OSTEOPOROSIS: IDENTIFICATION OF FACTORS ASSOCIATED WITH FRACTURE, BONE MINERAL DENSITY, BONE GEOMETRY AND BONE STRENGTH IN OLDER ADULTS

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Osteoporosis is a major public health problem in men and women. Low bone mineral density (BMD) is a major risk factor for fracture. Fractures have major implications for morbidity and mortality.

This research project evaluated correlates of trabecular and cortical vBMD at the radius and tibia in primarily Caucasian men aged 69 years or older using the Osteoporotic Fractures in Men study (MrOS). The correlation between serum 25-hydroxy vitamin D [25(OH)D] and bone density, content, geometry and strength was also assessed using Caucasian men from MrOS and men of African descent from the Tobago Bone Health Study, aged 65 years and older. We also investigated the longitudinal impact of serum leptin and adiponectin on bone loss and fractures in the Health Aging and Body Composition study (Health ABC) in men and women, aged 70 years and older, of African American and Caucasian descent.

Our findings have important public health implications. We have a better understanding of how different factors are correlated with trabecular and cortical and vBMD. Future research can generate hypotheses to evaluate these associations prospectively. Men of African descent had significantly higher 25(OH)D than Caucasians, which has never been reported. Serum 25(OH)D was positively correlated with indices of bone strength in Caucasian men, but not men of African descent. Serum 25(OH)D thresholds were identified at an estimated 20 ng/ml (lower

than previous consensus of 30 ng/ml), greater levels of 25(OH)D had minimal effects on bone measures. Significant correlations between 25(OH)D occurred only at cortical regions. Prospective studies that evaluate the impact of 25(OH)D on trabecular and cortical vBMD loss are needed. There was an association between higher adiponectin and greater risk of incident fracture in men only. We also report that adiponectin predicted greater hip areal BMD (aBMD) loss among women, but not men. The impact of leptin on bone loss and incident fractures was largely attenuated by BMI and weight change. This study provides strong evidence that higher levels of adiponectin may increase the risk of bone loss and fracture. Studies are needed to explain these differential associations for adiponectin in women and men.

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1.0 DISSERTATION OVERVIEW AND OBJECTIVES

Osteoporosis is defined as a systemic bone disease characterized by low bone mass and mircoarchitectural deterioration of bone tissue, with a subsequent increase in bone fragility and susceptibility to fracture.[1] The World Health Organization (WHO) defines osteoporosis as having a BMD of less than or equal to 2.5 standard deviations (SDs) below the mean BMD of a young adult.[2] Osteoporosis has major public health implications for aging men and women. In 1995, it was estimated that 18% of women, and 6% of men, 50 years of age or older in the United States (US) had this disorder.[2] An abundance of research has been conducted to better understand the etiology and prevention of osteoporosis in women. There is also growing recognition that osteoporosis and fractures in older men are significant public health problems that contribute to disability and premature death.[3] Men over the age of 50 have a 13% estimated risk of developing an osteoporotic fracture.[4] Mortality rate after a hip fracture has been shown to be greater in men than women.[1]

In past studies, BMD measurements were primarily made at the lumbar spine and hip by using dual energy x-ray absorptiometry (DXA). Two major limitations of the DXA technique is that it cannot distinguish cortical and trabecular bone and only provides measurement for the 2 dimensional areal BMD (aBMD) rather than the 3 dimensional volumetric BMD (vBMD). Bones of larger width and height tend to be thicker, and since bone depth is not factored into DXA, reliance on aBMD inherently underestimates the bone density.[5] Measurement of vBMD is

advantageous because it is independent of skeletal size. A method known as quantitative computed tomography (QCT) has been developed to assess vBMD, distinguish between cortical and trabecular bone, and provide information on geometry and bone strength.[6] Bone geometry is an important determinant of bone strength and fracture risk.[7] The two primary locations for QCT measurements are at the central (lumbar spine and proximal femur) and peripheral (radius and tibia) regions of the skeleton.[8] Two recent large studies in Tobago examined correlates of trabecular and cortical vBMD in men of African descent.[9,10] However, no comprehensive studies to date have examined correlates of vBMD in older white men.

Vitamin D is important for bone growth[11] and preservation.[12] Higher serum 25-hydroxyvitamin D levels enhance calcium absorption.[13] Calcium is the structural component of the bone and low levels in the body can lead to bone resorption that is directed by the parathyroid hormone (PTH).[14] Vitamin D deficiency can cause secondary hyperparathyroidism, resulting in high bone turnover and bone loss.[15] Adequate vitamin D levels are needed to maintain PTH levels and help prevent major bone loss.[16] Most comprehensive studies examining the association between serum 25(OH)D and BMD in men have evaluated this in participants of Caucasian descent.[17-20] Additionally, there is a dearth of studies that determine the association between serum 25(OH)D and bone geometry[21] and bone strength.

Leptin is a hormone secreted by adipose cells that have been shown to impact bone mineral density (BMD).[22] This molecule has been shown to be highly correlated with body fat mass[23] and been involved in fat metabolism and appetite control.[24] Leptin has been shown to play a role in the modulation of bone formation by enhancing differentiation of bone marrow stroma into osteoblasts and inhibiting its differentiation into osteoclasts and adipocytes.[25,26]

Adiponectin is another adipocyte derived hormone that regulates insulin sensitivity and has anti-inflammatory properties.[27] Unlike leptin, adiponectin levels are lower among individuals with obesity.[28] Adiponectin and its receptors are produced by human bone-forming cells, suggesting that adiponectin may be a hormone linking bone and fat metabolism[29]. Adiponectin may have harmful effects on bone by stimulating the receptor activator of nuclear factor-κB ligand (RANKL) pathway and inhibiting the production of the decoy receptor for RANKL, osteoprotegerin.[30] However, adiponectin administration has also been shown to increase osteoblast proliferation and differentiation, alkaline phosphatase activity (ALP), a marker of bone formation, and mineralization.[31] Few studies have examined the impact of these hormones on incident fractures[32,33] and changes in aBMD[34,35] and vBMD.

This dissertation aims to identify factors associated with fracture, bone mineral density, bone geometry, and bone strength in older adults. A cross-sectional analysis in a sample of primarily Caucasian men will examine correlates of trabecular and cortical vBMD of the radius and tibia and see if they differ. Another study, will test the hypothesis that higher levels of serum 25(OH)D will be positively associated with vBMD, bone geometry, and bone strength measures in men of Caucasian and African Heritage. Finally, a prospective study will examine the impact of baseline serum leptin and adiponectin on the risk of a fracture, hip aBMD and spinal vBMD loss, in men and women of Caucasian and African American descent.

