic and local cytokines, growth factors, and hormones.

The key regulator of osteoclast activity is the RANK/RANKL/Osteoprotegerin (OPG) system. Osteoclastogenesis begins with the differentiation of hematopoietic cells to osteoclasts. Cells of the osteoblastic lineage produce and express RANKL, a membrane-bound ligand for RANK (Receptor Activator of NF- κ B). RANK is expressed by hematopoietic cells, and the RANK/RANKL interaction is required for the differentiation of these cells to osteoclasts. Osteoblasts also produce OPG, a soluble decoy receptor for RANKL that blocks the RANK/RANKL interaction. Thus the balance between RANKL and OPG production determines the rate of bone resorption. [50, 51]

Other cytokines are involved in bone resorption. Interleukin-1 (IL-1) is released by activated monocytes and other cell types such as osteoblasts. It is a stimulator of osteoclast formation and activation. Its effects are mediated through RANKL. Tumor necrosis factor-alpha (TNF- α) is another factor that is functionally similar to IL-1. Interleukin-6 (IL-6) is a pleiotropic cytokine secreted by bone cells in response to hormones (parathyroid hormone (PTH), 1,25 dihydroxyvitamin D), and other cytokines (IL-1 and TNF- α). IL-6 stimulates osteoclast formation and is implicated in estrogen deficiency-related bone loss. In addition to these local factors, systemic hormones including PTH, calcitonin, 1,25 dihydroxyvitamin D, and sex steroid hormones are also involved in osteoclast formation and activation. [50]

The tight coupling of bone formation and resorption is further fostered by osteoclasts regulating osteoblast differentiation and activation, in tandem with the requirement for osteoblasts in osteoclastogenesis. Bone resorption results in the production of transforming growth factor- β (TGF- β) and bone morphogenic protein-2 (BMP-2), both potent stimulators of osteoblastogenesis. Osteoblast progenitor cells migrate to the resorption site and differentiate in response to chemokines secreted by activated osteoclasts such as Mip-1. [50, 51]

Under physiological circumstances, bone formation is coupled to preceding bone resorption so that the amount of bone lost during resorption is replaced during formation. Remodeling imbalance, whereby bone resorption and bone formation are not matched, results in bone loss. Different stressors such as menopause, aging, inflammation, and other disease processes can affect the balance of bone remodeling and result in bone loss.

At the time of menopause, a period of accelerated bone loss begins in which women lose an average of 2%-5% per year of their bone mass. This period lasts for around 5 years. Menopause-related bone loss primarily affects trabecular bone resulting in thinning of the

tabeculae and/or loss of trabecular connectivity. Estrogen deficiency plays a pivotal role in postmenopausal bone loss. Circulating estradiol levels decrease by 90% in the menopausal transition. While the exact mechanism is still unclear, estrogen deficiency is thought to result in increased bone resorption. Estrogen has a direct inhibitory effect on osteoclasts. Moreover, it suppresses the production of potent bone resorbing cytokines (RANKL, IL-1, TNF- α , IL-6) from osteoblasts and monocytes, increases the production of the anti-resorptive OPG, and thereby indirectly inhibits osteoclast function. Estrogen deficiency results in the reversal of these processes leading to osteoclast recruitment and activation.

Age-related bone loss starts in the fourth or fifth decades and continues throughout life. This type of bone loss is slow (0.5% per year); it affects men as well as women; and can occur at trabecular and cortical sites. The slow age-related bone loss is attributed to factors such as increased PTH levels that accompany aging in men and in women. Abnormalities in calcium metabolism (decreased intestinal absorption and renal reabsorption of calcium) that could result from vitamin D deficiency, lead to an increase in PTH levels, which in turn results in bone resorption. Senescence of osteoblasts impairs their function and additionally contributes to age-related bone loss. [52]

In addition to menopause and aging, several other factors have been implicated in bone loss. These include a number of diseases (inflammatory bowel disease, rheumatoid arthritis, celiac disease, lupus, hyperthyroidism, type 2 diabetes mellitus, etc), medications (corticosteroid therapy, anticonvulsants, heparin), and conditions (weight loss and low body weight, immobilization, sedentary life style, alcoholism, smoking, amenorrhea, and family history of osteoporosis). [53]

2.1.3 Assessment of Bone Mineral Density

Measurement of bone mass is the standard method of diagnosing osteoporosis. The World Health Organization has provided diagnostic criteria for osteoporosis and osteopenia based on BMD. Osteoporosis is defined as a value of BMD that is 2.5 or more standard deviations (SD) below the mean for young adults. Osteopenia is defined as a BMD value between 1 and 2.5 SD below the mean for young adults. [54] The value of BMD stems from being a primary predictive risk factor for osteoporotic fractures. In fact, the risk of fractures was found to increase by a factor of 1.5 to 2.6 for every standard deviation decrease in BMD. This predictive ability is paralleled by that of diastolic blood pressure for stroke, and exceeds that of serum cholesterol for predicting coronary heart disease. [55]

BMD may be measured noninvasively using different techniques including: radiographic absorptiometry, single-photon and single X-ray absorptiometry (SPA/SXA), dual-photon and dual X-ray absorptiometry (DPA/DXA), quantitative computed tomography (QCT), quantitative ultrasound, quantitative magnetic resonance, and magnetic resonance microscopy. Currently, DXA and QCT are the only modalities routinely used for measurement of bone density of the central skeleton.

DXA, introduced in 1987, is the most widely used technique for measuring bone density. It is a projectional technique that measures areal bone density (bone mineral content (BMC) divided by area [g/cm^2]) and allows for BMD determination at the lumbar spine, proximal femur, whole body, and peripheral sites. This technique has two main limitations: a) its 2-dimensional areal assessment of BMD which does not adjust for bone size; and b) its sensitivity to extra-osseous calcification such as degenerative osteoarthritic changes and aortic calcification, which get incorporated in the region of interest and lead to a falsely increased bone density of the spine.

[26] This is an important drawback, particularly in the elderly who have an increased prevalence of such degenerative conditions. [56]

QCT is the only three-dimensional bone mass measurement technique available. It can determine volumetric bone density (mg/cm^3), and allows for the differentiation between the 2 types of bone: cortical and trabecular. QCT is performed on standard clinical CT scanners with the addition of: a) an external bone mineral reference phantom for calibration of the CT number measurements to bone-equivalent values; and b) a special software for determination of regions of interest and analysis of image. Compared to DXA, QCT can selectively measure the metabolically active and structurally important trabecular bone in the center of the vertebral body, and is therefore a more sensitive measure of age-related bone loss and response to therapy. In addition, this modality adjusts for bone size and is more accurate in elderly subjects who typically have degenerative changes at the spine. However, QCT is less precise and involves a higher radiation dose than DXA. [57]

2.2 CARDIOVASCULAR DISEASE

2.2.1 Burden of Cardiovascular Disease

Cardiovascular disease is the leading cause of death in both women and men. In 2003, CVD was the underlying cause for 37.3% of all deaths in the United States; that is 1 of every 2.7 deaths was caused by CVD. Older people are at higher risk of CVD. Estimates from the National Health and Nutrition Examination Survey [NHANES 1999-2002] indicate that out of 71.3 million American adults affected with one or more types of CVD, 27.4 millions are age 65 and older. The average annual rate of the first major cardiovascular event in men is 7 per 1,000 at ages 35-

44 and increases to 68 per 1,000 at ages 85-94. In women, similar rates are achieved later in life lagging by around 10 years. [58]

Coronary heart disease (CHD) and stroke account for over 70% of cardiovascular mortality. The incidence rates of CHD are higher in men than in women in all age groups. During a 5-year follow-up in the Cardiovascular Health Study (CHS), the incidence rate of CHD in men was twice that in women (39.2/1000 person years vs. 19.7/1000 person-years). [59] The lifetime risk of developing CHD after age 40 is 49% for men and 32% for women. The age-adjusted incidence rates (per 1000 person-years) by race and gender groups are as follows: 12.5 for white men, 10.6 for black men, 5.1 for black women, and 4.0 for white women. In 2006, the direct and indirect cost of CHD is estimated at \$142.5 billion. [58]

The incidence rate of stroke is 1.25 times higher in men than in women. This difference diminishes with age and disappears in the older age groups. The male-to-female incidence ratio decreases from 1.59 in the 65-69 age group to 0.74 for the 80 and older group. The risk of first stroke is two times higher in blacks than whites. The age-adjusted incidence rates (per 10,000) by race and gender groups are as follows: 323 for black men, 260 for black women, 167 for white men, and 138 for white women. The cost of stroke is expected to reach \$57.9 billion in 2006. [58]

Factors such as increased longevity and the growing number of elderly people are expected to increase the burden of CVD in the future. Estimates from the US Census Bureau indicate that 40 million individuals will be over the age of 65 years in the U.S. in 2010. [60].

2.2.2 Pathogenesis of Atherosclerosis

Atherosclerosis is defined as “a disease of large and medium-sized muscular arteries and is characterized by endothelial dysfunction, vascular inflammation, and the buildup of lipids, cholesterol, calcium, and cellular debris within the intima of the vessel wall. This buildup results in plaque formation, vascular remodeling, acute and chronic luminal obstruction, abnormalities of blood flow, and diminished oxygen supply to target organs”. [61] Obstruction of vessels by atherosclerosis results in inadequate perfusion of tissues supplied by them, and depending on the degree of stenosis, could result in tissue ischemia and infarction. Clinical manifestations of atherosclerosis include CHD, cerebrovascular disease, and peripheral arterial disease (PAD). Atherosclerotic lesions begin with endothelial injury and dysfunction and progress from fatty streaks to fibrous plaques to advanced lesions. [62, 63] Inflammation plays a key role in all stages of the atherosclerotic disease process.

Endothelial injury and dysfunction constitute an early step in the pathogenesis of atherosclerosis. Factors that predispose to endothelial injury include increased cholesterol, reduced HDL, family history of CHD, byproducts of cigarette smoking, diabetes (insulin resistance), hypertension, obesity, hyperhomocysteinemia, high-fat diet, infectious agents, and hemodynamic factors such as turbulence and decreased shear stress. Endothelial dysfunction and inflammation resulting from injury lead to pathophysiologic changes in the endothelium that precede the formation of atherosclerotic lesions. These changes include increased endothelial permeability to plasma constituents including lipoproteins, expression of leukocyte adhesion molecules, migration of leukocytes into the arterial wall, release of cytokines such as TNF- α , interferon- γ , and interleukin-1, decreased availability of antithrombotic and vasodilating

cytokines leading to increased platelet adherence, and the release of growth factors that stimulate smooth muscle cell proliferation. [61-63]

The oxidation of LDL is an important step in the pathogenesis of atherosclerosis. In hypercholesterolemia, excess LDL infiltrates the endothelium and is retained in the intima. This initiates an inflammatory response which leads to oxidative and enzymatic modifications of LDL (the oxidation process modifies a lysine amino acid on the apolipoprotein B). Release of phospholipids from these processes causes the activation of endothelial cells and induces them to express leukocyte adhesion molecules and inflammatory genes. Blood cells (monocytes, lymphocytes) adhere to the site of endothelial activation, and are stimulated by chemokines to migrate through the endothelium into the subendothelial space. The production of the cytokine macrophage colony-stimulating factor in the inflamed media causes monocytes entering from the blood stream to differentiate into macrophages. Different molecules including endotoxins, apoptotic cell fragments, and modified LDL are internalized by scavenger receptors on the surface of macrophages and destroyed. Excess cholesterol derived from the uptake of oxidized LDL accumulates in the cytoplasm of macrophages. This eventually leads to the formation of foam cells. [64]

Fatty streak formation occurs subsequently. A fatty streak is the earliest atherosclerotic lesion. It consists of lipid-laden cells that accumulate beneath the endothelium. These cells include macrophages, T cells, and smooth muscle cells. [63, 64]

Fatty streaks could progress to more advanced lesions via the increased migration and proliferation of smooth muscle cells and accumulation of lipids and connective tissue. The smooth muscle cells deposit an extracellular connective tissue matrix and form a fibrous cap on

top of the lipid rich core that includes foam cells, extracellular lipids, and necrotic cellular debris. The lipid core can become calcified. [62, 63]

The progression of some fibrous plaques may gradually lead to significant stenosis and result in obstruction of blood flow to distal tissues. These are referred to as “stable” lesions. Alternatively, fibrous plaques can become “unstable” and cause tissue ischemia and infarction even before they produce significant luminal narrowing. Endothelial erosion or rupture of the fibrous cap exposes the thrombogenic core of the plaque to the blood, thus activating the clotting cascade and leading to thrombus formation on the surface of the plaque. Thrombus formation may lead to partial or complete occlusion of the blood vessel and can contribute to plaque progression. Molecules produced by activated macrophages, T-cells, and mast cells such as inflammatory cytokines, proteases (matrix metalloproteinase (MMPs) and cysteine proteases), radicals, and coagulation factors can cause destabilization of the plaque by attacking collagen in the cap, inhibiting the formation of a stable fibrous cap, and initiating thrombus formation. [61, 63, 64]

2.2.3 Pathogenesis of Atherosclerotic Calcification

Vascular calcification is the pathologic deposition of calcium phosphate mineral in cardiovascular structures, including blood vessels (arterial calcification). There are two major types of arterial calcification: 1) medial calcification, occurring in the tunica media layer of the arterial wall and associated with vascular stiffness, and 2) intimal calcification, occurring within the intimal layer of the artery and associated with atherosclerosis.

Traditionally, atherosclerotic (or intimal) calcification was considered a passive degenerative process occurring in advanced plaques. However, it is now regarded as an actively

regulated process that can be initiated by several non-mutually exclusive mechanisms including:

- 1) loss of normal mineralization inhibition, 2) apoptosis, 3) circulating nucleational complexes, 4) bone formation, and 5) elevated serum calcium (Ca) and phosphorous (P) levels.

One mechanism that results in spontaneous vascular calcification is the loss of mineralization inhibition. Blood vessels constitutively express inhibitors of calcification such as matrix gla (gamma carboxyglutamate) protein (MGP) and pyrophosphate which prevent mineral deposition in the arterial walls. Dysregulation of these molecules can initiate calcification. Second, apoptotic bodies derived from vascular smooth muscle cells are suggested to provide nucleation sites for apatite deposition when they are not effectively cleared by phagocytic cells. Third, circulating nucleational complexes generated at sites of bone resorption could travel in blood and occasionally lodge in arterial walls where they initiate calcification. Fourth, there is evidence that bone formation occurs inside blood vessels. This is demonstrated by the presence of bone forming machinery in calcified vascular lesions. [Refer to section 2.3.1, Biological Link]. Finally, elevated Ca and P levels can promote the growth of apatite crystals by increasing the $\text{Ca} \times \text{P}$ ion solubility product, and they can trigger calcification by initiating signaling cascades that promote matrix mineralization. [65-67]

Based on biomechanical stress considerations, calcification could initiate or prevent plaque rupture. Early or intermediate stages of calcification were suggested to increase the risk plaque rupture due to the high shear stress exerted on the interface between the calcified and the soft portions of the plaque. However, once calcified plaques coalesce or calcification becomes more extensive, this reduces the interface area, making the plaques more stable and less prone to rupture. [1, 68]

2.2.4 Assessment of Subclinical Atherosclerosis

Several noninvasive surrogate markers of atherosclerosis have been developed over the past few decades to identify subclinical disease in different vascular beds before the occurrence of clinical events. These noninvasive measures include ankle-arm index, coronary artery and aortic calcification determined by electron-beam computed tomography (EBCT), ultrasonographic measurement of carotid intima-media thickness and plaque, pulse wave velocity, brachial artery reactivity testing, cardiac magnetic resonance imaging, left ventricular echocardiography, and noninvasive assessment of coronary flow reserve. [69-72] These measures were shown to predict cardiovascular events. [73-80]

2.2.4.1 Ankle-arm index

The ankle-arm index is a ratio of Doppler-recorded systolic blood pressure in the lower and upper extremities. This noninvasive measure determines arterial narrowing of lower extremity blood vessels and is used as a marker of subclinical peripheral arterial disease (PAD). Epidemiological studies often define abnormal AAI (or low AAI) as a ratio <0.9 . Among community-dwelling men and women 65 years and older and free of clinical CVD, the prevalence of low AAI (<0.9) was reported to be around 10%. [81]

An inverse dose-response association was observed between AAI and the prevalence of subclinical and clinical CVD including MI, stroke, and CHF in older adults. [81] Additionally, low AAI was found to be an independent predictor of incident CVD, [73, 75, 76, 82] recurrent CVD, [76] CVD mortality, [73, 74, 76, 82, 83] and total mortality [73, 74, 76, 82, 83] in different populations.

2.2.4.2 Aortic and coronary artery calcification measured by EBCT

X-ray techniques such as computed tomography (CT) can be used to detect calcification in the coronary arteries and aorta. EBCT is a well-established CT technology for the noninvasive detection of calcification. Calcium load can be quantified using 2 scoring algorithms, Agatston scoring and volume scoring. Agatston scoring is the traditional method and is based on the maximum x-ray attenuation level and the area of the calcified lesion. Volume scoring is based on the number of voxels containing calcium for each region of interest and the volume of one voxel. [84]

Higher CAC was shown to be associated with the prevalence of clinical [85, 86] and subclinical CVD [85] and with the incidence of cardiovascular events [87, 88] and death. [88]

Aortic calcification (AC), measured by radiography, was shown to predict cardiovascular morbidity and mortality. [78, 89, 90] EBCT determined AC was associated with obstructive coronary artery disease on angiography [91, 92] and with CVD in patients with type 2 diabetes. [93]

2.3 LINK BETWEEN OSTEOPOROSIS AND CARDIOVASCULAR DISEASE

2.3.1 Biological Link

Atherosclerotic calcification and bone mineralization share a number of intriguing common features. It is now recognized that calcification of the arterial tissue is not merely a passive process of calcium phosphate precipitation or adsorption in end-stage atherosclerosis, but instead is a highly organized process that is regulated by mechanisms similar to those involved in bone mineralization [1].

The mineral observed in calcium deposits of atherosclerotic plaques has a very similar chemical composition to hydroxyapatite crystals which form the inorganic bone matrix. [3, 4] Calcifiable vesicles were isolated from human atherosclerotic aortas, [94] suggesting that these may be involved in mineral deposition, similar to “extracellular matrix vesicles” that are secreted from chondrocytes and osteoblasts and are involved in initial bone mineralization. Calcified plaques were also shown to express several bone matrix proteins such as type I collagen, gla (gamma carboxyglutamate)-containing proteins such as osteocalcin (bone-gla protein) and matrix gla protein, bone morphogenetic protein (BMP)-2 and -4, osteopontin, osteonectin, and bone sialoprotein. [2, 3, 5, 6] Osteogenic cells, called calcifying vascular cells (CVCs), were identified in atherosclerotic plaques. These are a subpopulation of vascular smooth muscle cells (VSMC) that are capable of osteoblastic differentiation. [3, 95] When stimulated by BMP-2 and BMP-4, these cells begin expressing osteoblast genes including alkaline phosphatase, collagen I, and osteocalcin which are needed for bone formation. Other cells involved in bone metabolism including osteoclast-like cells, chondrocyte-like cells, and hematopoietic bone marrow cells were also seen in plaques. [66]

2.3.2 Epidemiologic Link

2.3.2.1 Bone mass and cardiovascular mortality

Low BMD and bone loss appear to be risk factors for cardiovascular mortality in both women [8, 10-12] and men [7, 9] (Table A-1, Appendix). The Study of Osteoporotic Fractures (SOF) showed that an increase in BMD loss at the hip in the order of one standard deviation (SD) was associated with a 1.3-fold increase in CHD mortality among white women 65 years of age and older. Similarly, calcaneal bone loss was related to increased risk of death due to atherosclerosis

(HR= 1.2, 95%CI= 1.1-1.4) and CHD (HR= 1.3, 95%CI= 1.0-1.6). [10] In the same cohort, lower broadband ultrasound attenuation (BUA) and calcaneal BMD were related to higher cardiovascular death, [8] and decreased BMD of the proximal radius was related to increased risk of stroke mortality (HR= 1.91, 95% CI 1.25-2.92). [12] In a population of Danish women, low bone mineral content in the forearm at the menopause was associated with an increased cardiovascular death later in life (RR= 2.3 per SD decrease in BMD, 95%CI 1.0-4.9). In the same study, a prevalent vertebral compression fracture was independently associated with cardiovascular death in late postmenopausal women (RR= 2.0, 95%CI 1.4-3.3). [11]

Similar results were observed in men. Results from the NHANES I Epidemiologic Follow-up Study indicated that low phalangeal BMD was a significant predictor of subsequent cardiovascular mortality among white men aged 45 to 74 years (RR= 1.16, 95% CI 1.0-1.30). This association, however, was not present in white women or blacks. [7] In another prospective study, low bone density at the hip was found to be a significant predictor of cardiovascular mortality in a cohort of British men aged 65-76 years. [9]

Contrary to the above studies, Mussolino et al. did not find a significant association between BMD and stroke mortality in white men, white women, or blacks in NHANES I. [96]

2.3.2.2 Bone mass and cardiovascular morbidity

A number of studies have investigated the association between BMD and cardiovascular morbidity (Table A-2, Appendix). Results from the Multiple Outcomes of Raloxifene Evaluation (MORE) trial indicated that osteoporosis was a strong predictor of incident cardiovascular events in postmenopausal women independent of age and other traditional cardiovascular risk factors (adjusted HR= 3.9, 95%CI 2.0-7.7). [13] Osteoporosis was also associated with angiographically-determined coronary artery disease in a retrospective analysis of a population

predominantly of women referred for angiography and BMD assessment. [15] A report from the 30 year follow-up of the Framingham study found that metacarpal cortical area (MCA) predicts coronary heart disease in women free from CVD at baseline, with a significant trend of decreasing coronary heart disease risk with increasing MCA (HR for highest vs. lowest MCA quartile= 0.73, 95% CI 0.53-1.00, p for trend= 0.03). No association, however, was observed in men in this study. [16] In SOF, low calcaneal bone mass was significantly associated with stroke incidence (RR= 1.31 per SD, 95%CI 1.03-1.65). [19] In line with these findings, low femoral neck BMD was associated with an increased odds of stroke in women, but not in men, in a Norwegian population. [17]

Associations between BMD and CVD were also reported in men. Previous myocardial infarction was associated with low BMD in a multiethnic population of men in the Third National Health and Nutrition Examination Survey (NHANES III). [14] Additionally, in a study involving 18 men with asymmetrical symptomatic peripheral arterial disease, bone mineral content was shown to be significantly lower in the affected compared to the unaffected leg. [18]

In contrast to the above studies, and consistent with their mortality finding, Mussolino et al found no relationship between BMD and stroke incidence among white men, white women or blacks in NHANES I. [96]

2.3.2.3 Bone mass and subclinical atherosclerosis

An inverse relationship between bone mass and various measures of subclinical disease, especially in women, has been reported by many studies (Table A-3, Appendix). Cross-sectionally, vascular calcification, in both the aorta [20-22, 24, 26, 28] and the coronary arteries [29, 30] was found to be negatively correlated with bone density [20-22, 24, 26, 28] and directly related to vertebral and hip fractures, [20, 21] predominantly in white postmenopausal women.