2.0 INTRODUCTION

2.1 EPIDEMIOLOGY OF OSTEOPOROSIS AND OSTEOPOROTIC FRACTURES

2.1.1 Prevalence of osteoporosis and osteoporotic fractures

Approximately 22 million women and 12 million men in the US are considered to have low bone mass (osteopenia or osteoporosis).(2) In the United States, there are approximately 700,000 vertebral fractures, 300,000 hip fractures, 250,000 wrist fractures, and 300,000 fractures at other skeletal sites.(35) The estimated number of hip fractures worldwide is expected to increase from 1.66 million in 1990 to 6.26 million in 2050.(36) Currently, North America and Europe account for approximately half the hip fracture in the world, however by 2050 this estimate is expected to be reduced to 25% because of the large growth of fractures in Asia.(36) The mortality rate 1 year after having a hip fracture is estimated to be around 20%.(37) Among white women with osteoporosis, 40 to 50% are expected to have a hip fracture.(38;39) The highest incidence rates for hip fracture in men were found in Scandinavian countries while some of the lowest have been reported among blacks and Asians.(40;41) Men over the age of 50 have a 13% lifetime estimated risk of developing an osteoporotic fracture in the US, although in Sweden it is estimated to be 23%.(4) Men with low BMD have an estimated 50% lower fracture rate than women with low BMD.(2)

2.1.2 Mortality and Morbidity

Nearly one-third of hip fractures result in nursing home admissions and one-fifth of patients with hip fractures die within the year following their fracture.(42) The incidence of hip fractures in men is uncommon till after the age of 75.(1) However, mortality rate after a hip fracture is greater in men than women.(1) African Americans who sustain a hip fracture have increased morbidity and nearly twice the mortality as Caucasian Americans.(37;43)

2.1.3 Economic Burden

Fractures accounted for an estimated $17 billion (25% in men) in health care service usage in 2005, with costs likely to rise to $25 billion in 2025.(44) In terms of type of health service for osteoporotic fractures, 62.4% of the costs are attributed to inpatient hospitalization costs and 28.2% to nursing home care.(45) More than 20% of osteoporotic related costs were due to fractures.(44) Among men, Caucasians have accounted for an estimated 80% of all costs related to fractures.(44)

2.2 PATHOPHYSIOLOGY OF OSTEOPOSOSIS

As people get older, resorption of the minerals in the cortical layer can lead to an inexorable loss of trabecular bone and a widening of the bone cavity.(46) Also, bones tend to get thicker with age however they are not getting any denser as peak bone mass tends to decline.(47) Bone architecture and continual remodeling combine to have a huge impact on the pathophysiology of

osteoporosis. For example, having a wider femur at a young age might put individuals at risk for hip fractures late in life because wider bones tend to have thinner cortical layers which are more susceptible to resorption later in life.(48) Cellular pathophysiology is influenced by osteoclasts which pump protons into the extracellular space lowering the pH in their own microenvironment which results in dissolving of the bone mineral.(49) Osteoblasts are bone cells that lay down new bone material and facilitate mineralization of the bone. The balance between these activities by osteoblasts and osteoclasts results in whether bone is made, maintained or lost.(50) Any increase in remodeling activity tends to result in bone loss because bone formation takes longer to occur than resorption.(51) The balance between bone formation and resorption is primarily mediated by the interaction between receptor activator of nuclear factor kB ligand (RANKL) and osteoprotegerin (opg). Hormones such as parathyroid hormone (PTH)(52), and prostaglandins(53), bind to receptors on osteoblasts. This initiates the release of RANKL which binds to the RANK receptor on osteoclasts leading to the proliferation and differentiation of these cells followed by increased bone resorption.(6;54-57) OPG is a cytokine that is also released by osteoblasts which can bind to RANKL, preventing RANK and RANKL from coming into contact and thus reducing resorption of the bone.(58)

The osteoclast arises from hematopoietic stem cells and is a bone lining cell responsible for bone resorption. Produced by the stromal cell, macrophage colony stimulating factor (M-CSF) is responsible for the differentiation of hematopoietic stem cells into pre-oseteoclasts. The receptor activator of nuclear factor kappa B (RANK) receptor, and receptor activator of nuclear factor kappa B ligand (RANKL) is required for the differentiation of osteoclasts. When RANKL activates RANK receptors on the pre-osteoclasts, the cells fuse and differentiate into mature multinucleated osteoclasts. The mature osteoclasts solubilize the mineral and organic

constituents of bone matrix by synthesizing lysosomal enzymes and activating collagenase. High levels of calcium, magnesium, phosphate and products of collagen are released into the extracellular fluid as the osteoclasts tunnel into the mineralized bone.(31)

2.3 AGE-RELATED CHANGES IN TRABECULAR AND CORTICAL BONE

Past studies have shown that reduction in trabecular vBMD occurs before mid-life (age 20-49) in men and women and continues unabated through life.(6;56;57). In a recent study, (among women and men, aged <50 years) trabecular bone loss accounted for 37% and 42% of lifetime bone loss, respectively. In contrast, the amount of cortical bone lost before 50 years of age represented only 6% in women and 15% in men. Mechanisms for early onset of trabecular vBMD loss remain largely unknown. Riggs et al. hypothesized that decreases in paracrine production of the hormones IGF-1 and IGF-2 by osteoblasts may contribute to significant trabecular bone loss early on in life.(6) Prior findings show that major cortical bone loss occurs late in life (age ≥50).(6;54-57) This observation is likely related to being sex steroid deficient late in life.(6) In women, substantial cortical bone loss has been shown to occur after mid-life (age ≥50) in association with menopause and estrogen deficiency.(59) However, cortical bone loss in men seems to decrease at a constant rate in young adulthood until accelerating late in life (age ≥75).(6)

2.4 MEASUREMENT OF BONE STRENGTH

In past studies, BMD measurements were primarily made at the lumbar spine and hip using dual energy x-ray absorptiometry (DXA). A major limitation of the DXA technique is that it cannot distinguish cortical and trabecular bone and only provides measurement for the 2 dimensional areal BMD (aBMD) rather than the 3 dimensional volumetric BMD (vBMD). Bones of larger width and height tend to be thicker, and since bone depth is not factored into DXA, reliance on aBMD inherently underestimates the bone density.(46) Measurement of vBMD is advantageous because it is independent of skeletal size. A method known as quantitative computed tomography (QCT) has been developed to assess vBMD, distinguish between cortical and trabecular bone, and provide information on geometry or structure.(6) The two primary locations for QCT measurements are at the central (lumbar spine and proximal femur) and peripheral (radius and tibia) regions of the skeleton.

Bone geometry is an important determinant of bone strength and fracture risk.(7) The cross-sectional area of the medullary cavity increases in old age as endosteal resorption outpaces periosteal apposition, resulting in reduction in the cortical area and bone strength.(60) Reduction in cortical area has been shown to be more pronounced in women than in men. This is primarily due to the fact that men have greater periosteal apposition than women, despite having similar rates of endosteal resorption.(61) Researchers have found that resistance of bone to compressive (axial) loads primarily depends on the cortical area (CA), whereas resistance to bending and torsion depends on the distribution of cortical mass about the bone center.(62)

Bone strength is largely influenced by a combination of bone material properties and cross-sectional geometry.(7) There are several ways to measure bone strength using QCT.(63)

The bone's polar and axial moments of inertia measure the redistribution of cortical mass by combining the distribution and area of cortical bone as an index of bending and torsional strength.(64) The polar moment of inertia (I_{polar}, mm^4) of a bones cross-sectional area can determine the bones ability to resist torsion.(63) It is calculated by taking the $\int$ of the cross-sectional area of a voxel (A) multiplied by the square of the center of gravity distance to the cross-sectional area of the voxel (d^2), $\int d^2A$.(65) The axial moment of inertia ($I_{axial,}$ mm^4) is a measure of the distribution of cortical bone mass about the center of the cross-section of a tubular bone and is related to bending strength of bone.(66) Section Modulus (SM) is the maximum bending stress in a cross-section (mm^3). SM $=\int d^2A/d_{max}$, where d_{max}= the maximum distance of any of the voxels from the center of gravity.(65) As mentioned earlier, bone strength is influenced by bone material properties and cross-sectional geometry. A key bone material property is the elastic modulus or stiffness. It is difficult to measure stiffness in vivo however the cortical vBMD is approximately linear to the elastic modulus and can be used as surrogate for bone material property.(64) The polar or axial strength-strain indices (SSIp, SSI_x) take bone material into account by calculating the weighted bone density. The strength strain indices denote the density-weighted polar or axial section moduli and reflect the torsional and bending rigidity of the long bone shaft (mm^3).(67) SSI= $=\int d^2A*(vBMD_{vox}/vBMD_{max})/d_{max}$, where $vBMD_{vox}$=bone density of a voxel and $vBMD_{max}$=1200 mg/cm^3, maximal bone density.(65)

2.5 CORRELATES AND RISK FACTORS FOR LOWER BMD THAT HAVE BEEN IDENTIFIED

2.5.1 Demographic measures

Demographic risk factors for osteoporosis include older age, female sex, and white race.(35) African Americans have been shown to have higher BMD than Caucasians independent of body weight.(3;68;69) An association between genetics and BMD has been shown in several studies.(38;70-73) A negative association between parental history of fracture and lower BMD was found in two studies on older women.(38;71) Twin studies have shown that up to 80% of the variance for BMD is controlled by genetics.(35) Candidate genes researched that are known to influence bone metabolism include the vitamin D receptor (VDR)(74) the type I collagen α1 gene(75), the estrogen receptor(76), Apolipoprotein E (ApoE), transforming growth factor B 1 (TGFB1) and interleukin-6.(72) VDR is a receptor involved in generation of proteins which are involved in calcium absorption in the intestines. The absence (BB genotype) of BsmI restriction site for the VDR receptor in intron 8 is present in 17% of Caucasians and 13% of African Americans BsmI.(73) A study in Australia on women with a mean age of 52.5 years found that subjects with the BB genotype had significantly lower lumbar spine and femoral neck aBMD than those with the bb genotype.(74) The FokI polymorphism (ATG to ACG) found in 18% of Caucasians and 4-6% of African Americans has been shown to be associated with lower spinal and femoral neck BMD in three studies.(73;77) Subjects with the *APOE**4 allele product appear to have appear to have reduced levels of vitamin K and impaired carboxylation of osteocalcin compared to subjects with the *APOE**2 or *APOE**3 allele products.(73) Some studies have also shown that individuals with this allele have more likely to have lower BMD than others.(78;79)

2.5.2 Anthropometric measures

Greater weight has been shown to be associated with a lower probability of osteoporosis. Several hypotheses have been proposed for this observation. Larger skeletal frames and greater muscle and fat compartments in heavy individuals may increase the mechanical load on the skeleton promoting mineralization and structural adaptive responses that can strengthen bone.(80) In postmenopausal women, adipose tissue is the principal site for estrogen biosythnesis. Hormones leptin and insulin, have been associated with increased body weight and BMD.(81) On the other hand, heavier weight was related to lower cortical vBMD in some studies.(9;82) It is also possible that mechanical factors or differences in bone geometry may explain these findings. Increased mechanical loads can lead to bone microdamage, increased bone turnover, and a transient reduction in cortical vBMD.(83) Also, overweight individuals may adapt by increasing periosteal bone diameter requiring lower cortical density to maintain the same strength.(84)

Findings examining the relationship between height and BMD have been conflicting. Several studies have found an inverse relationship between height and BMD,(3;18;85) while others have found a positive association.(86;87) Taller individuals are known to experience larger height loss and have been shown to be a higher risk of fracture than others.(38;88;89)