The progression of aortic calcification was also linked to volumetric trabecular BMD loss in white postmenopausal women, [20] and to metacarpal bone loss in women in the Framingham study and in a Dutch population-based longitudinal study. [23, 24]

Ankle-arm index, was positively correlated with BMD in an elderly population of Chinese men and women [31] and in European postmenopausal women. [32] In SOF, women with the highest decline in AAI were shown to have the largest magnitude of bone loss. [33]

Femoral artery intima-media thickness was negatively related to calcaneal osteo-sono assessment index in a population of Japanese men and women. [34] In another small group of postmenopausal Japanese women, higher carotid plaque score was significantly associated with lower total BMD. [38] Low BMD was also related to echogenic calcified carotid artery plaques in a large population of Norwegian men and postmenopausal women. [35] And in a small case-control study in an Italian population of men and postmenopausal women, patients with atherosclerotic involvement of the carotid and/or femoral artery had low bone mass, and significantly lower osteocalcin and bone-specific alkaline phosphatase than controls. [36] In another Italian population of postmenopausal women, the prevalence of carotid atherosclerosis was higher among women with low BMD and osteocalcin levels above the median. [37]

Additionally, pulse wave velocity (PWV), a marker of early stage atherosclerosis, was inversely associated with calcaneal quantitative osteo-sono assessment index (OSI) in a large Japanese population with a median age of 50 years. This association was stronger in women than men and in pre-menopausal than postmenopausal women. [39] A recent report on forearm endothelial function and spine BMD in early postmenopausal Japanese women indicated that osteoporotic women had a lower maximal forearm blood flow response to reactive hyperemia than those with normal BMD or osteopenia. [40]

Other studies have failed to observe an association between BMD and subclinical measures of atherosclerosis. In SOF, no significant association was observed between aortic calcification and bone density at the hip, spine, or calcaneus after adjusting for age; only a weak association with radial BMD was noted. [25] These findings were consistent with others reported by Frye et. al. among women in Rochester, Minnesota, [27] by Aoyagi et. al. in a population of Japanese-American women, [97] and by Anderson et al in a British men and women. [98]

2.4 POTENTIAL MECHANISMS FOR THE LINK BETWEEN OSTEOPOROSIS AND CARDIOVASCULAR DISEASE

The nature of the putative link between osteoporosis and cardiovascular disease remains unclear. Traditionally, these two conditions were considered unrelated and their progression was attributed to independent age-related processes. [25, 27, 97] However, recent evidence from many studies points to a link between osteoporosis and CVD that cannot be explained by age alone. While this evidence has been consistent in older populations, further support for the role of factors other than age is derived from observations in younger populations. For instance, osteoporotic fractures and cardiovascular outcomes have been shown to coexist in young women with systemic lupus erythematosus (SLE), an autoimmune systemic inflammatory disease that predominantly affects young premenopausal women. The increased risk for both conditions in this young group suggests that factors beyond age are at play in the pathogenesis of osteoporosis and CVD. [29] Several hypotheses have been proposed to explain the link between the two conditions.

2.4.1 Shared Risk Factors

One hypothesis puts forth that the coexistence of osteoporosis and CVD is due to their shared etiological factors (such as smoking, physical activity, alcohol intake, menopause, hypertension, diabetes, etc), which may simultaneously promote or inhibit atherosclerosis and bone demineralization, and could partly explain the association between the two diseases. [9, 22, 99, 100]

2.4.2 Common Pathophysiological Mechanisms

Common pathophysiological mechanisms involving inflammatory cytokines, [36] endogenous sex hormones, [9, 39] oxidized lipids, [101] vitamin K deficiency, [102] and vitamin D [103] were implicated in the progression of the two conditions.

2.4.2.1 Inflammatory markers and cytokines

Inflammation is known to play a central role in all stages of atherogenesis from fatty streak formation to plaque rupture, [64] and there is evidence for its involvement in bone loss. Animal models suggest that osteopenia can be induced in rats by triggering a generalized inflammation through the subcutaneous administration of nonspecific irritants (such as magnesium silicate and cellulose). [104] This induced osteopenia was mainly due to inhibition of bone formation. [105] Chronic inflammatory diseases such as rheumatoid arthritis, lupus, and crohn's disease, are associated with a significant risk for secondary osteoporosis and fractures. The pathogenesis of osteoporosis in these settings is attributed to systemic inflammatory processes among other factors such as glucocorticoid therapy. [106]

Inflammation is a complex process that is mediated by many cytokines including IL-1, TNF- α , and IL-6. Aging is associated with increased levels of circulating inflammatory cytokines such as IL-6 and TNF- α . [107] IL-6 was shown to stimulate osteoclasts, thereby increasing the rates of bone remodeling and bone loss. [108] This cytokine was also observed to act as a marker of subclinical CVD in elderly people [109] and to predict CVD mortality in relatively healthy people aged 65 years and older. [110] TNF- α was also shown to stimulate bone resorption and inhibit bone formation. [111] Results from the Health ABC study indicated that TNF- α and IL-6 were significantly associated with prevalent clinical and subclinical disease, [112] as well as incident cardiovascular events. [113]

Other cytokines may be involved. The OPG/RANK/RANKL triad, a novel signaling pathway recognized as a key regulator of bone resorption, was also shown to play a role in vascular calcification. OPG deficient mice were found to develop early-onset osteoporosis and calcification of the aorta and renal arteries. [114] In another animal study, OPG was shown to be a potent inhibitor of warfarin- and vitamin D-induced arterial calcification at doses known to inhibit bone resorption. [115] In epidemiologic studies, low OPG levels were related to higher prevalence of osteoporosis and vertebral fractures. [116] Increased osteoprotegerin levels were also associated with higher prevalence of CAD, suggesting that elevated OPG may reflect a compensatory mechanism to prevent further vascular damage. [117]

2.4.2.2 Endogenous sex hormones

Estrogen deficiency has been identified as the major determinant of age-related bone loss in women and men. [118, 119] Despite recent evidence from randomized, placebo-controlled trials on the adverse effects or lack of effects of HRT on CVD outcomes, [120, 121] endogenous estrogen is known to have protective effects on the cardiovascular system in women. Estradiol

prevents endothelial dysfunction by increasing the proliferation of endothelial cells, regulating the production of endothelium-derived factors such as nitric oxide, and decreasing the expression of leukocyte adhesion molecules. It inhibits the proliferation and migration of smooth muscle cells. It is also known to improve the lipid profile. [122] Estrogen receptor alpha (ESR1) was shown to have an effect on CVD susceptibility in both women and men. [123] Estrogen may be involved in the pathogenesis of atherogenesis and bone loss, either directly, [122, 124] or through modulation of other factors including cytokines [125] and oxidized lipids. [122] The direct effect of estrogen is manifested by the expression of estrogen receptors on osteoblasts, osteoclasts, [126] and vascular endothelial and smooth muscle cells. [122]

Androgens also seem to have an effect on bone and vascular health. A positive correlation between testosterone levels and bone density has been observed in men and women. [127, 128] Androgens were also related to cardiovascular risk factors in perimenopausal women [129] and in men [130] and to aortic atherosclerosis in men. [131]

2.4.2.3 Lipid metabolism and oxidized lipids

Oxidized lipids have been suggested as a potential mechanism for the paradoxical occurrence of bone loss with vascular calcification. The role of oxidized lipids in atherogenesis is well established. [64, 132] In vitro, Parhami et al have observed that lipid oxidation products including, minimally oxidized LDL, ox-PAPC (oxidized 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphocholine), and the isoprostan iso-PGE₂, have opposite effects on the differentiation of calcifying vascular cells (CVCs) and bone cells. Oxidized lipids were found to stimulate osteoblast differentiation in CVCs as manifested by their induction of alkaline-phosphatase, a marker of osteoblastic differentiation, [133] and their promotion of the formation of extensive areas of calcification in CVCs. In contrast, the same lipids were observed to inhibit osteoblast

differentiation in bone by depressing the induction of alkaline phosphatase activity and reducing mineralization in preosteoblastic bone cells. This lead to the suggestion that the accumulation of oxidized lipids in the subendothelial space of arteries promotes arterial calcification, and its accumulation in the subendothelial space of osteons may inhibit bone mineralization. [101]

A growing body of evidence suggests a negative effect of an atherogenic lipid profile on bone formation. In a cohort of post-menopausal women, plasma levels of low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were negatively and positively related to BMD, respectively. [134] In animal studies, an atherogenic high-fat diet was found to reduce bone formation in mice. [135] The adverse effects of dyslipidemia are mediated by the resultant increase in lipid oxidation products. Increased levels of circulating lipids result in the diffusion of lipoproteins across the vascular endothelium and their accumulation inside the arterial wall and in highly vascular tissues such as the bone microenvironment. Once outside the plasma, these lipid products are subjected to oxidative modification, thus becoming biologically active molecules capable of affecting a variety of cellular processes that ultimately result in atherogenesis and bone loss. [135]

In line with the lipid hypothesis, a potent class of lipid lowering drugs, the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (commonly referred to as statins), is suggested to have an effect on bone health. [136, 137] Statins inhibit HMG-CoA reductase, the enzyme that catalyzes the rate-determining step of cholesterol biosynthesis, the reductive deacylation of HMG-CoA to mevalonate. In large clinical trials, statins have demonstrated the ability to markedly reduce total cholesterol, LDL-C, and triglycerides, to increases HDL-C, and to reduce the incidence of cardiovascular events and mortality. [138, 139] Recent evidence suggests that statin use is related to higher BMD [140] and reduced fracture risk. [138, 139] In

vitro and in animal studies, statins were found to stimulate bone formation and enhance osteoblast differentiation, by increasing the expression and production of BMP-2 by human osteoblasts. [141] Like other members of the BMP family, BMP-2, is known to enhance osteoblast differentiation. [142]

Another class of drugs, bisphosphonates, which inhibit bone resorption and are widely used for the treatment of osteoporosis, may have cardiovascular effects. Like statins, nitrogen-containing bisphosphonates also act on the cholesterol biosynthesis pathway, however; they target enzymes more distal in the mevalonic acid pathway than HMG Co-A reductase. [137] These drugs were found to have unexpected effects on lipids in postmenopausal women with osteoporosis. Chronic intravenous therapy with neridronate was shown to decrease LDL-C and apolipoprotein B and to increase HDL-C. [143]

2.4.2.4 Vitamin K deficiency

Vitamin K deficiency was suggested as a common denominator for atherosclerotic calcification and low bone mass. [102] Low vitamin K intake was related to low bone density [144] and increased risk of osteoporotic fracture. [145] Intake of menaquinone (vitamin K-2) was inversely associated with all-cause mortality, CHD mortality, and severe atherosclerosis in the Rotterdam study. [146] Impaired vitamin K status was also linked to increased atherosclerotic calcification in postmenopausal women. [147] Additionally, Jie and colleagues have observed an inverse association between markers of vitamin K status and bone mass in atherosclerotic women; whereas, no such association was found in the non-atherosclerotic group. [102] It is speculated that the effect of vitamin K on bone demineralization and vascular calcification is mediated by a vitamin K-dependent class of proteins, gla-containing proteins, which include matrix gla protein (MGP) and osteocalcin. Gla-containing proteins are thought to be involved in calcium

metabolism and in the process of calcification in bone and vascular tissues due to the calcium-binding properties of their gla residues.[102] These residues are acquired post-translationally by the action of vitamin K that functions as a coenzyme for glutamate carboxylase, an enzyme that mediates the conversion of glutamate to γ -carboxyglutamate (Gla). The exact physiological role of these proteins is still not clear. However, it is hypothesized that the undercarboxylation of MGP, a mineralization inhibitor, is a risk factor for vascular calcification, and that the undercarboxylation of osteocalcin, a marker of osteoblastic activity, disrupts the normal bone remodeling process mediated by osteocalcin and results in bone loss. [102]

2.4.2.5 Vitamin D

Imbalances in the calciferol endocrine system may also be involved. Excess vitamin D was shown to induce atherosclerosis and osteoporosis in humans and laboratory animals, and the use of vitamin D as a food supplement in some countries coincided with an increase in the incidence of both conditions. [103]

2.4.2.6 Hyperparathyroidism

Parathyroid hormone (PTH) is one of the main regulators of calcium homeostasis. It stimulates the release of calcium and phosphate from bones. Aging is associated with increased levels of PTH as a result of vitamin D deficiency and decreased calcium intake and absorption. Elevated PTH levels contribute to the age-related bone loss and bone fragility. [52, 148] Secondary hyperparathyroidism was also linked to increased risk for fractures, cardiovascular outcomes, and vascular calcification in end-stage renal disease. [148, 149]

2.4.2.7 Homocysteine

Homocysteine is a variant of the amino acid cysteine and is formed during the metabolism of methionine. Its degradation requires folic acid and vitamin B₁₂ as cofactors. Elevated levels of homocysteine could result from genetic or nutritional factors and may lead to osteoporosis and atherosclerosis. Homocystinuria, a genetic disorder of cystathionine β -synthase deficiency, results in early onset osteoporosis and cardiovascular events. There is considerable evidence that elevated plasma homocysteine levels are associated with an increased risk of vascular disease. Homocysteine was reported to enhance the proliferation of vascular smooth muscle cells, inhibit the regeneration of endothelial cells, and increase lipid oxidation. [150] High homocysteine levels were also associated with osteoporotic fractures [151] and reduced BMD. [152] Homocysteine was observed to impair bone mineralization [153] and inhibit collagen cross-linking. [154]

2.4.2.8 Other factors

Other factors implicated in the pathogenesis of atherosclerosis and bone loss include nitric oxide, endothelin-1, angiotensin converting enzyme activity, ascorbic acid, potassium, hyperphosphatemia, oxidative stress, and the preferential differentiation of bone marrow stromal cells into smooth muscle cells over osteoblasts.

2.4.3 Common Genetic Factors

The osteoprotegerin, matrix gla protein, and apolipoprotein E (ApoE) genes have been invoked in both atherogenesis and bone loss. Mice lacking the osteoprotegerin gene were found to develop early-onset osteoporosis and calcification of the aorta and renal arteries. [114] Similarly,

mice lacking the gene for matrix gla protein exhibited vascular calcification as well as osteopenia and fractures. [156] ApoE genotype was associated with atherosclerosis in the Framingham Study and in patients with end stage renal disease. [157, 158] The ApoE4 gene was also associated with reduced BMD and increased fracture risk. [159, 160]

2.4.4 Causal Association

Other hypotheses point to a causal association between the two conditions whereby one of them may lead to the other.

The reduced blood flow hypothesis assumes that atherosclerosis, by reducing blood flow to the lower extremities, could affect intraosseous blood circulation. This in turn alters bone metabolism in the hip and results in osteoporosis. This hypothesis is supported by a study which showed that in cases of asymmetrical peripheral arterial disease, hip bone mineral content was lower in the affected leg compared to the unaffected one. [18, 161] Consistent with this finding, low ankle-arm index was associated with low BMD at the femoral neck, but not at the spine in the Rotterdam Study. [32] Additionally, BMD at the hip, but not at the spine or radius, showed an inverse relation with aortic calcification- a condition thought to affect blood flow to the distal regions or reflect atherosclerosis in arteries directly responsible for blood supply to the hip. [22] In line with this theory, one histological study of 100 cadavers, reported the existence of atherosclerotic changes in intraosseous arteries and arterioles of the femur. [162]

Physical activity was also suggested to lie on the causal pathway between atherosclerosis and bone loss. CVD might limit physical activity and accordingly contribute to bone loss. [19]

It is also hypothesized that with the progressive bone loss leading to osteoporosis, calcium and phosphate salts get redirected from the bone matrix to the arterial wall. [25, 28, 30, 163, 164]

2.5 LIMITATIONS OF THE EXISTING EPIDEMIOLOGIC LITERATURE

Although several lines of evidence support a link between osteoporosis and CVD, the nature of this link and the mechanisms involved are still not clearly elucidated. Reports on this association have focused mostly on white postmenopausal women. [8, 10-13, 15-17, 19] [20-23, 25-27, 30, 33, 37] Less is known about the presence of such relationship in men and in other races. Furthermore, the majority of previous studies have not utilized state-of-the-art measures of subclinical atherosclerosis and BMD. In the existing literature, vascular calcification was mostly assessed using conventional radiography, [21-25, 27, 28, 97, 98] which is rather insensitive for the identification of small calcium deposits. Similarly, in a large number of studies, bone mass was determined using radiographic techniques, single-photon or single X-ray absorptiometry, or dual-photon absorptiometry. [7, 11, 12, 16, 19, 23, 24, 28, 35, 37, 39, 96-98] Some studies have employed DXA in bone determination; [8-10, 13-15, 17, 18, 21, 22, 25, 29-34, 36, 38, 40] however, this technique is limited by its 2-dimensional areal assessment of BMD which does not adjust for bone size. This is especially important in studies of different ethnic and gender groups since there are well-established differences in bone size by race and gender. [165, 166] DXA is also affected by the presence of extra-osseous calcium such as aortic calcification and degenerative osteoarthritic changes, which get incorporated in the region of interest and lead to a falsely increased bone density at the spine. [26] This is an important drawback, particularly in

the elderly who have an increased prevalence of such degenerative conditions. [56] Computed tomography allows for a three-dimensional volumetric determination of bone density, an assessment of purely trabecular bone, and a graded quantification of vascular calcification. This technique was used only in two studies for BMD and aortic calcification quantification, [20, 26] and in two others for coronary calcification assessment. [29, 30] Furthermore, the role of common pathophysiological factors including inflammatory cytokines, endogenous estradiol, and oxidized lipids in the link between low BMD and CVD has not been investigated by epidemiologic studies.

2.6 SPECIFIC AIMS

The aim of *Research Article 1* was to evaluate the association of volumetric and areal BMD measures with prevalent CVD and subclinical peripheral arterial disease (defined using ankle-arm index) in a biracial cohort of older men and women. It investigated whether such associations were a) independent of age, b) independent of shared risk factors between osteoporosis and CVD, or c) explained by the inflammatory cytokines IL-6 and TNF- α .

Research Article 2 evaluated the association between volumetric BMD and vascular calcification of the aorta and coronary arteries in a biracial cohort of middle-aged women. It assessed whether such associations were a) independent of age, b) independent of shared risk factors between osteoporosis and CVD, or c) explained by endogenous estradiol levels.

Research Article 3 examined the relation between baseline BMD measures and incident CVD events in older men and women with no prior history of CVD; and investigated whether

such associations were a) independent of age, b) independent of shared risk factors between osteoporosis and CVD, or c) explained by common pathophysiological factors such as oxidized LDL (oxLDL) or the inflammatory cytokines IL-6 and TNF- α .

3.0 BONE MINERAL DENSITY AND PREVALENT CARDIOVASCULAR DISEASE IN OLDER MEN AND WOMEN: THE HEALTH, AGING, AND BODY COMPOSITION STUDY

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3.1 ABSTRACT

This cross-sectional analysis investigated the associations of volumetric (vBMD) and areal (aBMD) bone mineral density measures with prevalent cardiovascular disease (CVD) and subclinical peripheral arterial disease (PAD) in a biracial cohort of older men and women, and evaluated whether such associations were independent of age and shared risk factors between BMD and CVD, or explained by inflammatory cytokines.

Data were from the baseline assessment of the Health, Aging, and Body Composition Study which included 3075 well-functioning white and black men and women (42% Black; 51% women), aged 68-80 years. Prevalent CVD was defined as the presence of coronary heart disease, PAD, congestive heart failure, cerebrovascular disease, or positive results on the Rose questionnaire for angina or claudication. Subclinical PAD was defined as a low ankle-arm index (<0.9) in the absence of CVD. Total hip, femoral neck, and trochanter aBMD were assessed using DXA. Spine trabecular, integral, and cortical vBMD measures were assessed using QCT in a subset ($n=1489$). Logistic regression examined associations of BMD measures (per standard deviation (SD)) with CVD and PAD.

The prevalence of CVD was 29.8%. Among participants without CVD, 10% had subclinical PAD. Spine vBMD measures were inversely associated with CVD in men [OR(integral)=1.34, 95%CI 1.10-1.63; OR(trabecular)=1.25, 95%CI 1.02-1.53; OR(cortical)=1.36, 95%CI 1.11-1.65]. In women, the odds of CVD was significantly increased by 28%, 27%, and 22% for each SD decrease in integral vBMD, cortical vBMD, or trochanter aBMD, respectively. Total hip aBMD was associated with subclinical PAD in men (OR=1.39, 95%CI 1.03-1.84) but not women. All associations were independent of age and shared risk factors between BMD and CVD, and were not influenced by inflammatory cytokines.

In conclusion, our results provide further evidence for an inverse association between BMD and CVD in men and women. Further research should investigate common pathophysiological links between osteoporosis and CVD.

3.2 INTRODUCTION

Cardiovascular disease (CVD) and osteoporosis are common age-related conditions. Mounting biological [1-6] and epidemiological evidence supports a link between the two diseases. In both cross-sectional and longitudinal epidemiologic studies, low bone mineral density (BMD) was related to higher cardiovascular mortality, [7-11] cardiovascular morbidity, [12-17] and subclinical measures of atherosclerosis. [18-31]

An inverse association between bone density and cardiovascular mortality was reported in both women [7-9] and men.[10, 11] In white women, low bone mass was related to higher incidence [12, 15, 17] and prevalence [13, 16] of coronary artery disease and stroke. In men, previous myocardial infarction was associated with low BMD in a multiethnic population of men in the Third National Health and Nutrition Examination Survey (NHANES III). [14] Lower bone mineral content was also related to asymmetrical symptomatic peripheral arterial disease in a small study involving 18 men. [32] Consistent with these findings, several studies reported inverse relationships between bone density and subclinical measures of atherosclerosis, including vascular calcification of the aorta [18-21] and coronary arteries, [22] carotid plaque and intima-media thickness, [23-26] pulse wave velocity, [27] and endothelial dysfunction. [28] Similarly, ankle-arm index was positively correlated with BMD in an elderly Chinese population [29] and in white postmenopausal women. [30, 31]

Several hypotheses have been proposed to explain the link between CVD and osteoporosis including: 1) their age-related independent progression, [33-35] 2) the presence of shared risk factors (such as smoking and physical inactivity), [36, 37] 3) the presence of common pathophysiological mechanisms that could lead to the development of both conditions and which may involve inflammatory cytokines or endogenous sex hormones, [15, 27] and 4) a cause-effect relationship whereby one condition may be leading to the other. For instance, atherosclerosis, by reducing blood flow to the lower extremities, could alter bone metabolism in the hip and result in osteoporosis. [31, 32]

The nature of the putative link between CVD and osteoporosis is still controversial. Reports on this association have focused mostly on white postmenopausal women. Less is known about the presence of such relationship in men and in other races. Additionally, the majority of previous studies have not utilized state-of-the-art assessments of bone mass involving quantitative computed tomography (QCT) and dual energy X-ray absorptiometry (DXA). [8-10, 15, 17, 20, 21, 23, 25, 27, 33] Furthermore, while inflammatory cytokines have been suggested as a common denominator in the association between osteoporosis and CVD, [15, 27] to our knowledge no study has actually examined their role.

The aim of the current study was to evaluate the association of volumetric and areal BMD measures with prevalent CVD and subclinical peripheral arterial disease (PAD) in a biracial cohort of men and women; and to investigate whether such associations were a) independent of age, b) independent of shared risk factors between osteoporosis and CVD, or c) explained by inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α).

3.3 MATERIALS AND METHODS

3.3.1 Subjects

Participants in this study were enrolled in the Health, Aging, and Body Composition (Health ABC) Study, a population-based prospective study investigating the association between changes in body composition and functional decline in the elderly. The cohort included 3075 well-functioning, community-dwelling men and women aged 68-80 years. The demographic distribution of the population was as follows: 729 black women, 855 white women, 552 black men, and 939 white men. Participants were identified from a random sample of white Medicare beneficiaries and all age-eligible black community residents in designated zip code areas surrounding Pittsburgh, PA, and Memphis, TN. Subjects who reported difficulty walking one quarter of a mile, climbing 10 steps, or performing basic activities of daily living, who had a life threatening illness in the 3 years prior to the study, or who were planning to move in the next 3 years were excluded. Written informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of the University of Pittsburgh and the University of Tennessee.

The current cross-sectional analysis utilized data from the baseline Health ABC examination, performed between April 1997 and June 1998. Areal BMD data was missing for 32 participants. Volumetric BMD was performed at the Pittsburgh site only and was therefore available for only 1489 participants (out of 1527). Ankle-arm index data was missing for 197 participants. Only people without CVD were included in the subclinical PAD analysis (n= 2045).

3.3.2 Prevalent Cardiovascular Disease

Prevalent disease algorithms were created by Health ABC investigators based on self-reported history and the use of selected drugs in the past 2 weeks. CVD was considered present if one or more of the following conditions were prevalent at the baseline examination: coronary heart disease (defined as self-report of surgical or percutaneous revascularization; self-report of myocardial infarction (MI) or angina and use of anti-anginal medications; or electrocardiographic evidence of previous MI), cerebrovascular disease (defined as self-report of transient ischaemic attack or stroke), peripheral arterial disease (defined as self-report of intermittent claudication or pain in legs, or self-report of bypass or angioplasty in leg arteries), congestive heart failure (defined as self-report of congestive heart failure and use of diuretic, and use of vasodilator or cardiac glycoside), or the presence of positive results on the Rose questionnaire for angina or claudication. Overall, 915 participants (29.8%) were identified in this group.