2.5.3 Lifestyle factors

Certain lifestyle factors have been found to be associated with BMD. Most studies have found that smokers are at an increased odds of having lower BMD compared to nonsmokers

independent of body weight.(55;90;91) In vitro studies have shown that the toxicity from nicotine may decrease osteoblast activity.(92;93) Smoking may also have a negative effect on BMD by decreasing calcium absorption in the intestines.(94) Smoking also may increase the odds of lower BMD by altering estrogen metabolism in women.(95) Very high levels of alcohol have been found to increase the risk for osteoporosis,(96;97) while low to moderate intake may benefit, or have no effect on BMD.(3;3;86;87;91;91;98-100) It has been hypothesized that alcohol may increase BMD by raising sex steroid levels.(98;99) High caffeine intake has been implicated as a risk factor for lower BMD in men(87) and women.(38;101-103) Caffeine may reduce BMD by decreasing intestinal calcium absorption, however, it may also be a marker for low calcium consumption.(104) Low physical activity has been identified as a risk factor for lower BMD. Lack of physical activity or immobility may cause bone loss by increasing bone resorption.(105-107) Two studies on postmenopausal women found that walking significantly reduced bone mineral density.(108;109) A study on young men from Sweden found a positive association between physical activity and greater trabecular volumetric bone mineral density vBMD.(110) The effects of calcium intake and vitamin D on BMD are well documented. In regards to calcium, several studies have found a positive association with BMD.(3;87;91;100) An adequate intake of calcium is needed to ameliorate the progressive loss of bone with age and avoid osteoporosis.(111) Most studies on vitamin D intake has also been found to have a positive effect on BMD.(112-116) Low levels of serum vitamin D reduce the rate of calcium absorption in the intestines which subsequently can lower BMD.(117)

2.5.4 Medical conditions

Several medical conditions have been consistently related to BMD. Diabetes mellitus, despite being related to higher fracture risk, has been associated with higher BMD, independent of weight, in most studies on older men(3;118;119) and women.(120;121) Factors associated with greater BMD in individuals with diabetes compared to non-diabetics include greater abdominal obesity, growth factors(3) and use of statins.(118) Depression has also been linked to lower BMD independent of the fact that depression may lead to lower weight.(122;123) Hypothyroidism can increase the risk of lower BMD in men(124) and women.(125;126) Excess thyroid hormone in the body has been known to decrease BMD by increasing bone resorption.(127) Diseases that result in cognitive impairment such as stroke and Parkinson's disease have also been shown to be risk factors for reduced BMD in women, though the mechanism is not well understood.(38;128;129) Most studies have a negative association between history of a gastrectomy and BMD.(86;130) The operational removal of the stomach had been known to result in loss of weight and reduction in calcium absorption, which subsequently decreases BMD.(130;131) A previous link between OA and higher BMD has been established.(3;132;133) Though the mechanism is not well understood it has been hypothesized that genetics,(3;133) sex steroid levels(3) and osteophyte(86) formation may increase BMD among those with OA. Two large studies have found a positive association between hypertension and BMD in men of Caucasian(3) and African descent.(134) This association though not completely understood may reflect higher insulin levels(3) or may reflect confounding by the use of certain medications such as thiazide (TZ) diuretics that may increase vBMD due to their ability to improve calcium retention.(135-138) Studies have found a negative correlation between RA and BMD(86;139;140) in men and women. The negative association

between RA and BMD can be explained by an increase in inflammatory activity among those with RA resulting in a greater rate of bone loss than normal.(139) Inflammatory activity influenced by C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) has been shown to increase bone resorption in individuals with RA.(141)

2.5.5 Medications

In addition to TZ diuretics, other medications may also increase or decrease BMD. Corticosteroid have been shown to decrease BMD.(142) Ant-depressive treatment, specifically selective serotonin re-uptake inhibitors have been linked to lower BMD in some studies independent of depression or weight.(3;143) Studies have hypothesized that SSRI use may reduce osteoblast activity by disrupting the serotonin transport gene leading to lower BMD.(143;144) Another study on mice found that those lacking the serotonin transporter gene have lower BMD than wild-type mice.(145) Statin use has been found to be associated with greater BMD in independent of MI and body weight, in study on postmenopausal women(146) and another on older men.(3) Past studies have showed that non-steroidal anti-inflammatory drug use was associated with greater BMD which may reflect a role of prostaglandin.(3;147)

2.5.6 Neuromuscular function

A positive association between neuromuscular function and BMD has been documented in several studies.(3;55;148-152) Though the rationale for this association is not entirely clear, it is thought that this may be due to the increased skeletal loading that occurs as a result of having

stronger muscles.(149) However, a recent study on men of African descent in Tobago found an inverse association between grip strength and BMD.(9)

2.5.7 Risk factors specific to women

Several traits related only to women have been examined in their role with BMD. Researchers have found that the association between parity and BMD is likely to be null though it may increase the risk of a hip fracture.(35;153) History of breastfeeding may decrease BMD initially, however in the period after cessation women are likely to recover this bone loss.(154;155) Other factors not associated with osteoporosis include earlier age of menopause onset, and history of a premenopausal oophorectomy.(38;156) Low levels of endogenous estrogens can increase the risk of osteoporosis among women by making calcium maintenance more difficult.(157;158) Also, osteoblasts contain estrogen receptors that are highly sensitive to estrogen levels.(159) In addition, estrogen can improve BMD, by increasing OPG and decreasing RANKL production by osteoblastic cells, and by suppressing production of TNF-α and other pro-resorptive cytokines, estrogen reduces osteoclastogenesis.(160) Estrogen therapy however has been shown to slow bone loss and reduce fracture risk.(161) However, once discontinued it does not appear to have long lasting effects on BMD loss and the risk of fracture. This is important to consider given the adverse effects of long-term estrogen therapy on cardiovascular disease, stroke and breast cancer.(162)

2.5.8 Risk factors specific to men

In men, low levels of testosterone have been associated with an increased risk of osteoporosis.(163;164) A cell culture study found androgen receptors on human osteoblast-like cells.(165) High Androgen levels may decrease the risk of osteoporosis by promoting osteoblast proliferation and differentiation, and inhibiting osteoclast recruitment.(166) The impact of testosterone on the bones may be mediated by aromatization to oestradiol based on case reports of osteoporosis in men with mutations in the oestrogen receptor or aromatase genes.(167;168) Estrogen therapy on these men had a large effect on the patient's bone growth and maturation.(167;168) Androgen replacement therapy has been shown to increase BMD in some studies.(169;170) Studies have also shown that androgen deprivation therapy or surgery to treat prostate cancer may result in hypogonadism which can increase an individual's risk of having osteoporosis.(3;171-173)

2.6 SERUM 25(OH)D AND BONE

2.6.1 25(OH)D

Vitamin D is a fat soluble vitamin that comes in 2 major forms, D_2 and D_3. Vitamin D_2 (ergocalcifirol) is derived from plants, but is not formed inside the body. Vitamin D_3 (cholecalciferol) is primarily found in animals and can be produced endogenously when ultraviolet rays from sunlight strike the skin and trigger vitamin D synthesis.(174) Few foods (fish, mushrooms, egg yolk) naturally contain vitamin D. The vitamin D obtained from sun

exposure, food, or supplements is biologically inactive and most undergo hydroxylation to become active. The liver converts vitamin D to 25-hydroxyvitamin D [25(OH)D] followed by further hydroxylation in the kidney which results in the physiologically functional 1,25-dihydroxyvitamin D [1,25(OH)2D] compound. The renal production of 1,25(OH)2D is tightly regulated by parathyroid hormone (PTH) levels and serum calcium and phosphorus levels.(175)

2.6.2 Definition of 25(OH)D deficiency, insufficiency and sufficiency

There is no clear consensus on what optimal levels of 25(OH)D in the serum are needed.(175) Vitamin D deficiency has been defined by most experts as 25(OH)D values of less than 20 ng/ml or 50 nmol/l.(176-178) Serum 25(OH) levels have been shown to be inversely associated with PTH levels until they reach 30-40 ng/ml or 75-100 nmol/l, at which point PTH levels begin to level off.(179;180) Also, intestinal calcium transport increased by 45 to 65% in women when 25 (OH)D levels were increased from an average of 20 to 32 ng/ml per or 50 to 80 nmol/l.(12) Based on these observations a level of 21-29 ng/ml or 52 to 72 nmol/l can be considered to indicate 25(OH)D insufficiency and 30 ng/ml or greater can be defined as having sufficient 25(OH)D.(181) However, recent cohort studies in older men and women found that hip fracture risk was reduced among participants with 25(OH)D levels >20 ng/ml.(182;183) In addition, a cohort study showed that men with 25(OH)D levels below 20 ng/ml experienced greater hip aBMD loss while rates were similar in higher levels.(184) All of this evidence points to a lower 25(OH)D threshold than previous research in regards to bone health.

2.6.3 25(OH)D levels in men of Caucasian and African descent

Serum levels of 25(OH)D are generally lower among blacks compared with Caucasian individuals in other studies.(185) African Americans typically have lower levels of serum 25(OH)D than Caucasians across all age groups and both genders (149;185-188) due at least in part to differences in skin pigmentation and dietary vitamin D intake. However, mean 25(OH)D concentrations in the African continent are relatively high, but vary considerably according to geography, climate, and other factors.(189)

2.6.4 25(OH)D and bone

Vitamin D is important for bone growth(10) and preservation.(11) Higher serum 25-hydroxyvitamin D levels enhance calcium absorption.(12) Calcium is the structural component of the bone and low levels in the body can lead to bone resorption that is directed by the PTH.(13) Without vitamin D, only 10 to 15% of dietary calcium and about 60% of phosphorus (another element that consists widely in bone) is absorbed.(175) Vitamin D deficiency can impact bone growth(10) and preservation(11) by increasing the risk of secondary hyperparathyroidism and decreasing calcium absorption, resulting in greater bone turnover, bone loss and increased risk of fracture.(14;190) Adequate vitamin D levels are needed to maintain PTH levels and help prevent major bone loss.(15) Chronic elevations in PTH have been shown to have primarily catabolic effects on cortical bone and anabolic effects on trabecular bone.(191) In cortical bone, chronic high levels of PTH can result in increased endocortical resorption, cortical thinning and cortical porosity.(192;193) Several studies have shown that PTH administration and elevated serum PTH levels were associated with preserved trabecular bone

structure.(194-198) PTH is likely to increase trabecular bone by increasing osteoblast bone differentiation.(199)

2.6.5 25(OH)D assays

The use of different serum 25(OH)D assays has made it difficult to compare studies. Binkley et al. found that they yield markedly different results after comparing radioimmunoassay (RIA) and competitive binding protein methods to high performance liquid chromatography (HPLC).(200) The use of RIA or competitive binding protein assay has also been shown to overestimate 25(OH)D levels and have poorer reliability compared to HPLC and liquid chromatography and analysis on a tandem mass spectrometer (LC-MS/MS).(201;202)

2.6.6 Past studies examining the association between 25(OH)D and bone density, geometry and strength measures

The prospective and cross-sectional findings examining the association between serum 25(OH)D and BMD in older men have been inconsistent. Some studies have found a positive(15;16;19;203-205) association while others reported null findings.(17;18;206-208) One study examined the effects of vitamin D on bone geometry.(20) The correlation between 25(OH) and bone strength measures such as polar/axial moment of inertia or strength strain index has not been evaluated. Few studies have examined the association of 25(OH)D with measures of bone health in men of African descent.(16;183;204) The association between higher levels of 25(OH)D and greater bone health has been established in Caucasians, however in men of African descent there is no clear consensus. Men of African descent have also been shown to

be resistant to the resorptive effects of PTH on bone and markers of bone resorption.(209;210) Also, polymorphisms in the vitamin D receptor (VDR) gene may explain this differential association.(189) The absence of the BsmI restriction site and the FokI polymorphism for the VDR receptor occur at a higher frequency in whites than blacks and were found to be associated with lower aBMD.(73;74;77) Table 2-1 below provides a summary of past findings.

Table 2-1 Characteristics of studies examining the association between serum 25(OH)D and BMD and geometry, primarily in men