3.3.3 Subclinical Peripheral Arterial Disease

Among participants who did not have CVD, subclinical PAD status was defined using the ankle-arm blood pressure index (AAI). AAI was defined as the ratio of either the right or the left ankle artery systolic blood pressure to the right upper arm systolic blood pressure, measured by a hand-held, 6-MHz Doppler probe placed directly over the artery and a conventional mercury sphygmomanometer. First, two AAI averages were calculated based on two ratios with the right leg and two ratios with the left leg. The lower AAI of the 2 averages was used. Participants were categorized as having subclinical PAD if they had low AAI, i.e. a ratio of less than 0.9. [38] Overall, 206 participants (10.1%) without CVD had subclinical PAD.

3.3.4 Areal Bone Mineral Density

Dual-energy X-ray absorptiometry (DXA) was used to assess areal BMD (g/cm²) of the total hip and hip subregions (femoral neck and trochanter) using a Hologic 4500A densitometer (Hologic 4500A, version 9.03; Hologic, Inc., Waltham, MA, USA). DXA quality assurance measures were performed at both study sites and identical scan protocols were used for all participants.

3.3.5 Volumetric Bone Mineral Density

Quantitative computed tomography (QCT) was used to obtain volumetric BMD measures (mg/cc) of the spine (General Electric 9800 Advantage, 80 kVp/140 mAs, 10-mm slice thickness; GE Medical Systems Milwaukee, WI). QCT images were acquired at the level of the L3 vertebra to obtain trabecular and integral BMD. Cortical BMD, which includes the cortical shell of the vertebral body and the posterior elements, was estimated by taking the difference in BMC between the integral and trabecular regions and dividing it by the difference in the volumes of these two regions. CT numbers were converted to equivalent tissue concentration of hydroxyapatite by the use of a reference calibration phantom placed under the lower back of the participant. Osteophytes were edited out of the external bone contour during image analysis. Scans were performed by certified technicians and analyzed with a standardized protocol at the University of California, San Francisco.

3.3.6 Inflammatory Cytokines

The concentrations of IL-6 and TNF- α were obtained from frozen stored serum samples collected via venipuncture after an overnight fast. Cytokines were measured in duplicate using commercial ELISA assays from R&D Systems (Minneapolis, MN). The lower detectable limit

was 0.10 pg/ml for IL-6 and 0.18 pg/ml for TNF- α , the detection range was 0.156-17.0 pg/ml for IL-6 and 0.5-32 pg/ml for TNF- α , and the interassay coefficient of variation was 10.3% for IL-6 and 15.8% for TNF- α . IL-6 and TNF- α were available for 2913 and 2872 participants, respectively.

3.3.7 Potential Confounders

Sociodemographic factors (age, gender, race, study site, education), smoking history, alcohol consumption, weekly physical activity from walking and exercise (Kcal/Kg/hour), medication use (including hormone therapy, statins, osteoporosis drugs, thiazide diuretics, systemic corticosteroids, calcium supplements, vitamin D supplements), and time since menopause were determined by an interview-administered questionnaire.

Medication use in the previous 2 weeks was coded using the Iowa Drug Information System (IDIS) ingredient codes. [39] Prevalent diabetes was defined as self-report of diabetes previously diagnosed by a physician, use of hypoglycemics medications, or a fasting glucose \geq 126 mg/dl. Prevalent hypertension was defined as self-report of hypertension and use of anti-hypertensive medications. Prevalent osteoporosis (total hip BMD 2.5 SD or more below the young adult mean) was defined using gender and race-specific T-scores determined from the NHANES III study population. [40]

Lower extremity physical function was assessed by the Health ABC performance battery, a supplemented version of the lower-extremity performance test used in the Established Populations for the Epidemiologic Studies of the Elderly (EPESSE; chair stands, standing balance, 6-m walk for gait speed) [41] with increased test duration, a single foot stand, and a narrow walk test of balance as previously described (score range 0-12). [42] Height and weight were obtained

using a Harpenden stadiometer (Holtain, Wales, UK) and a standard balance beam, respectively, and body mass index (BMI) was calculated as weight divided by height squared (Kg/m^2). Seated systolic and diastolic blood pressures were measured by a manual mercury sphygmomanometer using a standardized protocol.

Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride were measured by a colorimetric technique (Johnson & Johnson Vitros 950 analyzer, New Brunswick, New Jersey). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. [43] Plasma glucose was measured using an automated glucose oxidase reaction (YSI 2300 STAT Plus Glucose & Lactate Analyzer; YSI Life Sciences, Inc., Yellow Springs, Ohio). Serum insulin was assessed using a commercially available radioimmunoassay kit (Pharmacia, Uppsala, Sweden).

3.3.8 Data Analysis

All analyses were stratified by gender. Baseline characteristics and BMD measures of groups with or without prevalent CVD were compared using chi-square test for categorical variables and either 2-sample t-test or Wilcoxon rank-sum test for continuous data. Logistic regression was used to determine the associations of BMD measures with CVD. Separate logistic regression models were fitted for each BMD variable using gender-specific standard deviation (SD) scores (calculated as the deviation from the mean BMD divided by the standard deviation of the BMD measure in each gender) instead of raw values. Unadjusted, age-adjusted, and risk factors adjusted models were fitted. Variables were selected for entry into the multiple regression models if they were associated with CVD and any of the BMD variables at the 0.15 level of

significance in univariate analyses (performed using simple logistic regression for CVD and simple linear regression for BMD measures).

The effect of IL-6 and TNF- α on statistically significant associations between BMD measures and CVD was tested by introducing these variables, separately, into multiple regression models. Because of the skewed distribution of IL-6 and TNF- α , their log-transformed values were used in analyses.

Potential racial differences in the relationship of BMD with CVD were tested by entering product terms for race and BMD measures in the multiple logistic regression models. Associations between BMD measures and CVD were presented as unadjusted, age-adjusted, and risk factor-adjusted odds ratios (OR) and 95% confidence intervals per 1 SD decrease in BMD. The Hosmer and Lemeshow test was used to evaluate the goodness of fit of logistic regression models. The level of significance was considered to be 0.05 except for the initial identification of shared risk factors between CVD and BMD as described above. The same analytical approach was followed for subclinical PAD. In both CVD and PAD models, no significant interactions between race and BMD measures were observed, therefore no race-specific analyses were performed. Subclinical PAD analyses were repeated after excluding participants with high AAI (values ≥ 1.3 , N= 121, 5.9% of the population that had no prevalent CVD). This had no effect on the results, therefore this group was retained in the analyses. Data were analyzed using SAS version 8.01 (SAS Institute Inc, Cary, NC, USA).

3.4 RESULTS

3.4.1 BMD and Prevalent CVD

Overall, 29.8% (N= 915 out of 3075) of the study population had CVD. The gender-specific rates were 25.1% in women and 34.7% in men. In both women and men, participants with CVD had higher triglyceride, glucose, insulin, IL-6, and TNF- α levels, had lower HDL and Health ABC performance battery scores, and were more likely to be hypertensive, diabetic, and on statins (Table 3-1).

In women, none of the BMD variables were related to prevalent CVD in unadjusted or age-adjusted analyses. However, after controlling for shared risk factors between CVD and BMD, cortical vBMD, integral vBMD, and trochanter aBMD became significantly associated with CVD. For every standard deviation decrease in each of these BMD measures, the odds of CVD was increased by 28%, 27%, and 22%, respectively (Table 3-2). No associations were observed between CVD and trabecular vBMD of the spine, total hip aBMD, or femoral neck aBMD (Table 3-2).

In men, all vBMD measures (integral, trabecular, and cortical) of the spine were significantly associated with CVD in unadjusted, age-adjusted (except for trabecular), and risk factor-adjusted models. A one SD decrease in cortical, integral, or trabecular BMD increased the odds of CVD by 36%, 34%, and 25%, respectively. However, none of the areal BMD measures showed an association with CVD (total hip aBMD: adjusted OR= 1.14, 95% CI 0.96-1.36; femoral neck aBMD: adjusted OR= 1.00, 95% CI 0.87-1.15; trochanter aBMD: adjusted OR= 1.02, 95% CI 0.91-1.22) (Table 3-2).

We tested the effect of IL-6 and TNF- α on associations that were statistically significant after adjusting for shared risk factors between BMD and CVD. In women, adjusting for IL-6 did

not affect the associations between BMD measures and CVD. For instance, the adjusted OR for CVD per 1 SD decrease in integral BMD changed from 1.28 (95% CI 1.02-1.62, based on sample of 616 women with non-missing IL-6 values) to 1.30 (95% CI 1.03-1.64, N= 616) after controlling for IL-6. The same was observed for TNF- α . Similarly, in men, adjusting for IL-6 had no effect on the strength or statistical significance of the associations of vBMD measures with CVD. For example, the adjusted OR related to integral vBMD remained the same before (OR= 1.30, 95% CI 1.06-1.59, based on sample of 614 men with non-missing IL-6) and after (OR= 1.30, 95% CI 1.06-1.60, N=614) IL-6 was added to the logistic regression model. Similar results were observed for TNF- α .

3.4.2 BMD and Subclinical PAD

Among participants who did not have CVD, 10% (N= 206) had evidence of subclinical PAD. A low ankle-arm index was observed in 10.4% (N= 117) of women and 9.7% (N= 89) of men.

In women, no unadjusted differences in areal or volumetric BMD measures existed between participants with and without subclinical PAD, except for trochanter aBMD which was significantly and inversely associated with subclinical PAD (OR= 1.26, 95%CI 1.03-1.54). However, this association became not significant after controlling for age. In adjusted models, all aBMD measures of the hip showed inverse associations with PAD; however these did not reach statistical significance. None of the spine vBMD measures exhibited a significant relationship with subclinical PAD (Table 3-3).

In men, total hip aBMD was significantly associated with the outcome in unadjusted (OR= 1.28, 95% CI 1.02-1.60) and risk factor-adjusted models. In the full model, a one SD decrease in total hip aBMD was related to a 39% increased odds for subclinical PAD. Trochanter

and femoral neck aBMD were also inversely related with PAD, but these associations did not achieve statistical significance. Spine vBMD variables were not related to subclinical PAD (Table 3-3).

Adjusting for IL-6 minimally reduced the strength of the association between total hip aBMD and subclinical PAD. The adjusted OR was reduced from 1.38 (95% CI 1.02-1.87, $p=.04$, based on sample of 844 men with non-missing IL-6 values) to 1.34 (95% CI 0.98-1.82, $p=.07$, $N=844$) after controlling for IL-6. TNF- α had no effect on the magnitude and significance of the association.

3.5 DISCUSSION

In this cross-sectional analysis performed in a biracial cohort of older men and women, volumetric BMD measures of the spine were significantly associated with CVD in men and women, and areal BMD of the trochanter was related to CVD in women. Additionally, areal BMD of the total hip was related to subclinical PAD in men. These inverse relationships were not age-related, were independent of shared risk factors between BMD and CVD, and were not influenced by the inflammatory cytokines IL-6 or TNF- α .

Our results in men, regarding both CVD and subclinical PAD, make an important addition to the existing literature since only a few studies investigated the association of BMD with CVD and subclinical atherosclerosis in men. [10, 11, 14-16, 20, 27, 29, 30, 32] Moreover, most of the reports that found significant relationships in women failed to observe the same associations in men. [15, 16, 20, 30] In our study, trabecular, integral, and cortical vBMD measures of the spine were significantly associated with prevalent CVD in men. Results from

NHANES III showed a significant association, of comparable magnitude to ours, between MI and low BMD (OR= 1.39, 95% CI= 1.03-1.87) in a multiethnic cohort of 2,281 men, aged 50-79 years. [14] Additionally, BMD was inversely related to CVD mortality in white men in the NHANES I study and in a British population. [10, 11]

The associations of BMD measures with CVD in women confirm prior cross-sectional and longitudinal findings and extend them by including a cohort of black and white women and by using volumetric measures of BMD. In the Multiple Outcomes of Raloxifene Evaluation (MORE) trial, osteoporosis was found to be a strong predictor of future cardiovascular outcomes in white postmenopausal women with low bone mass, independent of age and other traditional cardiovascular risk factors (adjusted HR= 3.9, 95%CI 2.0-7.7). In a 30-year follow-up to the Framingham study, metacarpal cortical area (MCA) predicted coronary heart disease in white women free from CVD at baseline (HR for highest vs. lowest MCA quartile= 0.73, 95% CI 0.53-1.00, p for trend= 0.03). Additionally, low bone mass was associated with stroke incidence, prevalence, and mortality in older white women. [16, 17]

Notably, the associations between BMD and CVD in women were observed only after adjusting for risk factors. These associations seemed to be masked by negative confounders such as BMI, black race, diabetes, glucose level, and statin use, which were associated with higher BMD and increased prevalence of cardiovascular disease.

We observed a significant association between total hip aBMD and subclinical PAD in men. The hip subregions showed a trend for an inverse association with PAD; however these relationships did not reach statistical significance. Spine vBMD measures were not associated with PAD. Results from other large studies did not indicate significant relationships of BMD with PAD in men. [29, 30] However, in a small study of men with asymmetrical symptomatic

PAD, hip bone mineral content was lower in the affected leg as compared to the unaffected one. [32]

Similarly in women, aBMD measures of the total hip and hip subregions tended to be inversely associated with PAD, but these relationships did not achieve statistical significance. On the other hand, spine vBMD measures did not exhibit a relationship with PAD. In the Rotterdam study, which included a cohort of white women of similar age to our population, femoral neck aBMD was found to be associated with PAD. In line with our findings, no associations with spine BMD were reported. The lack of significant associations in women in our study may be explained by the lower prevalence of PAD (10%) compared to that in the Rotterdam study (16%). Also, due to the smaller sample size available for PAD analysis (n= 1011, compared to n= 2984 in the Rotterdam cohort), we had lower power to detect similar associations. [30]

A biological basis for the inverse association between hip BMD and PAD has been sought in the reduced blood flow hypothesis. It was suggested that atherosclerosis, by reducing blood flow to the lower extremities, could alter bone metabolism in the hip and result in decreased bone density. [19, 31] This hypothesis is supported by studies that observed site-specific associations between AAI and BMD of the hip, but not other areas. [29-31] In a Chinese cohort of older men and women, an increase in AAI of 1 SD was related to a 0.5% increase in BMD of the total hip, but not the spine. [29] In the Study of Osteoporotic Fractures, a decrease in the AAI was associated with increased bone loss at the hip and calcaneus. [31] In line with this notion, a study of asymmetrical peripheral arterial disease showed that hip bone mineral content was lower in the affected leg as compared to the unaffected one. [32] Our study lends additional support to the reduced blood flow hypothesis as there was evidence for an association between BMD and PAD at the total hip but not at the spine.

Traditionally, osteoporosis and CVD have been regarded as independent processes that occur with aging. Therefore, the association between them was attributed to their age-related independent progression. [33-35] Mounting biological observations [1-6] and epidemiologic evidence from this study and others [7-31] suggest a link between the two conditions that is independent of age. Laboratory studies indicate that atherosclerotic calcification and bone calcification share a number of common features. It is now understood that the arterial tissue is calcified in a highly regulated and organized process by mechanisms similar to those involved in bone mineralization.[1] Hydroxyapatite, a mineral that is present in bones, is also found in calcium deposits of atherosclerotic plaques. [4] In addition, calcified plaques express several bone matrix proteins such as GLA protein, bone morphogenetic protein-2, osteopontin, osteocalcin, and collagen I. [2, 3, 5, 6]

Other hypotheses proposed to explain the link between osteoporosis and CVD include: 1) shared etiological factors (such as smoking, physical activity, alcohol intake, hypertension, etc.), which may simultaneously promote or inhibit atherogenesis and bone demineralization, [11, 19, 36, 37] and 2) common pathophysiological mechanisms that could lead to the development of both conditions and which may involve inflammatory cytokines.

In our analysis, the observed inverse associations between BMD and CVD were present after controlling for age and other common etiological factors for osteoporosis and CVD including ethnicity, weight, physical activity, blood pressure, and lipids. Common pathophysiological factors may therefore be at play in the progression of the two conditions. Inflammatory cytokines, for instance, have been implicated in both atherogenesis and bone resorption. [15, 27] Aging is associated with increased levels of circulating inflammatory markers such as IL-6 and TNF- α . [44] These cytokines stimulate osteoclast formation, thereby

increasing the rates of bone resorption. [45, 46] IL-6 was shown to be a marker of subclinical CVD [47] and a predictor of CVD mortality in elderly people. [48] Previous analyses in the Health ABC cohort showed that IL-6 and TNF- α were significantly associated with prevalent clinical and subclinical disease, [49] as well as incident cardiovascular events. [50] In our analyses, IL-6 and TNF- α did not affect the associations of BMD with CVD. To our knowledge, no other study has investigated the role of IL-6 and TNF- α in these relationships. Other cytokines such as the osteoprotegerin (OPG)/ receptor activator of nuclear factor kappa B (NF- κ B) (RANK)/RANK ligand (RANKL) triad seem to play a dual role in bone physiology and vascular calcification and may therefore be implicated in the pathogenesis of CVD and osteoporosis. [51] Other factors that could be involved include endogenous sex hormones, oxidized lipids, [52] imbalances in the calciferol endocrine system, [53] vitamin K status, [54] and genetic factors. [51, 55]

Notably, all the associations we observed were consistently stronger in men. While this suggests that the potential pathophysiological mechanisms involved in the association between osteoporosis and CVD have an impact that may vary by gender, it can also point to the presence of other shared risk factors for the two conditions in men which we could not account for.

Our study has several advantages. The associations were investigated in a large and well-characterized biracial cohort of older men and women and were adjusted for a comprehensive set of shared risk factors for osteoporosis and CVD. Our study also had the benefit of utilizing QCT for volumetric BMD determination at the spine. In a large number of studies, bone mass was determined using radiographic techniques, single-photon or single X-ray absorptiometry, or dual-photon absorptiometry. [8-10, 15, 17, 20, 21, 23, 25, 27, 33] Some studies have employed DXA in bone determination; [7, 11-14, 16, 19, 22, 24, 26, 28-32, 34] however, this technique is limited

by its 2-dimensional areal assessment of BMD which does not adjust for bone size. This is especially important in studies of different ethnic and gender groups since there are well established differences in bone size by race and gender. [56, 57] DXA is also affected by the presence of extra-osseous calcium such as aortic calcification and degenerative osteoarthritic changes, which get incorporated in the region of interest and lead to a falsely increased bone density at the spine. [58] This is an important drawback, particularly in the elderly who have an increased prevalence of such degenerative conditions. [59] QCT, which allows for a three-dimensional volumetric determination of bone density, an adjustment for bone size, and an assessment of purely trabecular bone, was used only in one study. [18]

The main limitation of our analysis is its cross-sectional nature which does not allow the evaluation of a causal association between CVD and osteoporosis, and the elucidation of common mechanisms involved in the pathogenesis of both conditions. Additionally, our cohort included well-functioning, community-dwelling older adults, which may limit the generalizability of the results to other populations.

In conclusion, our results provide further evidence for the inverse association between BMD and CVD in both men and women. However, the establishment of a link between osteoporosis and atherogenesis requires more longitudinal evidence and will remain far from conclusive until further investigations into common pathophysiological mechanisms for the two conditions become available.

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Table 3-1 Comparison of baseline characteristics (% , mean \pm SD, median (IQR)) in women and men by prevalent cardiovascular disease status, the Health ABC Study

	Women			Men		
	No Cardiovascular Disease (N =1186)	Cardiovascular Disease (N = 398)	P-value	No Cardiovascular Disease (N = 974)	Cardiovascular Disease (N = 517)	P-value
Demographics						
Age	73.4 \pm 2.8	73.7 \pm 2.9	.116	73.6 \pm 2.9	74.0 \pm 2.8	.032
% Black	43.3	54.0	.002	38.4	34.4	.131
Life Style/ Diet						
% Current smoking	8.7	13.6	.005	11.4	9.7	.307
% Current alcohol drinking	43.2	38.9	.133	58.1	56.1	.468
Physical activity (Kcal/ week)*	351.2 (42.0-970.4)	182.9 (7.0-644.5)	<.0001	660.3 (163.8-1839.9)	702.8 (165.1-1738.5)	.737
% Calcium supplements	30.7	21.6	.0005	8.1	5.6	.073
% Vitamin D supplements	14.2	10.3	.049	3.6	3.7	.948
Anthropometric Measures						
BMI (kg/ms)	27.7 \pm 5.6	27.8 \pm 5.2	.726	27.0 \pm 4.0	27.2 \pm 3.9	.228
Weight (Kg)	70.5 \pm 14.8	70.8 \pm 14.4	.701	81.5 \pm 13.5	81.2 \pm 12.9	.658
Height (cm)	159.6 \pm 61.2	159.5 \pm 61.6	.822	173.7 \pm 66.4	172.6 \pm 65.4	.003
HABC performance score	6.7 \pm 1.6	6.4 \pm 1.7	.0003	7.6 \pm 1.6	7.2 \pm 1.6	<.0001
Lipids						
Total cholesterol (mg/dl)	214.0 \pm 38.1	210.1 \pm 42.3	.110	193.4 \pm 34.6	189.2 \pm 35.0	.028
LDL (mg/dl)	125.7 \pm 36.4	123.1 \pm 37.0	.213	119.3 \pm 31.9	114.8 \pm 32.1	.011
HDL (mg/dl)	61.1 \pm 17.3	57.5 \pm 17.5	.0004	48.3 \pm 14.4	46.3 \pm 13.1	.009
Triglyceride (mg/dl)*	119.0 (90.0-163.0)	125.0 (92.0-177.0)	.045	112.0 (84.0-154.0)	121.0 (87.0-173.0)	.004
Inflammatory Markers						
IL-6 (pg/ml)*	1.7 (1.1-2.7)	1.9 (1.4-3.0)	.003	1.8 (1.3-2.6)	2.2 (1.5-3.2)	<.0001
TNF- α (pg/ml)*	3.0 (2.3-3.9)	3.4 (2.5-4.4)	<.0001	3.1 (2.4-4.0)	3.5 (2.7-4.5)	<.0001
Blood Pressure (mmHg)						
Systolic blood pressure	135.8 \pm 20.4	138.6 \pm 23.2	.030	135.4 \pm 20.3	135.0 \pm 21.8	.734
Diastolic blood pressure	70.1 \pm 11.7	70.4 \pm 12.5	.684	73.5 \pm 11.3	71.3 \pm 11.7	.0004
Glucose level (mg/dl) *	92.0 (85.0-101.0)	95.0 (87.0-109.0)	<.0001	95.0 (89.0-107.0)	98.0 (90.0-115.0)	.009
Insulin level (uIU/ml) *	6.9 (4.8-10.2)	7.8 (5.1-11.2)	.003	6.6 (4.8-9.8)	7.1 (5.1-10.5)	.009

Table 3-1 (Continued)

Time since menopause (yrs)	27.4±7.6	28.8±8.2	.004	-	-	-
Medical History						
% History of hypertension	44.2	63.9	<.0001	32.2	53.5	<.0001
% History of diabetes	13.9	24.0	<.0001	20.3	25.9	.014
% History of osteoporosis	13.8	16.4	.20	3.4	3.4	.95
Medication Use						
% Oral estrogen	23.6	18.6	.040	-	-	-
% Osteoporosis medication	8.2	5.0	.035	0.6	1.4	.147
% Statins	10.8	20.4	<.0001	6.9	23.2	<.0001
BMD Measures						
Volumetric (mg/cc)						
Spine Integral	238.1±51.0	237.8±50.4	.95	264.3±56.7	253.6±51.1	.012
Spine Trabecular	111.6±40.1	111.6±39.7	.99	134.2±44.6	127.0±41.5	.032
Spine Cortical	278.2±53.8	277.1±53.8	.80	310.2±60.0	297.9±55.2	.006
Areal (g/cm²)						
Total Hip	0.81±0.14	0.80±0.15	.55	0.97±0.15	0.97±0.15	.94
Trochanter	0.62±0.12	0.60±0.12	.08	0.76±0.13	0.76±0.13	.60
Femoral neck	0.70±0.13	0.70±0.13	.65	0.80±0.14	0.79±0.14	.93

* P-value obtained using Wilcoxon rank-sum test since variables were not normally distributed

Table 3-2 Results of logistic regression models for prevalent CVD: unadjusted, age-adjusted, and risk factor-adjusted odds ratios (95% CI) per 1 SD decrease in BMD measures for women and men in the Health ABC Study

	Women [*]		Men ^{**}	
BMD [†]	N (cases)	Odds Ratio (95% CI)	N (cases)	Odds Ratio (95% CI)
Spine Integral vBMD				
Unadjusted	764 (216)	1.01 (0.86-1.18)	725 (261)	1.22 (1.04-1.43) ^a
Adjusted for Age	764 (216)	0.99 (1.00-1.54)	725 (261)	1.20 (1.03-1.41) ^a
Adjusted for shared risk factors between osteoporosis and CVD	649 (178)	1.27 (1.01-1.58) ^a	634 (224)	1.34 (1.10-1.63) ^b
Spine Trabecular vBMD				
Unadjusted	764 (216)	1.00 (0.85-1.18)	725 (261)	1.19 (1.01-1.39) ^a
Adjusted for Age	764 (216)	0.98 (0.83-1.15)	725 (261)	1.17 (1.00-1.37)
Adjusted for shared risk factors between osteoporosis and CVD	649 (178)	1.20 (0.96-1.49)	634 (224)	1.25 (1.02-1.53) ^a
Spine Cortical vBMD				
Unadjusted	764 (216)	1.02 (0.87-1.19)	725 (261)	1.24 (1.06-1.45) ^b
Adjusted for Age	764 (216)	1.00 (0.85-1.18)	725 (261)	1.22 (1.05-1.43) ^b
Adjusted for shared risk factors between osteoporosis and CVD	649 (178)	1.28 (1.02-1.59) ^a	634 (224)	1.36 (1.11-1.65) ^b
Total hip aBMD				
Unadjusted	1570 (396)	1.04 (0.92-1.16)	1473 (507)	1.00 (0.89-1.11)

Table 3-2 (Continued)

Adjusted for Age	1570 (396)	1.02 (0.91-1.14)	1473 (507)	0.98 (0.88-1.10)
Adjusted for shared risk factors between osteoporosis and CVD	1300 (323)	1.14 (0.96-1.36)	1289 (436)	1.05 (0.91-1.22)
Trochanter aBMD				
Unadjusted	1570 (396)	1.11 (0.99-1.24)	1473 (507)	1.02 (0.92-1.15)
Adjusted for Age	1570 (396)	1.10 (0.98-1.23)	1473 (507)	1.02 (0.92-1.14)
Adjusted for shared risk factors between osteoporosis and CVD	1300 (323)	1.22 (1.03-1.43)^b	1289 (436)	1.05 (0.91-1.22)
Femoral neck aBMD				
Unadjusted	1570 (396)	0.97 (0.87-1.09)	1473 (507)	1.01 (0.90-1.12)
Adjusted for Age	1570 (396)	0.96 (0.86-1.08)	1473 (507)	0.99 (0.89-1.11)
Adjusted for shared risk factors between osteoporosis and CVD	1300 (323)	1.04 (0.87-1.23)	1289 (436)	1.00 (0.87-1.15)

* Models in women were adjusted for age, ethnicity, study site (areal BMD models), educational level, time since menopause, BMI, physical activity, Health ABC physical performance score, smoking status, drinking status, systolic blood pressure, total cholesterol, HDL, triglyceride, plasma glucose level, serum insulin level, history of hypertension, history of diabetes, use of calcium and vitamin D supplements, statins, osteoporosis medications, and oral hormones.