Authors, Year and Journal	Study Design	Study Population	Major Findings
Dennison et al.1999. Osteoporos Int.	Longitudinal (4 years)	73 white men and 143 women aged 60-75	Null association for serum 25(OH)D and BMD of the lumbar spine and femoral neck in men and women.
Hannan MT et al. 2000. JBMR	Longitudinal (4 years)	1164 white men and women mean age 74 years	A null association at all sites including femoral neck, trochanter, Ward's area, radius and lumbar spine in men and women.
Szulc P et al. 2003. Calcified Tissue Int.	Cross-sectional	881 men aged 19-85 years that included 595 men aged 55-85 years.	Reported that 25(OH)D levels were significantly correlated with BMC but not BMD in older men.
Yan L et al. 2003. Bone	Cross-sectional	352 healthy volunteers, 60-83 years old, were studied in Shenyang, China (108 men, 110 women), and Cambridge, UK (67 men, 67 women)	No association was found between 25(OH)D and BMD. Marginally significant (p=0.05) association between femoral BMC and 25(OH)D in the UK sample.
Bischoff-Ferrari HA et al. 2004. Am J Med	Cross-sectional	13,432 men and women. Young and older white, Mexican American, and black	Men and women with higher 25 (OH)D levels had higher hip BMD for all racial groups.
Bischoff-Ferrari HA et al. 2005. Arthrites and Rheumatism	Cross-sectional	A total of 228 subjects (83 men and 145 women) mean age 74.4 years, with primary radiographic knee OA. Framingham study.	In the entire population, there was a positive significant trend for femoral neck aBMD across categories (deficient, hypovitaminosis D, and replete) of 25(OH)D, P = 0.04
Lauretani F et al. 2006. Bone.	Cross-sectional	372 Italian men and 435 women, aged 65-96	Cortical and trabecular bone area was positively associated with serum 25 (OH)D (nmol/l) levels in women.
Saquib et al.2006. Osteoporosis Int.	Cross-sectional	Rancho Bernardo study. 414 Caucasian men 45 or older	25(OH)D was positively associated with aBMD at the hip (p=0.01) and spine (p=0.001)
Hannan MT et al. 2008. J Clin Endocrinol Metab.	Cross-sectional	331 Black, 362 Hispanic, and 421 White men from Massachusetts with a mean age of 48 years	A significant association between 25(OH)D and hip and spinal aBMD among Caucasian men only.
Ensrud KE et al. 2009. J Clin Endocrinol Metab.	Longitudinal (4 years)	The Osteoporotic Fractures in Men Study, MrOS. 1279 community-dwelling men aged 65 yr or older with 25(OH)D levels	25(OH)D levels below 20 ng/ml had greater subsequent rates of hip bone loss, but rates of loss were similar among men with higher levels
Kuchuk NO et al. 2009. J Clin Endocrinol Metab.	Cross-sectional	Longitudinal Aging Study Amsterdam. 1319 subjects (643 men and 676 women) ages 65- 88	A significant association between 25(OH)D and total hip BMD in men and women.

2.7 SERUM LEPTIN AND BONE

2.7.1 Leptin

Leptin is a hormone secreted by adipose cells that have been shown to impact bone mineral density (BMD).(21) Leptin is a protein molecule secreted by white adipose tissue that is highly correlated with body fat mass.(22) Leptin has been known to be involved in fat metabolism and appetite control.(23) Leptin has been shown to play a role in the modulation of bone formation by enhancing differentiation of bone marrow stroma into osteoblasts and inhibiting its differentiation into osteoclasts and adipocytes.(24;25)

2.7.2 Leptin and bone

In vivo studies in mice have shown an increase in BMD after leptin administration.(211-213) A study in leptin knockout mice showed that BMD increased following administration of leptin.(211) A positive effect on BMD was also observed in another study after administration of leptin prevented the fall in plasma osteocalcin observed during 24-hour and 5-day starvation in male mice.(212) Hemrick et al. found that Leptin treatment induces loss of bone marrow adipocytes and increases bone formation in leptin-deficient ob/ob mice.(213)

Several in vitro findings reported that higher leptin can have a positive impact on BMD.(214-216) Takahashi et al. showed that leptin induced mitogen-activated protein kinase-

dependent proliferation of C3H10T1/2 cells and a multipotential stromal murine cell line(215). Another study found that leptin was associated with the development of a more mature osteoblastic cell line.(214) Also, Thomas et al. observed an increase in osteoblastic differentiation of human marrow stromal hMS2-12 cells after leptin administration, which led to an increase in the mineralization of the extracellular matrix.(216)

Several population studies reported that leptin is positively(34;217-222) associated with BMD, while others observed a negative association(223-229), or a null finding after adjusting for body weight.(227;230;231) Few studies have examined the association between leptin and fracture.(32;222) Table 2-2 summarizes some of these findings.

Table 2-2 Characteristics of studies showing the association between serum leptin, fractures and BMD

Authors, Year and Journal	Study Design	Study Population	Major Findings
Martini G et al. 2001.Bone.	Cross-sectional	123 healthy postmenopausal women aged 39-82 years	After adjustment for BMI, the association between leptin and BMD was no longer significant
Pasco et al. 2001. J Clin Endocrinol Metab	Cross-sectional	Nonobese Australian women aged 20-91 years	A significant positive association at the lateral spine, proximal femur sites, and whole body BMD
Thomas T et al. 2001. Bone	Cross-sectional	Sample of 137 premenopausal women (21-54 years), 165 postmenopausal women (34-93 years), and 343 men (23-90 years)	Serum leptin levels were significantly associated with hip, spine, and radial aBMD in the women, but not in the men.
Yamauchi M et al. 2001. Clin Endocrinol (Oxf)	Cross-sectional	139 postmenopausal women in Japan aged 48-78	Higher leptin levels were associated with a lower prevalence of vertebral fractures.
Blain H et al. 2002. J Clin Endocrinol Metab.	Cross-sectional	107 white postmenopausal women aged 50-90	Leptin was associated with whole body and femoral neck BMD.
Ruhl CE et al. 2002. JBMR	Cross-sectional	5815 adults from the Third U.S. National Health and Nutrition Examination Survey (NHANES III; 1988-1994	Inverse association between leptin and BMD in men after adjusting for BMI. Null associations observed in pre and post menopausal women
Blum M et al.2003. Calcif. Tissue Int.	Cross-sectional	153 premenopausal women	Leptin was inversely associated with BMD
Morberg CM et al. 2003. J Clin Endocrinol Metab	Cross-sectional	Denmark 327 non-obese men and 285 obese men	Null finding related to BMD in both groups non-obese and obese.
Sun AJ et al. 2003. Acta Diabetol.	Cross-sectional	50 healthy men, 18-66 years of age	Leptin (p=0.004) was negatively associated with whole body aBMD
Zoico et al. 2003. Clin Endocrinol (Oxf)	Cross-sectional	A cohort 92 men and 171 women, of Caucasian descent	A significant positive association between leptin levels, whole-body, total hip and

Table 2-2 continued.

		with ages ranging from 68 to 75 years	femoral neck BMC and BMD was found in both sexes
Kontogianni MD et al.2004. JBMR	Cross-sectional	Twenty-five premenopausal and 55 postmenopausal, healthy women (42-68 years old)	Leptin levels were negatively correlated with BMD of spine and total body only when insulin was included in the model
Schett G et al. 2004. Am J Med	Longitudinal (10 years)	Italy. 125 women and 125 men, mean age 58.5 years	Individuals in the highest tertile for leptin had 0.25 times the hazard of having a nontraumatic fracture than those in the lowest tertile.
Oh KW et al. 2005. Clin Endocrinol (Oxf)	Cross-sectional	80 male adults (mean age 54.5 years)	Log-transformed serum leptin showed a significant negative correlation with lumbar spine BMD after adjusting for age and BMI
Lorentzon M et al. 2006.JBMR	Cross-sectional	1068 Swedish young men (aged 18-20)	Negative association between leptin and areal BMD at the lumbar spine, trochanter and total body.
Weiss LA et al. 2006.J Bone Miner Res.	Longitudinal (4 years)	Community-dwelling white men (N = 498) and nonestrogen-using postmenopausal women (N= 411), 45–92 years of age	Leptin associated with greater femoral neck, total hip, lumbar spine and radial BMD among women at baseline, but not over 4 years.
Jurimae J et al. 2008. J Bone Miner Metab.	Cross-sectional	Healthy postmenopausal women (n = 88; age, 68.9)	No longer significant association between leptin and BMD after adjusting for body composition measures
Jurimae J et al. 2009. Eur J Endocrinol.	Longitudinal (1 year)	35 women (age: 69.7+/-6.0 years)	Leptin predicted lower femoral neck and total body BMD

2.8 SERUM ADIPONECTIN AND BONE

2.8.1 Adiponectin

Adiponectin is another adipocyte derived hormone that regulates insulin sensitivity and has anti-inflammatory properties.(26) Unlike leptin, adiponectin levels are lower among individuals with obesity.(27)

2.8.2 Adiponectin and bone

Adiponectin and its receptors are produced by human bone-forming cells, suggesting that adiponectin may be a hormone linking bone and fat metabolism(28). Adiponectin may have harmful effects on bone by stimulating the receptor activator of nuclear factor-κB ligand (RANKL) pathway and inhibiting the production of the decoy receptor for RANKL, osteoprotegerin.(30) However, adiponectin administration has also been shown to increase osteoblast proliferation and differentiation, alkaline phosphatase activity (ALP), a marker of bone formation, and mineralization.(30)

Several studies reported that lower levels of adiponectin have been linked with higher BMD.(33;217;220;232) while others have failed to find an association between adiponectin and BMD.(26;225;225;227;229;233-235) Few studies have examined the association between adiponectin and fracture risk.(31;236) Table 2-3 below summarizes these findings.

Table 2-3 Characteristics of studies showing the association between serum adiponectin, fractures and BMD

Authors, Year and Journal	Study Design	Study Population	Major Findings
Huang KC et al.2004. Clin Endocrinol (Oxf)	Cross-sectional	105 nondiabetic female adolescents mean age 15.4 years	No association between adiponectin and total body BMD and BMC
Kontogianni MD et al.2004. JBMR	Cross-sectional	Twenty-five premenopausal and 55 postmenopausal, healthy women (42-68 years old)	Adiponectin was not significantly correlated with BMD(L2-L4) nor with total body BMC
Oh KW et al. 2005. Clin Endocrinol (Oxf)	Cross-sectional	80 male adults (mean age 54.5 years)	Adiponectin was no longer significant with femoral neck or lumbar spine aBMD after adjusting for age and BMI
Jurimae J et al. 2007. Osteoporos Int.	Cross-sectional	White women aged 38-49 years	Negative association between adiponectin and femoral neck and lumbar spine BMD.
Richards JB et al. 2007. J Clin Endocrinol Metab.	Cross-sectional	1735 nondiabetic white women. Mean age 50.	Doubling of serum adiponectin was inversely related to a significant mean decrease in total hip femoral neck, forearm and spine BMD.
Gonnelli S et al. 2008. Calcif Tissue Int	Cross-sectional	137 men aged 55 years and older	No significant correlations between adiponectin and BMD at all skeletal sites
Michaelsson K et al. 2008. J Clin Endocrinol Metab	Longitudinal (15 years)	Swedish men (N=441) and women (N=457) aged 70 and above	There was no association between lower adiponectin levels and fracture risk after finding an inverse relationship for BMD
Jurimae J et al. 2008. J Bone Miner Metab.	Cross-sectional	Healthy postmenopausal women (n = 88; age, 68.9)	No longer significant association between adiponectin and BMD after adjusting for body composition measures
Zoico et al. 2008. J Endocrinol Invest	Cross-sectional	Thirty-six post-menopausal non-diabetic elderly women, with ages ranging from 66 to 77 years	After adjusting for fat mass only adiponectin maintained a significant relation with whole body and femoral aBMD
Basurto L et al. 2009. Eur J Endocrinol	Cross-sectional	92 healthy men aged 60-80 years	Significant correlations between adiponectin and femoral neck and spine BMD disappeared after adjustment for BMI
Jurimae J et al. 2009. Eur J Endocrinol	Longitudinal (1 year)	35 women (age: 69.7+/-6.0 years)	Lower adiponectin was associated with lower spine aBMD loss than greater adiponectin
Kanazawa I et al. 2009. Eur J Endocrinol.	Cross-sectional	231 men and 170 post-menopausal Japanese women with type 2 diabetes	An increase in one standard deviation of adiponectin was associated with higher odds of vertebral fracture in men but not women.

2.9 SPECIFIC AIMS

The aim of *Research Article 1* was to better understand and evaluate if correlates of trabecular and cortical vBMD differ in older men of primarily Caucasian descent. The potential correlates included demographic characteristics, anthropometric measures, lifestyle factors and medical history.

The aim of *Research Article 2* was to better understand and determine if the associations between serum 25(OH)D and measures of bone quality differ among men of Caucasian and African descent. We also sought to identify approximate 25(OH)D thresholds at which there is greater bone quality.

The aim of *Research Article 3* was to test the hypothesis that higher serum leptin and lower adiponectin will decrease the risk of a fracture and predict lower hip aBMD loss in men and women of Caucasian and African American descent.

We used longitudinal and cross-sectional data from the osteoporotic fractures in men study (MrOS), the Tobago Bone Health Study, and the health aging and body composition study (ABC) to perform analyses for these three research manuscripts. These observational cohort studies are based upon large community based populations of primarily older residents.