** Models in men were adjusted for age, ethnicity, study site (areal BMD models), BMI, physical activity, diastolic blood pressure, total cholesterol, HDL, triglyceride, plasma glucose level, serum insulin level, history of hypertension, history of diabetes, and use of statins.

† BMD SD scores: Women: total hip aBMD (SD= 0.15 g/cm²), femoral neck aBMD (SD= 0.13 g/cm²), trochanter aBMD (SD=0.12 g/cm²), integral vBMD (SD=50.82 mg/cc), trabecular vBMD (SD= 39.98 mg/cc), cortical BMD (SD= 53.80 mg/cc). Men: total hip aBMD (SD= 0.15 g/cm²), femoral neck aBMD (SD= 0.14 g/cm²), trochanter aBMD (SD=0.13 g/cm²), integral vBMD (SD=54.94 mg/cc), trabecular vBMD (SD= 43.59 mg/cc), cortical BMD (SD= 58.61 mg/cc).

^a P<0.05

^b P<0.01

Table 3-3 Results of logistic regression models for subclinical PAD: unadjusted, age-adjusted, and risk factor-adjusted odds ratios (95% CI) per 1 SD decrease in BMD measures for women and men in the Health ABC Study

BMD [†]	Women [*]		Men ^{**}	
	N (cases)	Odds Ratio (95% CI)	N (cases)	Odds Ratio (95% CI)
Integral vBMD				
Unadjusted	528 (48)	1.07 (0.79-1.44)	441 (38)	0.91 (0.66-1.25)
Adjusted for Age	528 (48)	1.05 (0.78-1.42)	441 (38)	0.85 (0.62-1.16)
Adjusted for shared risk factors between osteoporosis and PAD	493 (42)	1.04 (0.70-1.53)	423 (37)	1.07 (0.74-1.56)
Spine Trabecular vBMD				
Unadjusted	528 (48)	0.97 (0.72-1.30)	441 (38)	0.83 (0.62-1.14)
Adjusted for Age	528 (48)	0.95 (0.71-1.28)	441 (38)	0.77 (0.57-1.05)
Adjusted for shared risk factors between osteoporosis and PAD	493 (42)	0.94 (0.66-1.35)	423 (37)	0.96 (0.67-1.38)
Spine Cortical vBMD				
Unadjusted	528 (48)	1.10 (0.81-1.49)	441 (38)	0.95 (0.69-1.32)
Adjusted for Age	528 (48)	1.08 (0.80-1.47)	441 (38)	0.89 (0.65-1.23)
Adjusted for shared risk factors between osteoporosis and PAD	493 (42)	1.05 (0.71-1.54)	423 (37)	1.13 (0.77-1.65)
Total hip aBMD				
Unadjusted	1115 (116)	1.19 (0.98-1.45)	913 (89)	1.28 (1.02-1.60) ^a

Table 3-3 (Continued)

Adjusted for Age	1115 (116)	1.15 (0.94-1.41)	913 (89)	1.23 (0.98-1.54)
Adjusted for shared risk factors between osteoporosis and PAD	1011 (101)	1.26 (0.94-1.68)	881 (87)	1.39 (1.03-1.84)^a
Trochanter aBMD				
Unadjusted	1115 (116)	1.26 (1.03-1.54)^a	913 (89)	1.24 (0.99-1.56)
Adjusted for Age	1115 (116)	1.22 (1.00-1.50)	913 (89)	1.20 (0.96-1.51)
Adjusted for shared risk factors between osteoporosis and PAD	1011 (101)	1.28 (0.97-1.69)	881 (87)	1.27 (0.97-1.66)
Femoral neck aBMD				
Unadjusted	1115 (116)	1.01 (0.83-1.23)	913 (89)	1.07 (0.86-1.34)
Adjusted for Age	1115 (116)	0.98 (0.80-1.19)	913 (89)	1.03 (0.82-1.29)
Adjusted for shared risk factors between osteoporosis and PAD	1011 (101)	1.18 (0.88-1.58)	881 (87)	1.11 (0.84-1.46)

* Models in women were adjusted for age, ethnicity, study site (areal BMD models), educational level, time since menopause, BMI, physical activity, Health ABC physical performance score, smoking status, drinking status, systolic blood pressure, total cholesterol, plasma glucose level, history of hypertension, history of diabetes, use of calcium and vitamin D supplements, osteoporosis medications, and oral hormones.

** Models in men were adjusted for age, ethnicity, study site (areal BMD models), educational level, systolic blood pressure, physical activity, smoking status, BMI, history of hypertension, and history of diabetes.

† BMD SD scores: Women: total hip aBMD (SD= 0.15 g/cm²), femoral neck aBMD (SD= 0.13 g/cm²), trochanter aBMD (SD=0.12 g/cm²), integral vBMD (SD=50.82 mg/cc), trabecular vBMD (SD= 39.98 mg/cc), cortical BMD (SD= 53.80 mg/cc). Men: total hip aBMD (SD= 0.15 g/cm²), femoral neck aBMD (SD= 0.14 g/cm²), trochanter aBMD (SD=0.13 g/cm²), integral vBMD (SD=54.94 mg/cc), trabecular vBMD (SD= 43.59 mg/cc), cortical BMD (SD= 58.61 mg/cc).

^a P<0.05

4.0 VOLUMETRIC BONE MINERAL DENSITY AND VASCULAR CALCIFICATION IN MIDDLE-AGED WOMEN: THE STUDY OF WOMEN'S HEALTH ACROSS THE NATION

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4.1 ABSTRACT

This analysis investigated the association of spine volumetric bone mineral density (vBMD) with aortic (AC) and coronary artery (CAC) calcification in middle-aged women; and evaluated whether such associations were independent of age and shared risk factors between osteoporosis and cardiovascular disease (CVD), or explained by endogenous estradiol levels.

Vascular calcification and trabecular vBMD of the spine were measured using electron-beam computed tomography in 490 women free from clinical CVD in the Study of Women's Health Across the Nation. Women were aged 45-58 years, 61% were Caucasian, and 64% were peri-menopausal. Calcification scores were categorized into 3 levels ("no AC" (N=146), "moderate AC" (scores= 1-74, N=221), and "high AC" (N=123); "no CAC" (N=256), "moderate CAC" (score=1-7.54, N=111), and "high CAC" (N=123)). The highest categories were set at the 75th percentiles. Multinomial logistic regression was used to assess the association between vBMD (per standard deviation (SD)) and the AC and CAC levels, with "no calcification" as the reference group.

AC and CAC were detected in 70% and 48% of the population, respectively. Mean vBMD was 161.6 mg/cc (SD=37.2mg/cc). vBMD was associated with "high AC" in unadjusted, age-adjusted, and risk factor-adjusted analysis. Per one SD decrease in vBMD, the adjusted odds of "high AC", compared to "no AC", was significantly increased by 68% (95%CI 1.06-2.68). Estradiol did not influence this association. vBMD was related to "high CAC" in unadjusted (OR=1.35, 95%CI 1.08, 1.70) but not adjusted models. No associations of vBMD with "moderate AC or CAC" were observed.

In conclusion, lower vBMD was related to high AC, but not to CAC, in a biracial cohort of healthy middle-aged women independent of age and shared risk factors between osteoporosis

and CVD. Further research should investigate possible pathophysiological links between the two conditions, and the potential for common preventive and therapeutic interventions.

4.2 INTRODUCTION

Cardiovascular disease (CVD) and osteoporosis are common age-related conditions. Mounting biological [1-6] and epidemiological evidence supports a link between the two diseases. In both cross-sectional and longitudinal epidemiologic studies, low bone mass has been related to increased cardiovascular mortality, [7-11] cardiovascular morbidity, [12-18] and subclinical markers of atherosclerosis, including vascular calcification. [19-30] Cross-sectionally, a negative association was observed between bone density and calcification of the aorta (AC) [19, 20, 22] and coronary arteries (CAC). [23] Similarly, the presence of AC was associated with a higher prevalence and number of vertebral and hip fractures. [19] The progression of aortic calcification was also linked to trabecular BMD loss at the spine in Caucasian postmenopausal women [19] and to metacarpal bone loss in women in the Framingham study [21] and in a Dutch population-based longitudinal study. [22]

Several hypotheses have been proposed to explain the association between CVD and osteoporosis including: 1) their age-related independent progression, 2) the presence of shared risk factors (such as smoking and physical inactivity), 3) the presence of common pathophysiological mechanisms that could lead to the development of both conditions and which may involve endogenous hormones or inflammatory cytokines, and 4) a cause-effect relationship whereby one condition may be leading to the other. For instance, atherosclerosis, by reducing

blood flow to the lower extremities, could alter bone metabolism in the hip and result in osteoporosis.

Although several lines of evidence suggest a link between CVD and osteoporosis, the nature of this link and the mechanisms involved are still not clearly elucidated. Reports on this association focused mostly on Caucasian postmenopausal women and less is known about the presence of such relationships in younger women and in other ethnic groups. Furthermore, the majority of previous studies did not utilize state-of-the-art assessments of vascular calcification and BMD, such as computed tomography (CT) technology. CT allows for a graded quantification of vascular calcification, and a three-dimensional volumetric determination of bone density that adjusts for bone size and is unaffected by the presence of extra-osseous calcification. Additionally, although estrogen deficiency was suggested as a common denominator in the association between osteoporosis and CVD, [21-23, 31] to our knowledge no study has actually explored the role of endogenous estrogen.

The aim of this analysis was to evaluate the association of spine volumetric BMD (vBMD) with vascular calcification of the aorta and coronary arteries, all determined using electron-beam computed tomography (EBCT), in a biracial cohort of middle-aged women; and to investigate whether such associations were a) independent of age, b) independent of shared risk factors between osteoporosis and CVD, or c) explained by endogenous estradiol levels.

4.3 MATERIALS AND METHODS

4.3.1 Subjects

The current analysis utilized data from the baseline assessment of an ancillary study of subclinical atherosclerosis in the Study of Women's Health Across the Nation (SWAN). SWAN is a multi-center, multi-ethnic, longitudinal study designed to characterize the biological and psychosocial changes that occur during the menopausal transition in a community-based cohort. Details of the study design and recruitment have been previously published. [32] Briefly, SWAN is being conducted at seven sites: Boston, MA, Chicago, IL, Detroit, MI, Los Angeles, CA, Newark, NJ, Pittsburgh, PA, and Oakland, CA. A total of 3,302 women aged 42-52 years were enrolled from 1996 to 1997. At the time of enrollment, women had an intact uterus and at least one ovary and were not pregnant or breast-feeding. All participants were still menstruating, and women who used oral contraceptives or hormone replacement in the prior three months were excluded. Women were followed up annually and evaluated for a wide array of physiologic, physical, behavioral, and psychological measures.

The subclinical disease evaluation (SWAN Heart) was performed at the Pittsburgh and Chicago study sites. SWAN participants at their 04, 05, 06, or 07 follow-up visits were eligible for this ancillary study if they had a carotid ultrasound scan in conjunction with a previous SWAN visit. If they did not have a baseline carotid scan, women were eligible if they had no history of CVD (including angina, myocardial infarction, congestive heart failure, stroke, transient ischemic attacks, coronary revascularization, peripheral vascular surgery, or endarterectomy), were not taking exogenous hormones in the past 3 months, were not taking medications for diabetes at the time of screening, and had no hysterectomy and/or bilateral oophorectomy. A total of 608 women were enrolled in this ancillary study. They underwent a

battery of measures to identify subclinical cardiovascular disease including vascular calcification of the aorta and coronary arteries, in addition to an evaluation of volumetric BMD of the spine.

This analysis included 490 women (189 African-American and 301 Caucasian). Women were excluded for one or more of the following conditions: had history of clinical CVD (N=2), were hormone users (N= 69), had surgical menopause (N= 11), or did not have vBMD or vascular calcification measures (N= 41). None of the women had treated diabetes.

The SWAN study was approved by the institutional review boards of the participating institutions and all women signed informed consent prior to participation.

4.3.2 Aortic and Coronary Calcification

Subjects underwent electron-beam computed tomography (EBCT) for quantification of calcification in the coronary arteries and aorta. An Imatron C-150 Ultrafast CT Scanner (Imatron, San Francisco, CA) was used. The participant was positioned supine on the table with their head toward the gantry. After skin preparation, electrodes were placed and an optimum ECG tracing was obtained. Three passes were performed, and the participants were asked to hold their breath during the scanning. The first was a scout pass that allowed an evaluation of the patient's anatomy so that landmarks for the coronary and aortic scans could be identified. The second scan was for the coronary arteries in which 30 to 40 contiguous 3mm thick transverse images were obtained from the level of the aortic root to the apex of the heart. Images were obtained during a maximal breath hold using ECG triggering so that each 100 millisecond exposure was obtained during the same phase of the cardiac cycle (60% of R-R interval). The third scan was for the aortic evaluation. The scanner was set to acquire images from the aortic arch to the iliac bifurcation and cross-sectional 6mm images were taken with a 300 millisecond

exposure time. The scanner was set in CVS mode so that gating was not required. The participant was again asked to hold her breath during this time. All scan data was saved to an optical disc. The radiation exposure was 0.783 rads for the coronary scan and 2.45 rads for the aortic scan. Readings of coronary and aortic calcification were done centrally in Pittsburgh using a DICOM workstation and software by AcuImage, Inc (South San Francisco, CA). This software program implements the widely accepted Agatston scoring method. Coronary artery and aortic calcium lesions were considered to be present when 3 contiguous pixels greater than 130 Hounsfield units were detected overlying the vessels of interest. Scoring results in a total calcium score as well as the total number of calcifications. The coronary calcification score was obtained from the sum of the individual scores for the four major epicardial coronary arteries. Aortic calcification produced one score. Image analysis was performed by a single physician trained in EBCT to guarantee consistency.

4.3.3 Volumetric Bone Mineral Density

Trabecular volumetric BMD of the spine was determined from the aortic CT scan using an Imatron C-150 Ultrafast CT Scanner (Imatron, San Francisco, CA) and Mindways data acquisition software, calibration phantom, and patient phantom (Mindways Software, Inc, Austin, TX). BMD measurements (mg/cc) were acquired from single-slice cross-sectional images at the level of L3, L4, and L5 vertebral bodies. An average of the 3 BMD values was then obtained. Quality control measures were performed at both study sites including weekly scanning of the calibration phantom, and the use of identical scan protocols for all participants. BMD readings were done centrally at the University of Pittsburgh.

4.3.4 Potential Confounders

Covariates were obtained from the SWAN follow-up visit that corresponds to the SWAN Heart baseline assessment (i.e. 04, 05, 06, or 07 SWAN visits). Variables such as lipids, glucose and insulin levels, and physical activity were obtained from the baseline SWAN visit since they were not available for all follow-up SWAN visits that concurred with the baseline SWAN Heart assessment.

Sociodemographic factors (age, race, study site, education), smoking history, alcohol consumption, and physical activity, were determined using either an interview-administered or a self-administered questionnaire. Physical activity was assessed using an adaptation of the Baecke questionnaire. [33] This self-reported instrument assesses physical activity in different domains including sports, household, and daily routine, on the basis of frequency, intensity, and duration of the activity. Scores for each domain were calculated as the average responses to questions about various activities and ranged from 1 (lowest) to 5 (highest). A total physical activity score was calculated as the sum of the individual scores.

Menopause status was determined using self-reported bleeding patterns and categorized as pre-menopause (a menstrual period within the past 3 months with no change in regularity), early peri-menopause (a menstrual period within the past 3 months but with a self-reported change in cycles), late peri-menopause (no menstrual bleeding for at least 3 months but no more than 12 months), and post-menopause (no menstrual bleeding for at least 12 months).

Blood pressure was measured in the right arm using a standard mercury sphygmomanometer, with the participant seated following at least 5 minutes of rest. Three sequential blood pressure values were completed and the final two were averaged. Weight and height were measured without shoes and with participants wearing light clothing. Portable scales

were calibrated weekly and stationary clinic devices were calibrated monthly. Body Mass Index was calculated as weight in Kg divided by height squared (m^2). Hypertension was defined based on a self-report of a physician's diagnosis of the condition.

A fasting blood draw was targeted to the follicular phase of the menstrual cycle (days 2 to 5). All samples were maintained at 4° C until separated and then were frozen at -80° C and shipped on dry ice to a central laboratory. Standard cardiovascular risk factors were assayed at the Medical Research Laboratories (Lexington, Kentucky, USA), which is certified by the National Heart Lung and Blood Institute, Centers for Disease Control Part III program. Lipids were analyzed on EDTA treated plasma. Total cholesterol and triglycerides were analyzed by enzymatic methods on a Hitachi747 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN). High density lipoprotein (HDL) was isolated using heparin-2M manganese chloride. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. [34] Serum insulin was measured using the RIA (DPC Coat-a-count, Los Angeles, CA) procedure. Glucose was measured using a hexokinase-coupled reaction (Boehringer Mannheim Diagnostics, Indianapolis, IN). The estradiol (E2) assay was conducted at the University of Michigan SWAN Endocrine Laboratory with an ACS-180 automated analyzer (Bayer Diagnostics Corporation, Tarrytown, NY) utilizing a double-antibody chemiluminescent immunoassay with a solid phase anti-IgG immunoglobulin conjugated to paramagnetic particles, anti-ligand antibody, and competitive ligand labeled with dimethylacridinium ester (DMAE). E2 concentrations were measured with a modified rabbit anti-E2-6 ACS-180 immunoassay with increased sensitivity and a lower limit of detection of 1.0 pg/mL. Duplicate E2 assays were conducted with results reported as the arithmetic mean for each subject, with a CV of 3-12%.

4.3.5 Data Analysis

In this analysis, aortic and coronary calcification measures were treated as categorical rather than continuous variables because of their skewness and the large proportion of zero scores (29.8% and 52.2% of the population had 0 scores for AC and CAC, respectively) (Figure 4-1). Aortic calcification (Range: 0-2810, Median= 13) was categorized into 3 levels as follows: “no AC” (0 scores), “moderate AC” (scores between 1-74), and “high AC” (scores ≥ 75). Similarly, coronary calcification (Range: 0-311.4, Median= 0) was categorized into “no CAC” (0 scores), “moderate CAC” (1-7.54), and “high CAC” (≥ 7.55). The highest categories for both AC and CAC were set at the 75th percentile of the variable. Baseline characteristics of women in the different AC groups were compared using chi-square test for categorical variables and either ANOVA or Kruskal-Wallis test for continuous data. The difference in mean vBMD among the 3 AC groups was tested using ANOVA, and a test for linear trend was performed. Multinomial logistic regression was used to assess the association between volumetric BMD, expressed as standard deviation (SD) scores (calculated as the deviation from the mean of vBMD divided by the standard deviation of vBMD), and the 3 AC groups. "No AC" was considered the reference group. This regression approach was used instead of ordinal logistic regression because the assumption of proportional odds was not met. Unadjusted, age-adjusted, and risk factor-adjusted models were performed. Covariates were selected for entry into the multiple regression model if they were associated with both vBMD and AC in univariate analyses, using a 0.15 level of significance. The effect of estradiol on the association between vBMD and AC was tested by introducing this variable into the adjusted regression model. Potential racial differences in the relationship of vBMD with AC were tested by entering a product term for race and vBMD in the multiple multinomial logistic regression model. Associations between vBMD and AC levels

were presented as unadjusted, age-adjusted, and risk factors-adjusted odds ratios (OR) and 95% confidence intervals per 1 SD decrease in vBMD. The same analytical approach was followed for CAC. In both AC and CAC models, no significant interactions between race and BMD were observed, therefore race-specific analyses were not performed. Data were analyzed using SAS version 8.01 (SAS Institute Inc, Cary, NC, USA). Multinomial regression models were fitted using the CATMOD procedure in SAS.

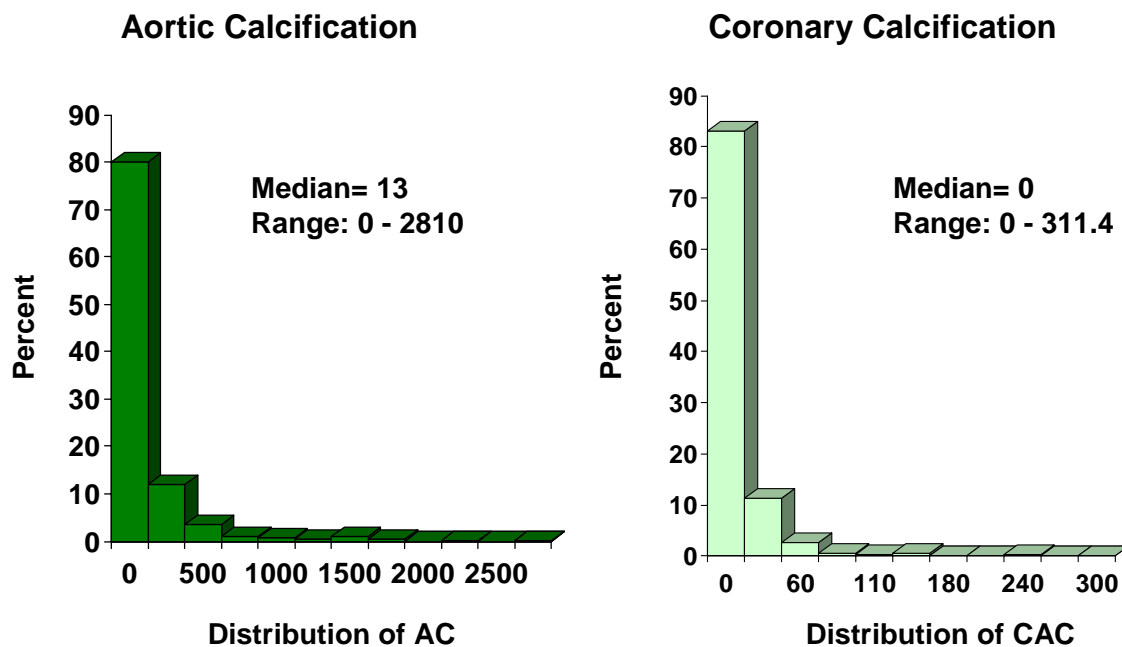


Figure 4-1 Distribution of original aortic and coronary calcium scores

4.4 RESULTS

4.4.1 Participant Characteristics

Figure 4-2 shows the proportion of women in each AC and CAC level. Seventy percent (N= 344) of the population (66% of Caucasian and 77% of African-American women) had AC, defined as an aortic calcium score >0; while 48% (N= 234) had CAC (41.9% of Caucasians and 57.1% of African-Americans), defined as a total coronary calcium score >0 (Figure 4-2).

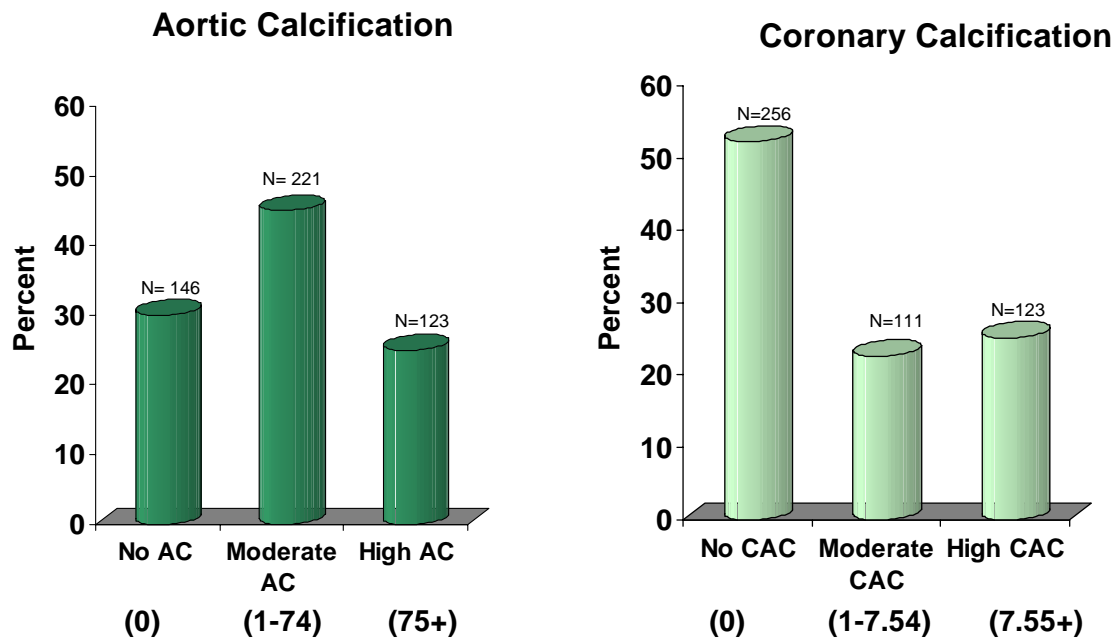


Figure 4-2 Proportion of women in each level of aortic and coronary calcification

The average bone density was 161.6 mg/cc (SD= 37.2 mg/cc) and the mean T-score was 0.26 (SD=1.43). By WHO criteria, 4.7% of the cohort had osteoporosis (T-score < -2.5), and 26.3% had low bone mass (T-score between -2.5 and -1). Participants with “high AC” scores were older, heavier, less physically active, and more likely to be postmenopausal, smokers, and

hypertensive compared to those with “moderate” or “no AC”. A significant decrease in HDL and increase in cholesterol, triglyceride, LDL, glucose, insulin, and systolic and diastolic blood pressure were observed with increasing AC levels (Table 4-1).

Similarly, participants with “high CAC” were older, heavier, and more likely to be hypertensive than those with “moderate” or “no CAC”. A significant increase in lipid levels, blood pressure, glucose, and insulin, and a decrease in HDL and estradiol were observed with increasing CAC levels (Table 4-2).

4.4.2 BMD and Aortic Calcification

There was a significant trend for decreasing vBMD with increasing AC levels (p for trend $<.0001$) (Figure 4-3). In unadjusted logistic regression analysis, lower vBMD was found to be associated with increased OR for “high AC”. The odds of “high AC” compared to “no AC” was significantly increased by 73% (OR=1.73, 95% CI 1.33-2.25), per one SD decrease in vBMD. This association remained significant after adjusting for age (OR=1.54, 95% CI 1.17-2.03) and with further controlling for shared covariates between vBMD and AC. A one SD decrease in vBMD was associated with a 68% increased odds for “high AC” (OR=1.68, 95% CI 1.06-2.68). On the other hand, the adjusted odds of “moderate AC” was increased by 33% per 1 SD decrease in vBMD (OR=1.33, 95% CI 0.93-1.90), however this association was not statistically significant (Table 4-3).

We tested the effect of estradiol on the association between BMD and high AC. Estradiol did not show a significant association with AC (Table 4-1), but was positively and significantly associated with vBMD (Spearman correlation= 0.26, $p <.0001$). After adding estradiol to the model, the adjusted OR for “high AC” per SD decrease in vBMD was only slightly reduced from

1.67 (95% CI 1.05-2.67, based on a sample of 347 women with non-missing estradiol levels, $p=.03$) to 1.62 (95% CI 1.00-2.61, $N=347$).

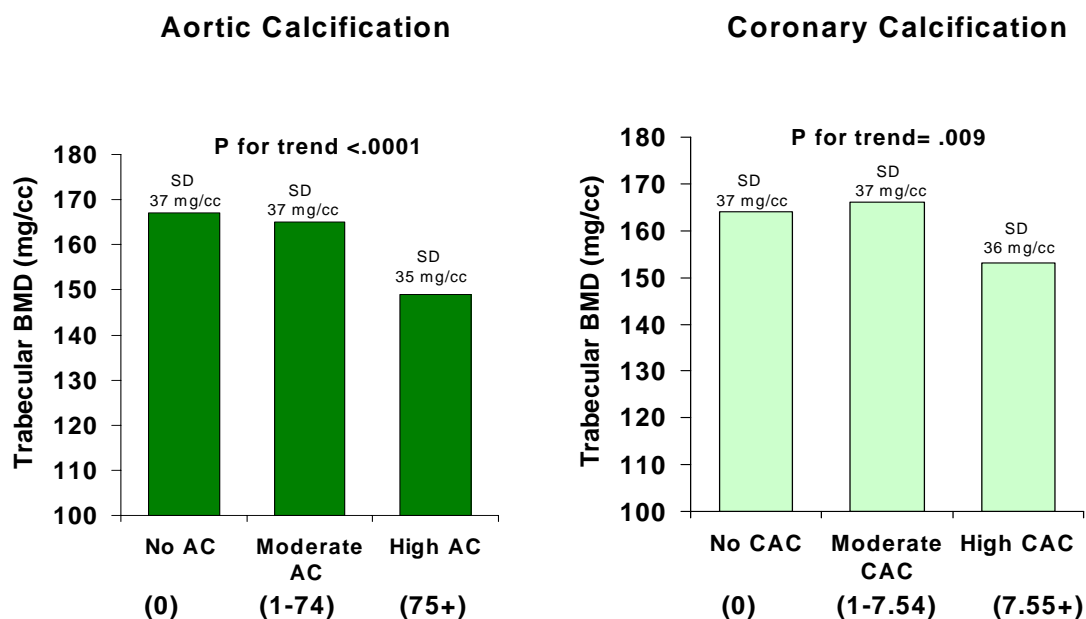


Figure 4-3 Average vBMD by aortic and coronary calcification levels

4.4.3 BMD and Coronary Artery Calcification

Participants with “high CAC” had the lowest vBMD (p for trend = .009) (Figure 3). In unadjusted regression analysis, vBMD was significantly associated with “high CAC” (OR=1.35, 95% CI 1.08-1.70). However, controlling for age reduced the strength of this association and rendered it non-significant (OR= 1.19, 95% CI 0.94-1.51). Additional adjustment for shared covariates between BMD and CAC did not affect the strength or significance of the relationship (OR= 1.19, 95% CI 0.81-1.74). No association between BMD and “moderate CAC” was observed in unadjusted, age-adjusted, or risk factors-adjusted models (Table 4-4).

Lower estradiol was found to be significantly associated with higher CAC levels in univariate analysis (Table 2). Adding estradiol to the model did not have an effect on the association between vBMD and CAC levels. The adjusted OR for high CAC per 1 SD decrease in vBMD changed from 1.24 (95% CI 0.83-1.86, based on a sample of 375 women with non-missing estradiol levels) to 1.21 (95% CI 0.81-1.82, N= 375) after adjusting for estradiol. The OR for moderate AC changed from 1.04 (95% CI 0.74-1.48, based on a sample with non-missing estradiol levels, N= 375) to 1.02 (95% CI 0.72-1.45, N= 375) after adding estradiol to the model. Estradiol itself became not significantly associated with CAC in the adjusted model (p-value= 0.22 for “High CAC”, p-value= 0.44 for “Moderate CAC”).

4.5 DISCUSSION

In this cross-sectional analysis performed in a biracial cohort of women during the menopause transition, lower trabecular BMD of the spine was significantly associated with higher AC levels. This association was not age-related, was independent of shared risk factors between BMD and AC, and was not influenced by estradiol. Additionally, lower vBMD was associated with high CAC levels; however this relationship was not significant after adjusting for age.

Our results on the association of BMD with AC confirm prior cross-sectional and longitudinal findings and extend them to a younger cohort of Caucasian and African- American women who were predominantly peri or pre-menopausal. Schulz et al observed that the presence of EBCT-determined AC was associated with lower BMD and higher prevalence of vertebral and hip fractures in Caucasian postmenopausal women. [19] In a cohort of Danish postmenopausal women, Tanko et al reported a negative correlation between hip BMD and

radiographically determined AC which was independent of age and common risk factors. [20] Longitudinally, the progression of AC was linked to a higher degree of bone loss. Schulz et al observed that the yearly rate of progression of AC explained 47% of the variance in the yearly rate of bone loss in Caucasian postmenopausal women. [19] In the Framingham Study, each percent decline in metacarpal cortical area was associated with a 7.3% increase in AC among Caucasian women followed for 25 years. [21] Similarly, in a Dutch population-based study, Hak et al reported a higher degree of metacarpal bone loss among women who had an AC progression through the menopause compared to those who did not. Other studies have failed to observe an association between BMD and AC. In the Study of Osteoporotic Fractures, no significant association was observed between aortic calcification and bone density at the hip, spine, or calcaneus after adjusting for age; only a weak association with radial BMD was noted. [35] Similar results were reported by Frye et al among women in Rochester, Minnesota, [36] and by Aoyagi in a population of Japanese-American women. [37]

Traditionally, osteoporosis and CVD have been regarded as independent processes that occur with aging. Therefore, the apparent association between them was thought to be due to their age-related independent progression. [35-37] Mounting biological observations [1-6] and epidemiologic evidence from this study and others [7-30] suggest a link between the two conditions that is independent of age. Laboratory studies indicate that atherosclerotic calcification and bone calcification share a number of common features. It is now considered that the arterial tissue is calcified in a highly regulated and organized process by mechanisms similar to those involved in bone mineralization. [1, 38] Hydroxyapatite, a mineral that is present in bones, is also found in calcium deposits of atherosclerotic plaques. [4] In addition, calcified

plaques express several bone matrix proteins such as GLA protein, bone morphogenetic protein-2, osteopontin, osteocalcin, and collagen I. [2, 3, 5, 6]

Several hypotheses have been proposed to explain the link between osteoporosis and CVD. One hypothesis puts forth that the co-existence of the 2 conditions is due to their shared etiological factors (such as menopause, smoking, physical activity, alcohol intake, hypertension, diabetes, etc.), which may simultaneously promote or inhibit subclinical atherosclerosis and bone demineralization. In our analysis, the observed inverse association between vBMD, a surrogate measure for osteoporosis, and AC, a marker of subclinical atherosclerosis, was present after controlling for age, ethnicity, and other common etiological factors for osteoporosis and CVD such as weight, physical activity, menopause status, blood pressure, and lipids. This suggests that common pathophysiological mechanisms may be at play in the progression of the two conditions. One such mechanism is estrogen deficiency. Estrogen deficiency has been identified as the major determinant of age-related bone loss in women and men. [39, 40] And despite recent evidence from randomized, placebo-controlled trials on the adverse effects or lack of effects of HRT on CVD outcomes, [41, 42] endogenous estrogen is known to have protective effects on the cardiovascular system in women, [43] either directly or through the modulation of other factors including cytokines, oxidized lipids, and endothelial nitric oxide synthase (eNOS), the main source of vascular NO. [31, 43] A beneficial effect of estrogen use on arterial calcification has also been suggested by some studies. [35, 44] In our analysis, estradiol was positively correlated with vBMD, however it was not associated with the extent of AC. Adding estradiol to the model minimally reduced the strength of the association between BMD and AC, and in itself, estradiol was not significantly associated with AC. This suggests that estrogen deficiency was not a major player in this association. Other factors may be involved in the link

between vascular calcification and bone demineralization including inflammatory markers, [12, 22] oxidized lipids, [45] imbalances in the calciferol endocrine system, [46] vitamin K deficiency, [47] or genetic factors. [48] Mice lacking the osteoprotegerin gene were found to develop early-onset osteoporosis and calcification of the aorta and renal arteries. [48]

We observed an association between BMD and CAC in unadjusted analysis; however, this association became non significant after adding age to the model. Only one other study has looked at the association of BMD with CAC but yielded inconsistent results. In a population of Caucasian postmenopausal women free from CAD, a negative correlation between CAC and BMD of the hip was observed, and women with lumbar spine osteoporosis had a significantly higher CAC score than controls. These results were not explained by age or common risk factors for CVD and osteoporosis, as the distribution of these variables was similar in women with osteoporosis and controls. [23] The prevalence of any CAC in our population (48%) was higher than that reported in the Healthy Women Study (HWS) (37%); however, the extent of CAC in our cohort was lower. Only 2.4% (N= 12) of this cohort had CAC scores ≥ 100 (range: 108-311) compared to 12.4% in HWS, which could be explained by the fact that the HWS cohort included postmenopausal women and was older (mean age in HWS= 59, mean age in SWAN Heart= 50). [49] Therefore, it is possible that we did not observe an association between BMD and CAC owing to the fact that our young cohort has not yet developed extensive CAC. It is known that aortic atherosclerosis begins earlier in life than coronary atherosclerosis, [49, 50] which might explain why we observed an association between BMD and AC but not CAC. Notably, in the study by Barengolts et al, which observed an association between BMD and CAC in postmenopausal women, calcium deposits (CAC scores >1) and osteoporosis were detected in

76% and 31% of the population, respectively. [23] Both proportions were much higher than those observed in our cohort (48% for CAC and 4.7% for osteoporosis).

Our study has several advantages. The associations were investigated in a well-characterized biracial cohort of middle-aged women who were free clinical CVD, and they were adjusted for a comprehensive set of shared risk factors for osteoporosis and CVD. Our study also had the advantage of using CT to simultaneously assess bone density and vascular calcification of the aorta and coronary arteries. In the existing literature, vascular calcification was mostly assessed using conventional radiography, [20-22, 35-37, 51] which has low sensitivity to the detection of small calcium deposits. Similarly, in a large number of studies, bone mass was determined using radiographic techniques, single-photon or single X-ray absorptiometry, or dual-photon absorptiometry. [8-10, 15, 17, 21, 22, 27, 29, 37] Some studies have employed DXA in bone determination; [7, 11-14, 16, 18, 20, 24-26, 28, 30, 35, 36] however, this projectional technique is limited by its 2-dimensional areal assessment of BMD which does not adjust for bone size. This is especially important in studies of women of different ethnic groups since there are well established ethnic differences in bone size. DXA is also affected by the presence of extra-osseous calcification such as aortic calcification and degenerative osteoarthritic changes, which get incorporated in the region of interest and lead to a falsely increased bone density of the spine. [51] CT technology, which allows for a three-dimensional volumetric determination of bone density, an assessment of purely trabecular bone, and a graded quantification of vascular calcification, was used only in one study for BMD and aortic calcification quantification, [19] and in another one for coronary calcification assessment. [23]

The main limitation of this study is its cross-sectional nature which does not allow the evaluation of a causal association between vascular calcification and low bone density and the

elucidation of common mechanisms involved in the pathogenesis of both conditions. Additionally, our cohort included healthy middle-aged women which may limit the generalizability of the results to other populations.

In conclusion, our results provide further evidence for a link between BMD and AC in women that is independent of age and shared risk factors. However, establishing a link between bone demineralization and vascular calcification requires more longitudinal evidence and further investigation into common pathophysiological mechanisms for the two conditions. Once confirmed, such association may lead to the early identification of subjects at risk for CVD or osteoporosis, and to the potential for common preventive and therapeutic interventions that target both conditions.

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Table 4-1 Participants Characteristics (% , mean \pm SD, or median (IQR)) by AC Levels, the SWAN Study

	No AC (Score= 0) N= 146	Moderate AC (Score= 1-74) N= 221	High AC (Score \geq 75) N= 123	P-value
Age (yrs)	49.3 \pm 2.6	50.1 \pm 2.9	50.7 \pm 2.8	<.0001
% African-American	30	44	39	.03
Menopause Status				
% Pre-menopause	9.9	12.0	6.6	
% Early peri-menopause	62.1	49.0	49.1	.10
% Late Peri-menopause	6.8	13.0	13.2	
% Post-menopause	21.2	26.0	31.1	
% Current Smoker	10.2	11.6	29.7	<.0001
% Hypertensive	8.3	13.2	18.8	.04
Physical activity score	8.4 (7.3, 9.6)	7.9 (6.8, 9.2)	7.6 (6.7, 8.8)	.008
Weight (Kg)	65.6 \pm 8.5	81.9 \pm 14.4	87.9 \pm 23.0	<.0001
Height (cm)	162.5 \pm 5.8	164.1 \pm 6.4	164.7 \pm 5.7	.005
Cholesterol (mg/dl)	179.2 \pm 29.9	194.3 \pm 32.9	205.0 \pm 37.4	<.0001
LDL (mg/dl)	102.4 \pm 27.0	118.9 \pm 28.8	127.2 \pm 34.1	<.0001
HDL (mg/dl)	60.0 \pm 14.4	54.2 \pm 12.9	52.5 \pm 14.3	<.0001
Triglyceride (mg/dl)*	76.5 (59.5, 98.0)	91.5 (66.0, 125.0)	105.5 (78.0, 154.0)	<.0001
Glucose (mg/dl)*	88.0 (83.5, 93.0)	91.0 (86.0, 99.0)	92.0 (86.0, 100.0)	<.0001
Insulin (uIU/ml)*	6.8 (5.4, 9.3)	8.8 (6.8, 14.0)	11.0 (7.7, 16.1)	<.0001
DBP (mmHg)	74.1 \pm 9.6	76.6 \pm 9.7	77.0 \pm 10.4	.02
SBP (mmHg)	114.5 \pm 14.8	119.9 \pm 15.5	124.0 \pm 18.1	<.0001
Estradiol level (pg/mL)*	35.1 (17.5, 106.9)	30.2 (15.8, 67.0)	27.3 (17.8, 68.6)	.34
vBMD (mg/cc)	167.2 \pm 37.0	165.2 \pm 37.2	148.6 \pm 34.7	<.0001
BMD T-score	-0.05 \pm 1.42	-0.12 \pm 1.43	-0.76 \pm 1.34	<.0001

* Medians, IQR, and p-value from Kruskal-Wallis test are reported because of the skewed distribution of the variable

Table 4-2 Participants Characteristics (%, mean \pm SD, or median (IQR)) by CAC Levels, the SWAN Study

	No CAC (Score= 0) N= 256	Moderate CAC (Score= 1-7.54) N= 111	High CAC (Score \geq 7.55) N= 123	P-value
Age (yrs)	49.6 \pm 2.7	50.2 \pm 3.1	50.9 \pm 2.6	<.0001
% African-American	31.6	46.0	46.3	.004
Menopause Status				
% Pre-menopause	11.1	10.9	7.2	
% Early peri-menopause	55.6	55.4	45.0	.11
% Late Peri-menopause	9.8	13.9	11.7	
% Post-menopause	23.5	19.8	36.0	
% Current Smoker	17.3	11.2	16.8	.36
% Hypertensive	7.0	16.7	22.3	.0001
Physical activity score	8.3 (7.2, 9.5)	7.6 (6.9, 9.0)	7.7 (6.6, 8.9)	.01
Weight (Kg)	68.7 \pm 11.2	85.2 \pm 14.4	93.0 \pm 20.0	<.0001
Height (cm)	163.2 \pm 6.0	163.8 \pm 6.3	164.9 \pm 6.0	.02
Cholesterol (mg/dl)	185.9 \pm 30.1	193.4 \pm 36.7	205.5 \pm 37.8	<.0001
LDL (mg/dl)	109.6 \pm 27.9	118.0 \pm 33.0	127.5 \pm 32.6	<.0001
HDL (mg/dl)	58.0 \pm 14.5	53.3 \pm 13.5	52.2 \pm 12.6	.0002
Triglyceride (mg/dl)*	79.0 (61.0, 108.0)	92.0 (68.0, 127.0)	107.0 (78.0, 164.0)	<.0001
Glucose (mg/dl)*	88.0 (84.0, 94.0)	92.0 (87.0, 99.0)	94.0 (88.0, 101.0)	<.0001
Insulin (uIU/ml)*	6.9 (5.3, 9.4)	10.3 (7.7, 13.6)	11.6 (7.8, 17.3)	<.0001
DBP (mmHg)	73.3 \pm 9.0	78.1 \pm 9.9	79.6 \pm 10.2	<.0001
SBP (mmHg)	114.4 \pm 13.6	123.1 \pm 16.0	126.3 \pm 18.5	<.0001
Estradiol level (pg/mL)*	42.4 (17.5, 102.6)	27.6 (16.4, 63.0)	23.1 (14.4, 41.9)	.0007
vBMD (mg/cc)	163.8 \pm 37.1	166.1 \pm 37.3	153.1 \pm 36.4	.01
T-score	-0.18 \pm 1.43	-0.09 \pm 1.43	-0.59 \pm 1.4	.009

* Medians, IQR, and p-value from Kruskal-Wallis test are reported because of the skewed distribution of the variable

Table 4-3 Results of the multinomial logistic regression models for AC: unadjusted, age-adjusted, and risk factors-adjusted odds ratios (95% CI) per 1 SD* decrease in vBMD

	AC Level		
	No AC	Moderate AC	High AC
	(0)	(1-74)	(≥75)
	Odds Ratio	Odds Ratio	Odds Ratio
	N	N	N
vBMD (unadjusted)	1.00	1.05	1.73 ^a
		(0.86, 1.30)	(1.33, 2.25)
	146	221	123
vBMD (adjusted for age)	1.00	0.98	1.54 ^b
		(0.78, 1.22)	(1.17, 2.03)
	146	221	123
vBMD (adjusted for age + shared risk factors)[†]	1.00	1.33	1.68 ^c
		(0.93, 1.90)	(1.06, 2.68)
	109	171	84

* vBMD SD= 37.2 mg/cc

[†] Adjusted for age, race, study site, menopause status, educational level, smoking status, physical activity score, weight, height, diastolic blood pressure, LDL, and triglyceride level.

^a P<0.0001

^b P<0.01

^c P<0.05

Table 4-4 Results of the multinomial logistic regression models for CAC: unadjusted, age-adjusted, and risk factors-adjusted odds ratios (95% CI) per 1 SD* decrease in vBMD

	CAC Level		
	No CAC	Moderate CAC	High CAC
	(0)	(1-7.54)	(≥7.55)
	Odds Ratio	Odds Ratio	Odds Ratio
	N	N	N
vBMD (unadjusted)	1.00	0.94	1.35 ^a
		(0.75, 1.17)	(1.08, 1.70)
	256	111	123
vBMD (adjusted for age)	1.00	0.88	1.19
		(0.70, 1.10)	(0.94, 1.51)
	256	111	123
vBMD (adjusted for age + shared risk factors)[†]	1.00	1.09	1.19
		(0.77, 1.53)	(0.81, 1.74)
	211	91	98

* vBMD SD= 37.2 mg/cc

[†] Adjusted for age, race, study site, menopause status, alcohol drinking, physical activity score, weight, height, diastolic blood pressure, LDL, and triglyceride level.

^a P<0.01

5.0 BONE MINERAL DENSITY AND INCIDENT CARDIOVASCULAR DISEASE IN OLDER MEN AND WOMEN: THE HEALTH, AGING, AND BODY COMPOSITION STUDY

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5.1 ABSTRACT

This analysis investigated the associations of volumetric (vBMD) and areal (aBMD) bone mineral density measures with incident cardiovascular disease (CVD) in a biracial cohort of older men and women with no prior history of CVD; and evaluated whether such associations were a) independent of age, b) independent of shared risk factors between BMD and CVD, or c) explained by inflammatory cytokines (IL-6 and TNF- α) and oxidized LDL (oxLDL).

The subjects were 2310 well-functioning white and black men and women (42% Black; 55% women), aged 68-80 years, enrolled in the Health, Aging, and Body Composition Study. CVD was defined as the incidence of coronary heart disease, cerebrovascular disease, carotid artery disease, or peripheral arterial disease during an average follow-up of 5.4 years. Total hip, femoral neck, and trochanter aBMD were assessed using DXA. Spine trabecular, integral, and cortical vBMD measures were assessed using QCT in a subset (n=1095). Cox proportional hazards regression was used to assess associations of BMD measures (per standard deviation (SD) decrease) with CVD. Multiple Cox regression models controlled for BMI, physical activity, lipids, blood pressure, and other important covariates.

During follow-up, 23% (241 out of 1040) of men and 14% (182 out of 1270) of women had incident CVD. Spine vBMD measures were inversely associated with incident CVD in white men [HR (integral)=1.39, 95%CI 1.03-1.87; HR (cortical)=1.38, 95%CI 1.03-1.84], but not in blacks. In women, with each SD decrease in femoral neck aBMD, the risk of CVD was significantly increased by 24%. In race-specific analyses, aBMD measures of the total hip (HR=1.36, 95% CI 1.03-1.78), femoral neck (HR= 1.44, 95%CI 1.10-1.90) and trochanter (HR=1.34, 95%CI 1.04-1.72) exhibited significant associations with CVD in black women, but not in whites. All associations were independent of age and shared risk factors between

osteoporosis and CVD. Inflammatory cytokines and oxLDL did not influence these relationships. In conclusion, our results provide epidemiologic evidence for an inverse association between BMD and the risk of CVD in women and white men. Further research should elucidate possible pathophysiological mechanisms linking osteoporosis and CVD, and the potential for common preventive and therapeutic interventions.

5.2 INTRODUCTION

Cardiovascular disease (CVD) and osteoporosis are common age-related conditions. Mounting biological [1-6] and epidemiological evidence suggests a possible link between the two diseases. In both cross-sectional and longitudinal epidemiologic studies, low bone mass has been related to increased cardiovascular mortality, [7-11] cardiovascular morbidity, [12-18] and subclinical measures of atherosclerosis. [19-29]

Osteoporosis was found to be a strong predictor of future cardiovascular outcomes in postmenopausal women with low bone mass, independent of age and other traditional cardiovascular risk factors (adjusted HR= 3.9, 95%CI 2.0-7.7). [12] It was also associated with angiographically-determined coronary artery disease in a retrospective analysis of a population predominantly of women referred for angiography and BMD assessment. [13] Lower bone mass was related to higher incident coronary heart disease, [15] incident stroke, [17] and prevalent stroke in white postmenopausal women. [16] Associations between BMD and CVD were also reported in men. Previous myocardial infarction was associated with low BMD in a multiethnic population of men in the Third National Health and Nutrition Examination Survey (NHANES III). [14] Lower bone mineral content was related to asymmetrical symptomatic peripheral

arterial disease in a small study involving 18 men. [18] Recently, in a cross-sectional analysis in the Health, Aging, and Body Composition (Health ABC) Study, we observed that volumetric BMD (vBMD) measures of the spine were significantly associated with prevalent CVD in women and men, and areal BMD (aBMD) of the trochanter was related to prevalent CVD in women. These inverse relationships were not age-related and were independent of shared risk factors between osteoporosis and CVD (unpublished results).

Although several lines of evidence support a link between CVD and osteoporosis, the nature of this association and the mechanisms involved are still not clearly elucidated. Most of the earlier work focused on white women. [7-9, 12, 13, 17, 20, 21, 23, 25] Less is known about the presence of this relationship in men and in black populations. Additionally, previous studies relied on prevalent CVD or subclinical markers of atherosclerosis, [13, 14, 16, 18-25, 27-29] and areal assessments of BMD. [7-19, 21-29] To our knowledge, no study assessed the association of volumetric BMD measures with incident CVD events. Furthermore, while inflammatory cytokines and oxidized LDL (oxLDL) were suggested as common denominators in the association between osteoporosis and CVD, none of the reported epidemiologic studies has actually examined their roles.

The purpose of this analysis was to longitudinally examine the association of baseline volumetric and areal BMD measures with incident CVD in a biracial cohort of men and women with no history of CVD; and to determine whether such associations were a) independent of age, b) independent of shared risk factors between osteoporosis and CVD, or c) explained by common pathophysiological factors such as oxLDL or the inflammatory cytokines tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6).

5.3 MATERIALS AND METHODS

5.3.1 Subjects

Participants were enrolled in the Health, Aging, and Body Composition (Health ABC) Study, a population-based prospective study investigating the association between changes in body composition and functional decline in the elderly. The cohort included 3075 well-functioning, community-dwelling men and women aged 68-80 years. The demographic distribution of the population was as follows: 729 black women, 855 white women, 552 black men, and 939 white men. Participants were recruited between April 1997 and June 1998 and were identified from a random sample of white Medicare beneficiaries and all age-eligible black community residents in designated zip code areas surrounding Pittsburgh, PA, and Memphis, TN. Subjects who reported difficulty walking one quarter of a mile, climbing 10 steps, or performing basic activities of daily living, who had a life threatening illness in the 3 years prior to the study, or who were planning to move in the next 3 years were excluded. Written informed consent was obtained from all participants. The study protocol was approved by the Institutional Review Boards of the University of Pittsburgh and the University of Tennessee.

The current analyses included 2310 participants. We excluded participants who had one or more cardiovascular diseases (N= 765) at the baseline visit including coronary heart disease (CHD) (N= 559), cerebrovascular disease (N= 223), or peripheral arterial disease (PAD) (N= 158). Areal BMD data was missing for 25 participants. Volumetric BMD was collected at the Pittsburgh site only and was therefore available for 1095 participants (out of 1124).

5.3.2 Incident Cardiovascular Disease

Incident CVD was defined as the onset of one or more of the following conditions between study entry and June 30, 2004: coronary heart disease (CHD) (defined as coronary death or any overnight hospitalization in an acute care hospital due to myocardial infarction, angina pectoris, symptomatic coronary insufficiency, or other ischemic heart disease, N= 277); cerebrovascular disease (defined as cerebrovascular death or any overnight hospitalization due to stroke or transient ischemic attacks, N= 150), PAD (defined as overnight hospitalization due to lower extremity claudication, atherosclerosis, or thrombosis/ embolism, N= 32), or carotid artery disease (defined by vascular or surgical procedure to improve flow to the ipsilateral brain, symptomatic disease with abnormal findings (>50% stenosis on carotid angiogram or Doppler), or symptomatic disease with carotid artery disease listed on discharge summary, N=33).

Participants were contacted every 6 months, alternating clinic visits and telephone interviews, to elicit information about events. For each reported event, hospital records were collected and abstracted. Cardiovascular events were reviewed locally by a study physician, and verified using standard criteria. Dates and causes of death were verified by a committee made up of 4-6 Health ABC physicians, using death certificates, hospital records, and proxy interviews. Adjudicated events occurring through June 30, 2004 were available. Follow-up time was calculated from the date of the first study visit to the date of the first cardiovascular event, or last contact with the patient (for participants who did not develop CVD or were lost to follow-up), or death (for participants who died from non-CVD causes). The average follow-up time was 5.4 years.

5.3.3 Areal Bone Mineral Density

Baseline areal BMD (g/cm²) measures of the total hip and hip subregions (femoral neck and trochanter) were determined using dual-energy X-ray absorptiometry (DXA) (Hologic 4500A, version 9.03; Hologic, Inc., Waltham, MA, USA). DXA quality assurance measures were performed at both study sites and identical scan protocols were used for all participants.

5.3.4 Volumetric Bone Mineral Density

Baseline volumetric BMD measures (mg/cc) of the spine were determined using quantitative computed tomography (QCT) (General Electric 9800 Advantage, 80 kVp/140 mAs, 10-mm slice thickness; GE Medical Systems Milwaukee, WI). QCT images were acquired at the level of the L3 vertebra to obtain trabecular and integral BMD. Cortical BMD, which includes the cortical shell of the vertebral body and the posterior elements, was estimated by taking the difference in BMC between the integral and trabecular regions and dividing it by the difference in the volumes of these two regions. Scans were performed by certified technicians and analyzed with a standardized protocol at the University of California, San Francisco.

5.3.5 Inflammatory Cytokines and Oxidized LDL

The concentrations of IL-6, TNF- α , and oxLDL were obtained from frozen stored serum samples collected via venipuncture after an overnight fast at baseline. IL-6 and TNF- α were measured in duplicate using commercial ELISA assays from R&D Systems (Minneapolis, MN). The lower detectable limit was 0.10 pg/ml for IL-6 and 0.18 pg/ml for TNF- α , the detection range was 0.156-17.0 pg/ml for IL-6 and 0.5-32 pg/ml for TNF- α , and the interassay coefficient of variation was 10.3% for IL-6 and 15.8% for TNF- α . Plasma levels of oxLDL were measured using a

monoclonal antibody (4E6)–based competition enzyme-linked immunosorbent assay. The interassay coefficient of variation of oxLDL is 12%. IL-6, TNF- α , and oxLDL were available for 2197, 2152, and 2282 participants, respectively.

5.3.6 Potential Confounders

Potential confounders of the association between BMD and CVD were identified and adjusted for. Sociodemographic factors (age, gender, race, study site, education), smoking history, alcohol consumption, weekly physical activity from walking and exercise (Kcal/Kg/hour), medication use (including hormone therapy, statins, osteoporosis drugs, thiazide diuretics, systemic corticosteroids, calcium supplements, vitamin D supplements), and time since menopause were determined by an interview-administered questionnaire. Medication use in the previous 2 weeks was coded using the Iowa Drug Information System (IDIS) ingredient codes. [30]

Lower extremity physical function was assessed by the Health ABC performance battery, a supplemented version of the lower-extremity performance test used in the Established Populations for the Epidemiologic Studies of the Elderly (EPSE; chair stands, standing balance, 6-m walk for gait speed) [31] with increased test duration, a single foot stand, and a narrow walk test of balance as previously described (score range 0-12). [32] Height and weight were obtained using a Harpenden stadiometer (Holtain, Wales, UK) and a standard balance beam, respectively, and body mass index (BMI) was calculated as weight divided by height squared (Kg/m^2). Seated systolic and diastolic blood pressures were measured by a manual mercury sphygmomanometer using a standardized protocol.

Prevalent diabetes was defined as self-report of diabetes previously diagnosed by a physician, use of hypoglycemics medications, or a fasting glucose ≥ 126 mg/dl. Prevalent

hypertension was defined as self-report of hypertension and use of anti-hypertensive medications. Prevalent osteoporosis (total hip BMD 2.5 SD or more below the young adult mean) and low bone mass (total hip BMD between 1.0 and 2.5 SD below the young adult mean) were defined using gender and race-specific T-scores determined from the NHANES III study population. [33]

Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride were measured by a colorimetric technique (Johnson & Johnson Vitros 950 analyzer, New Brunswick, New Jersey). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. [34] Plasma glucose was measured using an automated glucose oxidase reaction (YSI 2300 STAT Plus Glucose & Lactate Analyzer; YSI Life Sciences, Inc., Yellow Springs, Ohio). Serum insulin was assessed using a commercially available radioimmunoassay kit (Pharmacia, Uppsala, Sweden).

5.3.7 Data Analysis

All analyses were stratified by gender. Baseline characteristics and BMD measures of groups with or without incident CVD were compared using chi-square test for categorical variables and either 2-sample t-test or Wilcoxon rank-sum test for continuous data. Cox proportional hazards regression was used to estimate the hazard ratio (HR) of incident CVD per SD decrease in BMD (calculated as the deviation from the mean BMD divided by the standard deviation of the BMD measure in each gender). Unadjusted, age-adjusted, and risk factors adjusted models were fitted. Variables were selected for entry into the multiple Cox models if they were associated with incident CVD and any of the BMD variables at the 0.15 level of significance in univariate analyses. Separate Cox regression models were fitted for each BMD variable. The proportional

hazards assumption was checked by testing the significance of interaction terms of BMD variables with time. Racial differences in the relationship of BMD with incident CVD were tested by entering product terms for race and BMD measures in the adjusted Cox regression models. In cases where significant race interactions were observed at the 0.1 level of significance, the analyses were further stratified by race. In women, interactions between race and aBMD measures of the total hip (p-value for BMD x race= 0.05), femoral neck (p-value= 0.04), and trochanter (p-value= 0.05) were observed. In men, interactions between race and integral (p-value= 0.07), cortical (p-value= 0.08), and trabecular (p-value= 0.10) vBMD measures of the spine were observed. Therefore, the Cox models specific for these BMD measures were further stratified by race.

Associations between BMD measures and CVD were presented as unadjusted, age-adjusted, and risk factors-adjusted hazard ratios (HR) and 95% confidence intervals per 1 SD decrease in BMD.

The effects of IL-6, TNF- α , and oxLDL on associations between BMD measures and CVD were tested by introducing these variables, separately, into adjusted Cox models. Because of the skewed distribution of IL-6, TNF- α , and oxLDL, their log-transformed values were used in analyses. Data were analyzed using SAS version 8.01 (SAS Institute Inc, Cary, NC, USA).

5.4 RESULTS

5.4.1 Participant Characteristics

During an average follow-up of 5.4 years, 23% (241 out of 1040) of men and 14% (182 out of 1270) of women had an incident CVD event. Compared to men who did not develop CVD, those

who did were older, less educated, more likely to have diabetes, had lower HDL and Health ABC physical performance score, and had higher levels of glucose, systolic blood pressure, and inflammatory markers. In women, participants who had incident CVD were heavier, more likely to be hypertensive or diabetic, had higher levels of glucose, systolic blood pressure, and inflammatory markers, had a lower Health ABC physical performance score, and were less likely to be calcium users (Table 5-1).

5.4.2 BMD and Incident CVD in Women

In women, none of the areal or volumetric BMD variables were significantly associated with incident CVD in unadjusted or age-adjusted analyses. However, after controlling for shared risk factors between CVD and BMD, femoral neck aBMD was found to be significantly associated with CVD. A decrease in femoral neck aBMD by 1 SD below the mean was related to a 24% increased risk for incident CVD (HR= 1.24, 95% CI 1.02-1.52) (Table 5-2).

In race-specific adjusted models, we observed that aBMD measures of the total hip and hip subregions were significantly associated with CVD in black women. A 1 SD decrease in aBMD of the total hip, femoral neck, and trochanter was related to an increased CVD risk in the order of 36%, 44%, and 34%, respectively. On the other hand, none of these measures showed associations with CVD in white women (Table 5-3).

We looked at the effects of IL-6, TNF- α , and oxLDL on the associations of BMD with CVD in black women. IL-6 level was significantly higher among black women who had CVD (median IL-6= 1.97 pg/ml) compared to those who did not (median IL-6= 1.82 pg/ml, $p=.003$). However, IL-6 was positively correlated with aBMD measures of the hip (total hip: $r=0.17$, $p<.0001$; femoral neck: $r=0.19$, $p<.0001$; trochanter: $r=0.14$, $p=.0009$). Adding IL-6 to the

adjusted models, did not affect the strength or significance of the association between BMD measures and CVD (Table 4). For instance, the adjusted HR for incident CVD per 1 SD decrease in femoral neck aBMD was similar before (HR= 1.51, 95% CI 1.14-1.99) and after adjusting for IL-6 (HR= 1.49, 95% CI 1.13-1.96). Similarly, adjusting for TNF- α or oxLDL had no effect on the associations of aBMD measures with incident CVD (Table 5- 4).

5.4.3 BMD and Incident CVD in Men

In men, none of the BMD measures showed statistically significant associations with CVD in unadjusted or adjusted analyses. However, there was a trend for higher CVD incidence with decreased integral (HR= 1.17, 95% CI 0.93-1.48), cortical (HR= 1.18, 95% CI 0.94-1.48), and trabecular (HR=1.10, 95% CI 0.87-1.39) vBMD measures of the spine (Table 5-2).

In adjusted models stratified by race, we observed that spine integral and cortical vBMD measures were significantly associated with CVD in white men. The risk of incident CVD was increased by 39% and 38%, respectively, with every SD decrease in integral and cortical vBMD measures (Table 5-3). On the other hand, none of these measures showed associations with CVD in black men.

We looked at the effects of IL-6, TNF- α , and oxLDL on the associations of BMD with CVD in white men. White men with CVD had higher IL-6 and TNF- α levels than those who did not have CVD, whereas no significant difference in oxLDL was observed between the 2 groups. IL-6, TNF- α , and oxLDL were not correlated with integral or cortical BMD measures in white men, and adding them, separately, to adjusted Cox models did not affect the associations of BMD with CVD (Table 5-5).

5.5 DISCUSSION

This prospective analysis examined the association of BMD measures with incident CVD in a biracial cohort of older men and women who had no evidence of cardiovascular disease at baseline. Volumetric BMD measures of the spine were significantly associated with incident CVD in white men. Areal BMD of the femoral neck was related to CVD in women, and in race-specific analyses, aBMD measures of the total hip, trochanter, and femoral neck were associated with CVD in black women. These inverse relationships were independent of age and shared risk factors between BMD and CVD, and were not explained by inflammatory markers or oxLDL.

Our findings in men make an important addition to the existing literature as this is the first longitudinal study to report an association between BMD and cardiovascular events in men with no history of CVD. These associations were consistent with previous findings. In a cross-sectional analysis in this cohort, we observed significant inverse associations between spine vBMD measures and prevalent CVD in men. A one SD decrease in cortical, integral, or trabecular vBMD was associated with 36%, 34%, and 25% increased odds for CVD, respectively (unpublished results). In NHANES III, men with a history of MI had a significantly higher prevalence of low BMD (OR= 1.39, 95% CI 1.03-1.87). [14] In a study involving 18 men with asymmetrical symptomatic peripheral arterial disease, bone mineral content was shown to be significantly lower in the affected compared to the unaffected leg. [18] Additionally, BMD was inversely related to CVD mortality in white men in the NHANES I study and in a British population. [10, 11]

Interestingly, the associations of BMD measures with CVD were observed in white men, but not in blacks. This might be explained by the lower BMD and the higher incidence of CVD in white men compared to blacks. In this cohort, the incidence of CVD was 23% in white men

and 16% in black men. Spine vBMD measures were significantly lower in whites than blacks (mean integral vBMD= 248.6 mg/cc in whites vs. 285.2 mg/cc in blacks, $p<.0001$; mean cortical vBMD= 294.8 mg/cc in whites vs 330.0 mg/cc in blacks, $p<.0001$). Also, due to the smaller sample size available for analysis in black men, we had limited power to detect associations similar to those observed in whites.

In women, we observed that a decrease in femoral neck aBMD by 1 SD below the mean was related to a 24% increased risk for incident CVD. Notably, this association was observed only after adjusting for risk factors and it seemed to be masked by negative confounders such as BMI and glucose level which were associated with higher BMD and increased risk for cardiovascular disease. In race-specific analyses, lower aBMD measures of the total hip, femoral neck, and trochanter were associated with higher CVD risk in black women, but not in white women. To our knowledge, none of the previous reports have explored associations between BMD and CVD in black women separately. Studies have focused on white women, [7-9, 12, 13, 17, 20, 21, 23, 25] and blacks have been excluded from analyses due to their reduced risk for osteoporosis and fractures. [7, 8, 17] A higher than expected prevalence of low BMD was observed among black women in our cohort: 13% were osteoporotic, and 44.5% had low bone mass. Estimates from NHANES III indicate that the prevalence of osteoporosis and osteopenia in black women aged 50 years and older are 8% and 28%, respectively. [35] The high prevalence of osteoporosis and low bone mass in this group of black women may have lead to more pronounced associations between BMD and CVD.-

Unlike other cross-sectional and longitudinal studies, we did not observe an association between BMD and incident CVD in white women. In the Multiple Outcomes of Raloxifene Evaluation (MORE) trial, osteoporosis was found to be a strong predictor of future

cardiovascular outcomes in postmenopausal women with low bone mass, independent of age and other traditional cardiovascular risk factors (adjusted HR= 3.9, 95%CI 2.0-7.7). [12] In the MORE study, 53% of the women were osteoporotic and the remaining 47% had low bone mass. In another study of white postmenopausal women, the presence of aortic calcification (AC) was associated with lower BMD and higher prevalence of vertebral and hip fractures. The progression of AC was also linked to increased trabecular BMD loss at the spine. In this study, 70% of the women had osteoporosis. [20] In our cohort, the prevalence of osteoporosis in white women was 15%, consistent with estimates from NHANES III. [35] However, the incidence of CHD in this population (15/1,000 person-years) was lower than that reported in the Cardiovascular Health Study (CHS) for white women of similar age (18.6/1,000 person-years). [36] This suggests that the relationship between BMD and CVD may be more evident in populations at high risk. In addition, longer follow-up may be needed to detect associations between BMD and CVD in white women. A report from the Framingham study, based on a 30-year follow-up period, found that metacarpal cortical area (MCA) predicts coronary heart disease in women free from CVD at baseline, with a significant trend of decreasing coronary heart disease risk with increasing MCA (HR for highest vs. lowest MCA quartile= 0.73, 95% CI 0.53-1.00, p for trend= 0.03). [15] Other studies have related low bone density to stroke incidence, prevalence, and mortality in older white women. [8, 16, 17] In a cross-sectional analysis using this cohort, we observed that aBMD of the trochanter and spine vBMD measures of the integral and cortical regions were inversely associated with prevalent CVD in women. Although this analysis combined white and black women, we did not observe a significant interaction between BMD measures and race (unpublished results).

The lack of association of BMD with CVD in white women may also be explained, in part, by selective survival bias. We observed that white women who died from non-CVD causes had lower baseline aBMD of the total hip, compared to the rest of the group (mean aBMD= 0.77 vs. 0.72, respectively, p-value= .03). This suggests that white women with low BMD were at higher risk of death from non-CVD causes, and were therefore eliminated from the CVD risk group.

Traditionally, osteoporosis and CVD have been regarded as independent processes that occur with aging. However, mounting biological observations and epidemiological evidence from this study and others suggest a link between the two conditions that is independent of age. Several hypotheses have been proposed to explain the link between CVD and osteoporosis including: 1) shared etiological factors (such as menopause, smoking, physical activity, alcohol intake, hypertension, and diabetes), which may simultaneously promote or inhibit atherogenesis and bone demineralization, and 2) common pathophysiological mechanisms that could lead to the development of both conditions and which may involve inflammatory cytokines or oxidized lipids. In our analysis, the observed inverse associations between BMD and incident CVD were present after controlling for age and other common etiological factors for osteoporosis and CVD including weight, physical activity, blood pressure, and lipids. This suggests that common pathophysiological mechanisms may be at play in the progression of the two conditions. Inflammatory markers and oxidized lipids have been implicated in the link between osteoporosis and CVD. Aging is associated with increased levels of circulating inflammatory markers such as IL-6 and TNF- α . [37] IL-6 was shown to stimulate osteoclasts, thereby increasing the rates of bone remodeling and bone loss. [38] Previous analyses in the Health ABC cohort have shown that IL-6 and TNF- α were significantly associated with prevalent clinical and subclinical disease,

[39] as well as incident cardiovascular events. [40] The role of oxidized lipids in atherogenesis is well established. [41] In vitro, Parhami et al observed that lipid oxidation products including minimally oxidized LDL have opposite effects on bone and vascular cells; they were observed to inhibit osteoblast differentiation in bone cells and stimulate it in calcifying vascular cells. [42]

In our analysis, oxLDL and the inflammatory markers IL-6 and TNF- α did not explain the associations of BMD measures with CVD. It is possible that other cytokines are involved. The osteoprotegerin (OPG)/ receptor activator of nuclear factor kappa B (NF- κ B) (RANK)/RANK ligand (RANKL) triad seems to play a role in bone physiology and vascular calcification. [43] Other mechanisms involving elevated homocysteine levels, [44] low endogenous sex hormones, imbalances in the calciferol endocrine system, [45] vitamin K status, [46] and genetic factors [43, 47] may also have a role in the link between low BMD and CVD.

Our study extends previous findings by longitudinally examining the association of BMD with CVD in a healthy cohort with no history of CVD, in separate gender and race groups, and across a wider range of BMD values, not just the lower range. This study also had the benefit of utilizing QCT for volumetric determination of BMD at the spine. This technique adjusts for differences in bone size, an important confounder in studies that involve multiethnic groups and different genders. However, since this study included a well-functioning cohort of older adults, our findings may not be generalizable to other populations.

In summary, we observed a longitudinal inverse association between BMD measures and incident CVD in a healthy cohort of older women and white men with no prior history of cardiovascular disease. These findings provide further support for a link between osteoporosis and CVD that is independent of age and shared risk factors, and suggest that older men and women with lower BMD are potential targets for interventions to prevent cardiovascular

outcomes. Further research should assess these associations in separate race groups and investigate common pathophysiological links between osteoporosis and CVD.

5.6 ACKNOWLEDGEMENTS

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Table 5-1 Comparison of baseline characteristics (% , mean \pm SD, or median (IQR)) in women and men by incident cardiovascular disease status, the Health ABC Study

	Women			Men		
	No Cardiovascular Disease (N = 1088)	Incident Cardiovascular Disease (N = 182)	P- value	No Cardiovascular Disease (N = 799)	Incident Cardiovascular Disease (N = 241)	P- value
Demographics						
Age	73.4 \pm 2.8	73.3 \pm 2.8	.68	73.4 \pm 2.8	74.1 \pm 3.1	.001
% Black	43.4	50.0	.10	40.0	34.4	.12
% Pittsburgh	47.8	53.8	.13	49.6	45.6	.29
% Post-secondary education	38.8	35.4	.61	48.1	38.9	.04
Life Style/ Diet						
% Smoking	8.6	11.0	.27	11.4	11.2	.95
% Alcohol drinking	43.8	40.1	.35	58.9	55.4	.34
Physical activity (Kcal/ week)*	365.8 (47.2-959.7)	215.1 (18.5-938.1)	.06	635.8 (170.6-1737.5)	701.1 (129.1-1969.1)	.98
% Calcium supplements	31.6	24.2	.04	8.8	6.7	.30
%Vitamin D supplement	14.9	11.0	.16	4.1	2.5	.24
Anthropometric Measures						
BMI (kg/ms)	27.5 \pm 5.6	28.6 \pm 5.3	.02	27.0 \pm 4.0	27.2 \pm 4.0	.36
Weight (Kg)	70.2 \pm 15.0	72.2 \pm 13.4	.08	81.5 \pm 13.5	82.0 \pm 13.3	.62
Height (cm)	159.6 \pm 6.1	159.1 \pm 6.3	.29	173.7 \pm 6.7	173.4 \pm 6.2	.54
HABC performance score	6.8 \pm 1.6	6.5 \pm 1.6	.03	7.6 \pm 1.6	7.2 \pm 1.7	.0004
Lipids						
Total cholesterol (mg/dl)	213.0 \pm 37.4	217.2 \pm 39.9	.16	192.9 \pm 35.4	196.2 \pm 31.4	.18
LDL (mg/dl)	124.9 \pm 35.7	128.8 \pm 38.7	.21	118.5 \pm 32.7	122.7 \pm 28.9	.06
HDL (mg/dl)	61.0 \pm 16.8	60.3 \pm 18.9	.62	48.9 \pm 14.8	46.3 \pm 12.8	.01
Triglyceride (mg/dl)*	118.5 (90.0-162.5)	127.0 (92.0-166.0)	.17	112.0 (83.0-151.0)	119.0 (89.0-165.0)	.11
Inflammatory Markers and oxLDL						
IL-6 (pg/ml)*	1.7 (1.1-2.5)	2.2 (1.3-3.2)	<.0001	1.7 (1.2-2.5)	2.0 (1.4-3.0)	.0004
TNF- α (pg/ml)*	3.0 (2.3-3.8)	3.1 (2.6-4.3)	.007	3.1 (2.4-3.9)	3.4 (2.6-4.3)	.002
oxLDL (mg/dl)	1.1 (0.8-1.6)	1.3 (0.9-1.7)	.09	1.1 (0.8-1.5)	1.2 (0.9-1.6)	.10
Blood Pressure (mmHg)						
Systolic blood pressure	135.3 \pm 20.4	138.6 \pm 21.2	.04	134.5 \pm 19.9	137.7 \pm 21.5	.03
Diastolic blood pressure	70.0 \pm 11.6	71.2 \pm 11.7	.19	73.3 \pm 11.4	73.8 \pm 11.5	.56

Table 5-1 (Continued)

Glucose level (mg/dl)*	91.0 (85.0-101.0)	94.0 (87.0-104.0)	.007	95.0 (90.0-120.0)	97.0 (90.0-119.5)	.004
Insulin level (uIU/ml)*	6.8 (4.8-10.0)	7.6 (5.0-10.8)	.05	6.6 (4.8-10.0)	7.0 (4.8-10.0)	.49
Time since menopause (yrs)	27.4±7.4	27.9±8.8	.46	-	-	-
Medical History						
% Hypertension	43.3	53.8	.008	31.3	36.7	.12
% Diabetes	12.8	23.3	.0002	18.2	28.8	.0004
% Osteoporosis	14.0	15.5	.61	3.0	4.6	.23
Medication Use						
% Oral estrogen	23.9	22.0	.56	-	-	-
% Osteoporosis medication	8.3	7.1	.59	0.6	0.8	.73 ^a
% Statins	10.9	13.7	.26	7.2	5.4	.35
% Thiazide	21.3	30.2	.008	12.7	15.9	.20
% oral steroid	2.8	5.0	.12	1.2	0.8	.74 ^a
% Diabetes drugs	8.9	15.9	.003	10.5	17.6	.004
BMD Measures						
Volumetric (mg/cc)						
Spine Integral	237.6±51.5	241.5±49.2	.49	265.6±56.5	254.3±54.9	.07
Spine Trabecular	110.9±41.2	114.1±36.6	.48	134.9±43.9	128.4±45.7	.19
Spine Cortical	277.9±54.3	280.6±51.7	.65	311.5±59.8	299.2±58.2	.06
Areal (g/cm²)						
Total Hip	0.81±0.15	0.81±0.14	.84	0.97±0.15	0.96±0.16	.40
Trochanter	0.62±0.12	0.61±0.11	.81	0.76±0.14	0.75±0.14	.30
Femoral neck	0.70±0.13	0.69±0.12	.76	0.80±0.14	0.79±0.14	.43

* P-value obtained using Wilcoxon rank-sum test since variables were not normally distributed

^a Fisher's Exact Test

Table 5-2 Results of Cox regression models for incident CVD: unadjusted, age-adjusted, and risk factors-adjusted hazards ratios (95% CI) per 1 SD decrease in baseline BMD measures for women and men in the Health ABC Study

BMD[†]	Women[*]		Men^{**}	
	N at risk (events)	Hazard Ratio (95% CI)	N at risk (events)	Hazard Ratio (95% CI)
Total Hip aBMD				
Unadjusted	1257 (181)	0.98 (0.85-1.14)	1028 (237)	1.06 (0.93-1.20)
Adjusted for Age	1257 (181)	0.99 (0.85-1.14)	1028 (237)	1.04 (0.91-1.18)
Adjusted for shared risk factors between osteoporosis and CVD	1208 (176)	1.18 (0.97-1.43)	976 (217)	1.04 (0.89-1.22)
Femoral Neck aBMD				
Unadjusted	1257 (181)	1.02 (0.88-1.18)	1028 (237)	1.05 (0.92-1.19)
Adjusted for Age	1257 (181)	1.02 (0.88-1.18)	1028 (237)	1.02 (0.90-1.16)
Adjusted for shared risk factors between osteoporosis and CVD	1208 (176)	1.24 (1.02-1.52)^a	976 (217)	1.04 (0.89-1.21)
Trochanter aBMD				
Unadjusted	1257 (181)	1.01 (0.88-1.17)	1028 (237)	1.08 (0.94-1.22)
Adjusted for Age	1257 (181)	1.02 (0.88-1.18)	1028 (237)	1.06 (0.93-1.20)
Adjusted for shared risk factors between osteoporosis and CVD	1208 (176)	1.16 (0.97-1.39)	976 (217)	1.07 (0.92-1.25)
Spine Integral vBMD				
Unadjusted	599 (94)	0.92 (0.76-1.13)	496 (103)	1.20 (0.98-1.47)
Adjusted for Age	599 (94)	0.94 (0.76-1.15)	496 (103)	1.18 (0.96-1.44)
Adjusted for shared risk factors between osteoporosis and CVD	574 (93)	1.02 (0.80-1.28)	475 (95)	1.17 (0.93-1.48)
Spine Trabecular vBMD				
Unadjusted	599 (94)	0.93 (0.76-1.13)	496 (103)	1.14 (0.93-1.40)
Adjusted for Age	599 (94)	0.94 (0.77-1.15)	496 (103)	1.11 (0.91-1.37)

Table 5-2 (Continued)

Adjusted for shared risk factors between osteoporosis and CVD	574 (93)	0.99 (0.79-1.24)	475 (95)	1.10 (0.87-1.39)
Spine Cortical vBMD				
Unadjusted	599 (94)	0.95 (0.77-1.16)	496 (103)	1.20 (0.98-1.48)
Adjusted for Age	599 (94)	0.96 (0.78-1.18)	496 (103)	1.19 (0.97-1.46)
Adjusted for shared risk factors between osteoporosis and CVD	574 (93)	1.04 (0.83-1.31)	475 (95)	1.18 (0.94-1.48)

* Models in women were adjusted for: age, race, study site, physical activity, Health ABC physical performance score, BMI, cholesterol, systolic blood pressure, glucose level, history of hypertension, and use of diabetes drugs, calcium supplements, and oral estrogen.

** Models in men were adjusted for: age, race, study site, education, physical activity, Health ABC physical performance score, BMI, HDL, LDL, systolic blood pressure, glucose level, history of hypertension, and use of diabetes drugs.

† BMD SDs: Women: total hip aBMD= 0.15 g/cm², trochanter aBMD=0.12 g/cm², femoral neck aBMD= 0.13 g/cm², integral vBMD= 51.14 mg/cc, trabecular vBMD= 40.53 mg/cc, cortical vBMD= 53.87 mg/cc. Men: total hip aBMD= 0.16 g/cm², trochanter aBMD= 0.14 g/cm², femoral neck aBMD= 0.14 g/cm², integral vBMD=56.3 mg/cc, trabecular vBMD= 44.3 mg/cc, cortical BMD= 59.7 mg/cc.

^a p<0.05

Table 5-3 Adjusted hazard ratios (95% CI) for incident CVD per 1 SD decrease in baseline BMD measures for black and white women and men in the Health ABC Study

BMD	Black Women *		White Women *	
	N at risk (events)	Hazard Ratio (95% CI)	N at risk (events)	Hazard Ratio (95% CI)
Total Hip aBMD	526 (86)	1.36 (1.03-1.78) ^a	682 (90)	1.02 (0.76-1.37)
Femoral Neck aBMD	526 (86)	1.44 (1.10-1.90) ^b	682 (90)	1.05 (0.78-1.41)
Trochanter aBMD	526 (86)	1.34 (1.04-1.72) ^a	682 (90)	1.00 (0.77-1.32)
	Black Men **		White Men **	
	N at risk (events)	Hazard Ratio (95% CI)	N at risk (events)	Hazard Ratio (95% CI)
Spine Integral vBMD	187 (30)	0.86 (0.58-1.28)	288 (65)	1.39 (1.03-1.87) ^a
Spine Trabecular vBMD	187 (30)	0.84 (0.57-1.24)	288 (65)	1.27 (0.94-1.72)
Spine Cortical vBMD	187 (30)	0.87 (0.59-1.28)	288 (65)	1.38 (1.03-1.84) ^a

* Models in women were adjusted for: age, study site, physical activity, Health ABC physical performance score, BMI, cholesterol, systolic blood pressure, glucose level, history of hypertension, and use of diabetes drugs, calcium supplements, and oral estrogen.

** Models in men were adjusted for: age, study site, education, physical activity, Health ABC physical performance score, BMI, HDL, LDL, systolic blood pressure, glucose level, history of hypertension, and use of diabetes drugs.

^a p<0.05

^b p<0.01

Table 5-4 Effect of controlling for IL-6, TNF- α , or oxLDL on the adjusted associations of aBMD measures with incident CVD in black women

BMD	N at risk (events)	Adjusted for Risk factors* Hazard Ratio (95% CI)	Adjusted for Risk factors + IL-6, TNF-α, or oxLDL** Hazard Ratio (95% CI)
Total Hip aBMD			
<i>IL-6</i>	502 (84)	1.39 (1.06-1.83)^a	1.39 (1.06-1.82)^a
<i>TNF-α</i>	486 (77)	1.32 (0.99-1.76)	1.33 (1.00-1.77)
<i>oxLDL</i>	524 (86)	1.32 (1.02-1.72)^a	1.35 (1.03-1.77)^a
Femoral Neck aBMD			
<i>IL-6</i>	502 (84)	1.51 (1.14-1.99)^b	1.49 (1.13-1.96)^b
<i>TNF-α</i>	486 (77)	1.46 (1.09-1.96)^a	1.48 (1.10-1.98)^b
<i>oxLDL</i>	524 (86)	1.42 (1.09-1.86)^b	1.44 (1.09-1.89)^b
Trochanter aBMD			
<i>IL-6</i>	502 (84)	1.36 (1.05-1.77)^a	1.35 (1.05-1.74)^a
<i>TNF-α</i>	486 (77)	1.32 (1.01-1.73)^a	1.31 (1.01-1.72)^a
<i>oxLDL</i>	524 (86)	1.32 (1.02-1.69)^a	1.34 (1.03-1.72)^a

* Adjusted for same risk factors listed in Tables 2 and 3

** oxLDL models did not include cholesterol level due to the high correlation between the 2 measures.

^a p<0.05

^b p≤0.01

Table 5-5 Effect of controlling for IL-6, TNF- α , or oxLDL on the adjusted associations of vBMD measures with incident in white men

BMD	N at risk (events)	Adjusted for Risk factors* Hazard Ratio (95% CI)	Adjusted for Risk factors + IL-6, TNF-α, or oxLDL** Hazard Ratio (95% CI)
Integral vBMD			
<i>IL-6</i>	280 (62)	1.37 (1.01-1.86)^a	1.38 (1.02-1.88)^a
<i>TNF-α</i>	276 (63)	1.40 (1.04-1.89)^a	1.40 (1.04-1.89)^a
<i>oxLDL</i>	292 (66)	1.39 (1.04-1.87)^a	1.41 (1.05-1.89)^a
Cortical vBMD			
<i>IL-6</i>	280 (62)	1.37 (1.02-1.85)^a	1.38 (1.02-1.86)^a
<i>TNF-α</i>	276 (63)	1.39 (1.03-1.86)^a	1.38 (1.03-1.85)^a
<i>oxLDL</i>	292 (66)	1.39 (1.04-1.85)^a	1.41 (1.05-1.88)^a

* Adjusted for same risk factors listed in Tables 2 and 3

** oxLDL models did not include LDL level due to the high correlation between the 2 measures.

^a p<0.05

^b p<0.01

6.0 GENERAL DISCUSSION

6.1 SUMMARY OF FINDINGS

This dissertation investigated the association of BMD with prevalent CVD, incident CVD, and subclinical measures of atherosclerosis including ankle-arm index and vascular calcification of the aorta and the coronary arteries. The analyses were conducted in two populations: a cohort of older men and women participating in the Health ABC Study, and a cohort of middle-aged women enrolled in the SWAN Study.

In the cross-sectional Health ABC analysis, volumetric BMD measures of the spine were significantly associated with CVD in men and women, and areal BMD of the trochanter was related to CVD in women. Additionally, areal BMD of the total hip was related to subclinical PAD (defined as AAI <0.9) in men.

In the SWAN analysis, we observed an inverse cross-sectional association between trabecular BMD of the spine and aortic calcification. Meanwhile, no associations with coronary artery calcification were noted after adjusting for age.

In the longitudinal Health ABC analysis, vBMD measures of the spine were associated with incident CVD in white men, but not in blacks. In women, aBMD of the femoral neck was associated with incident CVD in the full cohort. In race-specific analyses, aBMD measures of the total hip, femoral neck, and trochanter exhibited significant relationships with incident CVD in black women, but not in whites.

Our findings from the three analyses provided support for an inverse association between BMD and CVD that is independent of age and shared risk factors for osteoporosis and CVD. The potential role of common pathophysiological factors in the observed relationships was investigated. Particularly, inflammatory cytokines (IL-6 and TNF- α), oxidized LDL, and estradiol levels did not appear to explain these associations.

Our results confirm previous findings from epidemiologic studies and extend them in several ways.

Most of the previous reports relied on white postmenopausal women [8, 10-13, 15-17, 19-23, 25-27, 30, 33, 37] and smaller populations. [9, 11, 15, 17, 18, 23, 26, 27, 29, 30, 34, 36-38, 40] Our associations were investigated in large and well-characterized biracial cohorts of men and women.

Unlike other studies that combined men and women in analyses, [15, 34-36] we looked at associations of BMD with CVD separately in each gender. This is particularly relevant given that previous reports failed to demonstrate strong evidence for the presence of such associations in men. The majority of studies investigating the relation of BMD with CVD and subclinical atherosclerosis in men [7, 9, 14, 16-18, 23, 31, 32, 39, 96] reported no associations. [16, 17, 23, 31, 32, 96] To our knowledge, no other study has examined the association of different BMD measures with incident cardiovascular events in men and women with no previous history of CVD.

Our study populations included black and white participants. In the longitudinal Health ABC analysis, the associations were assessed in separate ethnic groups. Most of the previous research focused on white women, and blacks have been excluded from analyses due to their reduced risk for osteoporosis and fractures. [8, 10, 12, 19, 25, 33] Given the well-known racial

differences in the burdens of CVD and osteoporosis, an investigation into the association between the two diseases in separate ethnic groups is warranted.

Recruitment of our cohorts was community-based, minimizing the chances for selection bias. Other studies have selected their participants from patients referred by their physicians for BMD testing and evaluation of osteoporosis; [15, 20, 30] hence the high prevalence of osteoporosis and osteopenia in such populations. In a study which found osteoporosis to be a strong predictor of future cardiovascular events, participants were selected from the placebo arm of the MORE randomized clinical trial. Enrollment in the MORE trial required participants to have osteoporosis (defined using low bone mineral density or radiographically apparent vertebral fractures), as the study was testing the effect of raloxifene in the primary and secondary prevention of incident vertebral fractures in postmenopausal women. [13] Our analyses included healthy populations and were not limited to high risk groups. Bone density was evaluated across a wide spectrum and was not restricted to the low BMD range. Additionally, the associations of BMD with incident CVD, vascular calcification, and subclinical PAD were investigated in cohorts who had no previous history of CVD. The majority of other studies on subclinical atherosclerosis and incident CVD did not exclude people with baseline CVD from analyses. [7-10, 12, 13, 17, 19, 20, 22-25, 31-33, 35, 37, 96, 97] Therefore, those associations might have been confounded by factors such as reduced physical activity ensuing from CVD, which in itself contributes to lower BMD.

We had the advantage of using QCT for volumetric determination of bone density. In a large number of studies, bone mass was determined using radiographic techniques, single-photon or single X-ray absorptiometry, or dual-photon absorptiometry. [7, 11, 12, 16, 19, 23, 24, 28, 35, 37, 39, 96-98] Some studies have employed DXA in bone determination; [8-10, 13-15, 17, 18,

21, 22, 25, 29-34, 36, 38, 40] however, this technique is limited by its 2-dimensional areal assessment of BMD which does not adjust for bone size. This is especially important in studies of different ethnic and gender groups since there are well-established differences in bone size by race and gender. [165, 166] DXA is also affected by the presence of extra-osseous calcium such as aortic calcification and degenerative osteoarthritic changes, which get incorporated in the region of interest and lead to a falsely increased bone density at the spine. [26] This is an important drawback, particularly in the elderly who have an increased prevalence of such degenerative conditions. [56] QCT allows for a three-dimensional volumetric determination of bone density, an adjustment for bone size, and an assessment of purely trabecular bone. Only two studies have utilized QCT for BMD assessment. [20, 26]

Similarly, we used EBCT for determination of aortic and coronary artery calcification. In the existing literature, aortic calcification was mostly assessed using conventional radiography, which has low sensitivity to the the detection of small calcium deposits. [21-25, 27, 28, 97, 98] EBCT which allows for a graded quantification of vascular calcification, was used only in one study for aortic calcification quantification [20] and in two others for coronary calcification assessment. [29, 30]

All the observed relationships were adjusted for a comprehensive set of common risk factors for CVD and osteoporosis. Other studies did not sufficiently control for important covariates including physical activity, lipids, blood pressure, and the use of medications such as statins. [7, 8, 10, 12, 13, 17-20, 23-26, 28-33, 36, 38, 40, 96-98]

We investigated the role of common pathophysiological factors in the link between CVD and osteoporosis. The inflammatory cytokines IL-6 and TNF- α , oxidized LDL, and estradiol levels did not appear to explain these associations. Given the complex pathogenetic processes

underlying both diseases, it is quite likely that multiple mechanisms are involved. This however does not completely rule out a role for these factors. Measurements of inflammatory cytokines were done at a single time point in our study. However, these cytokines (especially IL-6) are known to have diurnal variations and considerable intra-individual variability over time. [167, 168] Therefore, a single measurement may not be reflective of long-term exposure, and more than one assay may be needed to adequately classify a person's level.

Bone density was measured in body regions containing different proportions of trabecular and cortical bone. In women, there was no apparent relationship between the type of bone and CVD, as the associations were observed at both spine and hip sites. In men, however, associations seemed to be specific to spine BMD which is composed primarily of trabecular bone. This is probably a reflection of the greater magnitude of trabecular bone loss, as compared to cortical bone loss, in men after the age of 70 years. [169]

Limitations of our analyses include that they were conducted in healthy cohorts restricting their generalizability to other populations. Also, the associations of BMD with vascular calcification were cross-sectionally investigated. This does not allow the evaluation of a causal association between low bone density and vascular calcification.

6.2 PUBLIC HEALTH SIGNIFICANCE

CVD and osteoporosis are major public health problems. CVD is the leading cause of mortality in both men and women. In 2003, 910.6 thousand deaths were attributed to CVD. Osteoporotic fractures are a major cause of long-term disability and mortality. Up to one third of individuals who suffer a hip fracture can become totally dependent. [45] The financial burden incurring from

these conditions is overwhelming. In 2006, the direct and indirect cost for CVD is expected to total \$403.1 billion in the US. [58] The direct cost attributed to fractures is estimated at \$20 billion per year. The overall burden of these conditions on society is expected to rise dramatically in the coming years because of increased longevity and the growing number of elderly people.

New paradigms for treatment and prevention of both CVD and osteoporosis may emerge from investigating the link between the two conditions and elucidating the mechanisms involved in their progression. An understanding of the biological linkages may set the stage for dual-purpose preventive and therapeutic interventions aimed at reducing bone loss and the progression of atherosclerosis. For instance, there is a growing body of evidence suggesting that statins, cholesterol-lowering drugs, may have beneficial skeletal effects. Similarly, bisphosphonates, a class of antiresorptive agents, are shown to have protective cardiovascular effects.

Additionally, confirming the presence of an association between low BMD and clinical and subclinical CVD may allow the identification of groups at higher risk for cardiovascular events in which primary prevention would be of benefit. This is especially important in the elderly where traditional risk factors for CVD become less predictive of cardiovascular outcomes. [170] By the same token, the presence of clinical or subclinical cardiovascular disease may allow the identification of groups at higher risk for osteoporosis in which skeletal evaluations would be indicated.

6.3 FUTURE RESEARCH

Additional longitudinal studies are needed to confirm our findings of an inverse association between BMD and CVD. Specifically, racial differences in this association deserve further investigation. In fact, if the presence of a racial difference is confirmed, racially-targeted interventions may allow the provision of the most benefit to the population at highest risk for both conditions.

Examination of the relation between bone loss and the progression of vascular calcification is certainly warranted and may provide additional insight into their temporal association. This will allow a better assessment of a cause-effect relationship or, more likely, the identification of common causes. Subclinical assessment of either condition may therefore allow risk stratification for the other and trigger targeted interventions.

The most exciting future endeavor is the investigation and further elucidation of the common underlying mechanisms in the link between osteoporosis and CVD. Among the most promising targets for investigation is the elucidation of the role of osteoprotegerin/RANK/RANKL cytokine system, as well as the examination of the value of bone turnover markers.

Once the mechanisms linking both conditions are clearly elucidated, the stage will be set for the potential use of preventive and therapeutic interventions targeted at both conditions.

6.4 CONCLUSION

CVD and osteoporosis are major causes of morbidity, mortality, and disability. Both diseases increase with aging. Traditionally, these two conditions were considered unrelated and their

coexistence was attributed to independent age-related processes. Recently, an increasing body of evidence provided support for a link between the two conditions beyond age. These diseases share many risk factors which were suggested to account for their association. Furthermore, common molecular, cellular, and biochemical processes were implicated in their pathogenesis. In these analyses, we sought to determine whether an association exists between BMD and CVD and subclinical atherosclerosis above and beyond age and a host of risk factors that they are known to share. Indeed, we observed inverse cross-sectional and longitudinal associations between BMD, cardiovascular disease and subclinical atherosclerosis. These associations were not explained by age and shared risk factors. We further evaluated the role of common pathophysiological factors in these associations. The inflammatory cytokines IL-6 and TNF- α , oxLDL, and endogenous estradiol did not seem to explain these associations. Owing to the complex multifactorial pathogenesis of both conditions, it is likely that more than one biological mechanism is involved in their link. Overall, our findings provide epidemiological evidence for the presence of an association between BMD and CVD. Future research should investigate potential common mechanisms underlying atherogenesis and osteoporosis. Once these are elucidated, preventive and therapeutic approaches that target both conditions may be undertaken.

APPENDIX

SUMMARY OF EPIDEMIOLOGIC STUDIES ON BMD AND CVD

Table A-1 Summary of epidemiologic studies of BMD and cardiovascular mortality

Author	Design	Study	Population	BMD Measurement	Mortality	Result	Comment
Mussolino et al, 2003	Prospective (median follow-up= 18.5 years)	NHANES I Epidemiologic Follow-up Study	White and black, men and women, 45-74 years, n=3501	Phalangeal BMD (RA)	Mortality (total, cardiovascular, non-cardiovascular)	-1 SD lower BMD in white men was associated with 14% increase in CVD mortality, 16% increase in all-cause mortality, and 21% non-cardiovascular mortality. -1 SD lower BMD in white women was associated with 26% increase in non-cardiovascular mortality. -1 SD lower BMD in blacks was associated with 22% increase in all-cause mortality, and 41% increase in non-cardiovascular mortality.	Adjusted for age, smoking, alcohol, diabetes, heart disease, education, BMI, physical activity and blood pressure medications
Mussolino et al, 2003	Prospective	NHANES I	White and black, men and women, 45-74 years, n=3402	Phalangeal BMD (RA)	Stroke mortality	No association between BMD and stroke mortality	Adjusted for age, smoking, alcohol consumption, history of diabetes, history of heart disease, education, BMI, physical activity, and blood pressure medications.
Bauer et al, 2002	Prospective (average follow-up= 5 years)	SOF	White, postmenopausal women, 70 years and older, n= 5816	- Broadband ultrasound attenuation (BUA) (QUS) - Total hip BMD (DXA) - Calcaneal BMD (SXA)	Total and cause-specific mortality (CVD, cancer)	- 1 SD decrease in BUA was associated with 19% increase in CV mortality (95% CI 1.04-1.37) - 1 SD decrease in calcaneal BMD was associated with 17% increase in CV mortality (95% CI 1.01-1.37) - BUA, calcaneal and hip BMD associated with total mortality	Adjusted for age, weight, height, health status, smoking, physical activity, history of diabetes, hypertension, cancer, CVD, and stroke
Trivedi et al, 2001	Prospective (average follow-up= 6.7 years)	The Cambridge General Practice Health Study	White men, 65-76 years, n= 1002	Total hip BMD (DXA)	Mortality (all-cause, cardiovascular)	-1 SD increase in BMD associated with 28% reduction in CVD mortality and 29% reduction in all-cause mortality	Adjusted for age, BMI, smoking, cholesterol, SBP, past history of MI, stroke, or cancer, physical activity, alcohol, and general health status
Kado et al, 2000	Prospective (average follow-up= 3.2 years)	SOF	White, postmenopausal women, 65 years and older, n= 6046	- Calcaneal bone loss (SPA) (for a mean of 5.7 years). - Hip bone loss (DXA) (for a mean of 3.5 years)	Mortality (CHD, stroke, atherosclerosis, cancer , all other causes)	- 1 SD increase in hip BMD loss associated with CHD mortality (RH=1.3), total mortality (RH= 1.3), and pulmonary disease mortality (RH=1.6) - 1 SD increase in calcaneal bone loss associated with CHD (RH=1.3), atherosclerosis (RH=1.2), and all causes mortality (RH= 1.1)	Adjusted for age, baseline BMD, diabetes, hypertension, incident fractures, smoking, physical activity, health status, weight loss, calcium use.

Table A-1 (Continued)

von der Recke et al, 1999	Retrospective cohort	Danish Study	White, early postmenopausal (5,216 years of follow-up) and late postmenopausal (6,292 years of follow-up) women, n= 1,063	-Bone mineral content of the distal forearm (SPA) -Vertebral fractures (Radiography)	Mortality (cerebrovascular disease, heart disease, vascular disease, cancer)	- In early postmenopausal women: 1 SD decrease in BMC associated with increase in total mortality (RR= 1.4) and cardiovascular death (RR= 2.3). - In late postmenopausal women: 2 SD decrease in BMC associated with CVD mortality (RR= 3.2, p=.005), cardiovascular mortality (RR= 5.2, p=.002), and MI mortality (RR= 4.2, p=.01); a prevalent vertebral compression fracture associated with CVD death (RR= 2.0).	Adjusted for age, systolic blood pressure, diastolic blood pressure, BMI, cholesterol levels, smoking.
Browner et al, 1991	Prospective (average follow-up= 2.8 years)	SOF	White, postmenopausal women, 65 years and older (n= 9704)	Distal radius, proximal radius, and calcaneal BMD (SPA)	Mortality (all-cause, stroke)	- 1 SD decrease in proximal radius BMD was associated with 1.91-fold increase in stroke mortality (95% CI 1.25-2.92). - Calcaneal and proximal radius BMD were significantly associated with all-cause mortality in age-adjusted analysis. Associations became not significant after adjusting for covariates including measures of general health.	Stroke mortality: adjusted for history of previous stroke, hypertension, postmenopausal use of estrogen, thiazide diuretic treatment, diabetes mellitus, and smoking

Table A-2 Summary of epidemiologic studies of BMD and cardiovascular morbidity

Author	Design	Study	Population	BMD Measurement	CVD endpoint	Result	Comment
Tanko et al, 2005	Prospective (4-years follow-up)	MORE Study	2,576 postmenopausal women assigned to the placebo arm of the MORE trial, mean age= 66.5 years.	- Osteoporosis (=having a vertebral fracture or a total hip BMD t-score of -2.5 or less) - Low bone density (having a t-score greater than -2.5 but less than -1.0 without vertebral fractures)	Incidence of fatal and non-fatal cardiovascular events (coronary events and cerebrovascular events)	- Women with osteoporosis had a 3.9-fold increased risk for cardiovascular events, compared to those with low bone mass - Presence of at least 1 vertebral fracture, versus no vertebral fracture, was associated with a 3.0-fold increased risk for cardiovascular events	- Did not exclude prior CVD - 53% had osteoporosis, rest had low bone mass - Did not adjust for physical activity
Magnus et al, 2005	Cross-sectional	NHANES III	5,050 African-American, Mexican-American, and Caucasian men and women. Aged 50-79 years	Total hip BMD (DXA)	Myocardial infarction	- Previous MI was associated with low BMD in the total group (OR= 1.28, 95% CI 1.01-1.63) and in men (OR= 1.39, 95% CI= 1.03-1.87). - No association in women.	- Associations present only after adjusting for covariates
Marcovitz et al, 2005	Retrospective	Ambulatory adult patients	209 patients, 89% women, 91% white, average age= 67 years	Spine, femur, ultradistal radius, and 1/3 distal radius (DXA)	Angiographically-determined coronary artery disease ($\geq 50\%$ luminal narrowing in a major artery)	- Osteoporosis was an independent predictor of CAD (OR= 5.6, 95%CI 2.6-12.0).	- Most of patients (75%) were diagnosed with osteoporosis/ osteopenia. - 56% had significant CAD. - DEXA and coronary angiogram performed within a 12-month period
Samelson et al, 2004	Prospective (30- year follow-up)	The Framingham Study	White, men and women, 47-80 years, (n= 2,059)	Relative metacarpal cortical area (Radiogrammetry)	Incident CHD	- In women, highest MCA quartile was related to a 73% reduced risk of CHD incidence compared to lowest quartile. - No association in men	Adjusted for age, education, BMI, smoking, alcohol, systolic blood pressure, cholesterol, HDL, and diabetes.
Jørgensen et al, 2001	Case-control	Norwegian Study	White men and postmenopausal women, age ≥ 60 years, n= 260	Femoral neck BMD (DXA)	Acute stroke	- 1 SD decrease in BMD was associated with 1.9 fold increase in odds of stroke. - No significant association in men.	Adjusted for BMI, alcohol, previous MI, and medication for hypertensive
Mussolino et al, 2003	Prospective	NHANES I	White and black, men and women, 45-74 years, n=3402	Phalangeal BMD (RA)	Stroke incidence	Incidence of stroke was not associated with a decrease in BMD in white men, white women, or blacks.	Adjusted for age, smoking, alcohol consumption, history of diabetes, history of heart disease, education, BMI, physical activity, and blood pressure medications.
Laroche et al, 1994	Cross-sectional		18 men	BMC of legs (DXA)	Symptomatic peripheral arterial disease	BMC of the more severely affected leg was lower significantly lower than BMD of the less affected leg	

Table A-2 (Continued)

Browner et al, 1993	Prospective (1.98 years follow-up)	SOF	White, postmenopausal women, 65 years and older, n= 4024	Calcaneal BMD (SPA)	Incident stroke	-1 SD decrease in calcaneal BMD was associated with 1.31 fold increase in stroke	Adjusted for age, follow- up time, diabetes, systolic blood pressure, alcohol, smoking, HRT use, cognitive ability, grip strength, and functional ability
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Table A-3 Summary of epidemiologic studies of BMD and subclinical measures of atherosclerosis

Author	Design	Study	Population	BMD Measurement	Subclinical Atherosclerosis Measure	Result	Comment
Aortic Calcification							
Schulz et al, 2004	- Cross-sectional - Retrospective cohort (2.1 years of follow-up on average)	Study at Loma Linda University Medical Center	White postmenopausal women, 50 years and older, n= 2348 for cross-sectional and 228 for longitudinal	- Trabecular volumetric BMD (EBCT) - Vertebral and hip fractures (CT radiographs)	Aortic calcification (AC) (EBCT)	- BMD significantly associated with AC, adjusted for age (AC predicted 26% of the variance in BMD). - The odds ratios for vertebral and hip fractures in those with calcification, compared to those without AC, were 4.8 (95%CI 3.6-6.5) and 2.9 (95%CI 1.8-4.8), respectively. - Yearly rate of change in aortic calcification significantly related to yearly rate of bone change ($r^2=0.471$, $p<.001$)	- 70% of population had osteoporosis, 30% had at least one vertebral fracture - 76% had AC. - Sample selected from review of medical records
Tanko et al, 2004 (abstract)	Cross-sectional	Prospective Epidemiological Risk Factor Study, Denmark	Postmenopausal women, aged 60-85 years, n=5409	Hip, spine, and radius BMD (DXA)	Aortic calcification (Radiography)	- Age adjusted BMD was inversely related to AC severity at the hip and forearm. - RR of vertebral fractures was increased by 29% in the highest compared with the lowest AC quartile	- Age adjusted - 10% had manifest CVD
Tanko et al, 2003	Cross-sectional	Prospective Epidemiological Risk Factor Study, Denmark	Postmenopausal women, aged 60-85 years, n= 963	Hip, spine, and radius BMD (DXA)	Aortic calcification (Radiography)	- AC contributed significantly and independently to variations in hip BMD. - No association between spine or radius BMD and AC	Adjusting for intermittent claudication did not alter the association between AC and hip BMD
Kiel et al, 2001	Prospective cohort (25 year follow-up)	Framingham Heart Study	White, men and women, 47-80 years, (n= 554)	Relative metacarpal cortical area (Radiogrammetry)	Aortic calcification (Radiography)	- Significant association between percent change in MCA and change in AC in women (for each % decline in MCA, the AC index increased by 7.3%, $p=0.01$). - No association in men	Adjusted for recognized risk factors for atherosclerosis

Table A-3 (Continued)

Hak et al, 2000	-Longitudinal (9 years of follow-up) - Cross-sectional	Dutch Study	White premenopausal (n=236) and postmenopausal women (n=720), 45-64 years old	Relative metacarpal cortical area (Radiogrammetry)	Aortic calcification (Radiography)	- Significant association between the extent of aortic calcification and metacarpal bone mass - Metacarpal bone loss was higher in premenopausal women (at baseline) with progression of AC than women with no progression (adjusted change in MCA= -3.5 mm ² vs. -2.0 mm ² , respectively, p<.01) - BMD (mean SD for all 3 sites) was not significantly associated with AC after adjusting for age.	In women already postmenopausal at baseline, no association was found between progression of aortic calcification and metacarpal bone loss
Aoyagi et al, 2001	Cross-sectional	Hawaii Osteoporosis Study	Japanese-American women, n= 524	BMD at distal and proximal radius and calcaneus (SPA)	Aortic calcification (Radiography)	- Associations between BMD and AC were significant before adjusting for age - Age, SBP, physical activity, and smoking were independently associated with AC.	- Adjusted for age, BMI, estrogen use, smoking, exercise, and diabetes. - Significant association with radial BMD was attributed to Type I error
Vogt et al, 1997	Cross-sectional	SOF	White postmenopausal women, 65 years and older, n= 2051	- Hip and spine BMD (DXA) - Calcaneal, proximal and distal radius BMD (SPA)	Aortic calcification (Radiography)	- All sites, except spine, were significantly associated with AC in unadjusted analysis. - After adjusting for age and other risk factors, all associations become not significant, except for BMD at the proximal and distal radius	- Purpose of study was to look at degenerative change and extra-osseous calcification in general, not AC specifically. - Higher DPA spine BMD was found in women with spinal degenerative calcification - Adjusted for age, time since menopause, weight and height
Banks et al, 1994	Cross-sectional	Therapeutic RCT for prevention of postmenopausal bone loss	White early postmenopausal women, age 49-64 years, n for AC analysis= 70	- Hip and spine BMD (DPA) - Spine BMD (QCT)	Aortic calcification (defined using combination of radiography and CT)	- Women with aortic calcification had lower QCT spine BMD and DPA hip BMD compared to those without calcification	- Purpose of study was to look at degenerative change and extra-osseous calcification in general, not AC specifically. - Higher DPA spine BMD was found in women with spinal degenerative calcification - Adjusted for age, time since menopause, weight and height
Frye et al, 1992	Cross-sectional	Study in Rochester, Minnesota	White women, 50 years and older, n= 200	- Vertebral fracture - BMD	Aortic calcification (Radiography)	- In age-adjusted analysis, AC was positively correlated with BMD at lumbar spine only. - The association between AC and vertebral fractures and BMD at other sites were not significant after adjusting for age	

Table A-3 (Continued)

Boukhris et al, 1972	Cross-sectional	Study in George Washington University	White and Black women (n=290) and White and Black men (n=299)	Osteoporosis of the lumbar spine (normal, moderate, severe) (Radiography)	Aortic calcification (Radiography)	Positive correlation between osteoporosis and AC in all race and gender groups	Adjusted for age only
Anderson et al, 1964	Cross-sectional		Men and women attending bone clinic, n= 823	- Spine osteoporosis (defined using relative vertebral density) and metacarpal osteoporosis (defined using cortico/medullary ratio) (Radiography)	Aortic calcification (Radiography)	- Significant associations between prevalence of osteoporosis and AC in both genders - Associations were eliminated after stratifying by age, except for AC and hand osteoporosis in men 70-79 years old	
Coronary Calcification							
Ramsey-Goldman et al, 2001	Cross-sectional	Pilot study	13 women with Systemic Lupus Erythematosus, mean age= 45 years, 40% menopausal, 95% white.	Lumbar spine and total hip BMD (DXA)	Coronary artery calcification (EBCT)	- Correlation between CAC and spine BMD= -0.57 (p=0.04) - Correlation between CAC and hip BMD= -0.55 (p=0.05)	Unadjusted results
Barengolts et al, 1998	Cross-sectional		Postmenopausal women, n=45	Lumbar spine and hip BMD (DXA)	Coronary calcification (CAC) (EBCT)	- CAC was significantly higher in the osteoporotic women compared with the control group - Negative correlation between CAC and hip BMD (r=-0.34, p=.002)	Unadjusted results
Ankle-arm Index							
Wong et al, 2005	Cross-sectional	Mr. and Ms Os (Hong Kong)	3,998 Chinese men and women, 65 years and older	Lumbar spine and total hip BMD (DXA)	Ankle-arm index (<0.9)	- A 1 SD increase in AAI was associated with an increase in hip BMD of 0.5%. - No significant association between AAI and spine BMD	- Associations were not significant when stratified by gender. - Did not exclude CVD but adjusted for it.
van der Klift et al, 2002	Cross-sectional	Rotterdam Study	Men and women, age 55 years and older, n=5268	Femoral neck and spine BMD (DXA)	Ankle-arm index (<0.9)	- Low femoral neck BMD was associated with PAD in women (OR= 1.35, 95%CI 1.02-1.79)† - No association between spine BMD and PAD in men or women	- Did not exclude those with history of CVD. But results did not change after excluding subjects with prevalent MI, intermittent claudication, or current use of diuretics - †Adjusted for age, BMI, SBP, smoking, cholesterol, walking, age at menopause, estrogen use

Table A-3 (Continued)

Vogt et al, 1997	Cross-sectional and longitudinal (6 years of follow-up)	SOF	White, postmenopausal women, 65 years and older, n= 1292	- Hip and spine BMD (DXA) - Calcaneal and radius BMD (SPA)	Ankle-arm index	- AAI was positively correlated with BMD at the total hip, calcaneus and the distal and proximal radius in age-adjusted analysis (a decrease in AAI of 2 SD was associated with a 3.7% decrease in hip BMD (95%CI 1.7-5.8; age-adjusted). - Significant age-adjusted positive correlation between annual change in hip BMD and annual change in AAI. - Rate of bone loss in the hip and calcaneus was higher in women whose annual AAI decrease was more than 1 SD above the mean, compared to those whose change was within 1 SD around the mean (association remained after adjustments)	- The age-adjusted associations between AAI and BMD measures disappeared after adjusting for smoking and BMI. -AAI >1.5 was excluded from analysis.
Intima-Media Thickness and Carotid Plaque							
Yamada et al, 2005	Cross-sectional	Healthy Japanese population	106 males and 154 females, mean age= 51.4 years	- Spine BMD (DXA) - Calcaneal OSI (QUS)	Carotid and femoral artery IMT	- Femoral artery IMT was significantly associated with calcaneus OSI. - No associations with spine BMD	Adjusted for gender, age, BMI, SBP, smoking, LDL, physical functioning
Jørgensen et al, 2004	Cross-sectional, Population-based	Trømso Study, Norway	Men (n=2,543) and postmenopausal women (n=2,726), aged 55-74 years	Distal and ultradistal forearm BMD (SPA)	- Carotid atherosclerotic plaque score (B-mode ultrasonography) - Plaque echogenicity	- Prevalence of echogenic plaques was lower in highest BMD quartile compared to lowest (OR=0.51, 95%CI 0.31-0.83) - No association between BMD and echolucent plaques	- Pooled results for men and women - Results for ultradistal forearm were similar to distal and were not reported - Did not exclude those with history of CVD
Pennisi et al, 2004	Case-control	Italian Study	36 white men and postmenopausal women with peripheral atherosclerosis, 30 age and gender-matched controls	- Lumbar spine, total body, and total hip BMD (DXA) - BUA (QUS) - Bone turnover markers	- Common carotid and femoral artery IMT (B-mode ultrasound imaging) - Plaque score - Plaque echogenicity	- High occurrence of osteoporosis in cases - Osteocalcin and bone-specific alkaline phosphatase were lower in cases than controls	

Table A-3 (Continued)

Montalcini et al, 2004	Cross-sectional	Italian Study	White postmenopausal women, aged 45-75 years, n= 157	Calcaneal BMD (QUS)	- Carotid intima-media thickness - Carotid plaque.	The prevalence of carotid atherosclerosis was increased in women with low BMD and osteocalcin levels above the median compared to women with low BMD and osteocalcin levels below the median (61% vs 29%, p<.05)	Women with low BMD did not have higher prevalence of atherosclerosis
Ramsey-Goldman et al, 2001	Cross-sectional	Pilot study	65 women with Systemic Lupus Erythematosus, mean age= 45 years, 40% menopausal, 95% white.	Lumbar spine and total hip BMD (DXA)	Carotid plaque index and IMT (B-mode ultrasonography)	- Women in the middle and lowest tertiles of hip BMD had higher carotid plaque index than those in the highest tertile of BMD - No association with IMT was observed	Unadjusted results
Uyama et al, 1997	Cross-sectional	Japanese Study	Postmenopausal women, 67-85 years, n=30	Lumbar spine and total BMD (DXA)	Carotid atherosclerotic plaque score (B-mode ultrasonography)	- Total BMD negatively correlated with plaque score in unadjusted (r=0.55, p<.0002) and adjusted analysis (r=0.54, p<.01). - No association with spine BMD	Total cholesterol was also correlated with plaque score in adjusted analysis
Pulse Wave Velocity							
Hirose et al, 2003	Cross-sectional	Japanese study	Men and women, 21-81 years, n= 7865	Calcaneal OSI (QUS)	Brachial-ankle pulse wave velocity	- OSI negatively correlated with PWV in both genders	All subjects had normal ankle-arm index
Endothelial Function							
Sanada et al, 2004	Cross-sectional	Japanese study	Postmenopausal women, average age 53.8 years, without a history of smoking or diabetes, n= 110	Lumbar spine BMD (DXA)	Endothelial function: forearm blood flow (FBF) at baseline, during reactive hyperemia, and after the administration of sublingual nitroglycerine	Women with osteoporosis had a lower maximal FBF response to reactive hyperemia than those with normal BMD or osteopenia	ANCOVA adjusted for age, BMI, time since menopause, and basal FBF

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