3.0 CORRELATES OF TRABECULAR AND CORTICAL VOLUMETRIC BONE MINERAL DENSITY OF THE RADIUS AND TIBIA IN OLDER MEN: THE OSTEOPOROTIC FRACTURES IN MEN STUDY

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3.1 ABSTRACT

Quantitative computed tomography (QCT) can estimate volumetric bone mineral density (vBMD) and distinguish trabecular from cortical bone. Few comprehensive studies have examined correlates of volumetric bone mineral density (vBMD) in older men. This study evaluated the impact of demographic, anthropometric, lifestyle, and medical factors on vBMD in 1172 men, aged 69-97, and enrolled in The Osteoporotic Fractures in Men Study (MrOS). Peripheral quantitative computed tomography (pQCT) was used to measure vBMD of the radius and tibia. The multivariable linear regression models explained up to 10% of the variance in trabecular vBMD and up to 9% of the variance in cortical vBMD. Age was not correlated with radial trabecular vBMD. Correlates associated with both cortical and trabecular vBMD were age (-), caffeine intake (-), total calcium intake (+), non-trauma fracture (-), and hypertension (+). Higher body weight was related to greater trabecular vBMD and lower cortical vBMD. Height (-), education (+), diabetes with TZD use (+), rheumatoid arthritis (+), using arms to stand from a chair (-), and anti-androgen use (-) were only associated with trabecular vBMD. Factors only associated with cortical vBMD included clinic site (-), androgen use (+), grip strength (+), past smoker (-) and time to complete 5 chair stands (-). Certain correlates of trabecular and cortical volumetric BMD differed among older men. An ascertainment of risk factors associated with trabecular and cortical vBMD may lead to better understanding and preventive efforts for osteoporosis in men.

3.2 INTRODUCTION

There is growing recognition that osteoporosis and fractures in older men are significant public health problems that contribute to disability and premature death.(1) Osteoporosis is defined as a systemic bone disease characterized by low bone mass and mircoarchitectural deterioration of bone tissue, with a subsequent increase in bone fragility and susceptibility to fracture.(2) Low bone mineral density (BMD) is an important risk factor for fractures in men. Men over the age of 50 have a 13% estimated risk of developing an osteoporotic fracture.(3)

One major limitation of dual x-ray absorptiometry (DXA) is its inability to distinguish cortical and trabecular bone. Bones tend to differ in their composition of trabecular and cortical bone. For example, the spine is almost entirely trabecular bone.(4) Therefore, identifying correlates of trabecular bone can lead to a better understanding of what factors might be associated with spine fracture. Also, DXA only provides a measurement in 2-dimensions and thus reports an "areal" BMD (aBMD). Bones of larger width and length tend to have greater depth (e.g., in men compared to women), and since bone depth is not accounted for in DXA scanning, reliance on aBMD inherently underestimates bone density.(5) Volumetric bone mineral density (vBMD) has been shown to be a stronger predictor of vertebral fractures than aBMD in some studies.(6;7) Quantitative computed tomography (QCT) assesses vBMD and distinguishes cortical from trabecular bone.(8) The correlates of DXA-measured BMD have been well established in men.(1;9-13) However, few studies have examined the correlates of vBMD, especially in older men.(8;11) The aim of this study was to identify correlates of trabecular and cortical vBMD using peripheral quantitative computed tomography (pQCT) and examine if they differ.

3.3 METHODS

3.3.1 Study population

From March 2000 through April 2002, 5,995 men aged 65 years or older, were recruited using population-based listings from six US clinical sites to participate in the Osteoporotic Fractures in Men Study, "MrOS".(14;15) Peripheral QCT measurements were obtained at the Minneapolis, MN and Monongahela Valley, PA study sites during the second clinic visit between March 21, 2005 and April 11, 2006, for 1172 individuals (46.1% from MN, and 53.9% from PA), aged 69-97. The institutional review board (IRB) at each center approved the study protocol, and written informed consent was obtained from all the participants.

3.3.2 Peripheral QCT

Peripheral QCT examinations were performed on the Sratec XCT scanner series (2000, or 3000). Trabecular vBMD of the radius and tibia was measured by obtaining an ultra distal slice at 4% the length of the ulna proximal to the radial endplate and at 4% of the tibia length proximal to the tibial endplate, respectively. Cortical vBMD of the radius and tibia was measured by obtaining a distal slice at 33% the length of the ulna proximal to the radial endplate and at 33% of the tibia length proximal to the tibial endplate, respectively. A scoutview (an anatomic reference line for the relative location of subsequent scans) was obtained prior to the pQCT scan at the tibia and radius. Precise assessment of bone mineral properties by pQCT was assured by minimizing participant movement. A quality assurance (QA) phantom scan was used to monitor the stability

of the pQCT scanner. A cross-calibration check was also performed between the centers. The European Forearm Phantom was scanned 3 times at each site at 200, 100 and 50 mg/cc, respectively. Voxel size was 0.5 mm and the scan speed was 25 mm/s. To determine the trabecular (mg/cm^3) vBMD, all radius and tibia scans were analyzed using identical parameters for contour findings and separation of trabecular and cortical bone (contour mode 2, T=169 mg/cm^3; peel mode 1, area=45%). All proximal radius and tibia shaft scans were analyzed using identical parameters for contour finding and separation of total and cortical bone (contour mode 2, T=169 mg/cm^3;cortmode 1, T=710 mg/cm^3) to determine the vBMD of the cortical (mg/cm^3) rich bone compartment. Coefficients of variation (CV) were determined for pQCT scans by replicating measurements on 15 subjects (CV $\leq$ 2.1%).(8)

3.3.3 Other measurements

Self-administered and interviewer administered questionnaires were used by trained clinical staff to obtain demographic, medical and lifestyle information from the participants. Information on anthropometric measures, neuromuscular function, and medication use was also obtained at the clinic.(14)

Information on covariates were obtained from visit 2, with the exception of race/ethnicity, education, alcohol intake, calcium and vitamin D intake, history of a non-trauma fracture after the age of 50, self-report of diabetes and fasting glucose level, whether a subject ever had a gastrectomy, and testosterone injection use which were abstracted at baseline.

Race or ethnicity was determined by self-declaration and included these categories: Caucasian, African American, Hispanic, Asian, and other. The participants were divided into 2

categories of education for analysis: greater than a high school education and high school education or lower.

A calibrated balance beam scale was used to measure weight in kilograms (kg). Participants had to wear indoor clothing and remove their shoes when their weight was being measured. Height was measured in meters (m) using a Harpenden Stadiometer (Dyfed, UK).

Gait speed (m/s) was determined as the time to complete a six meter walk. Grip strength (kg) was measured twice using a handheld dynamometer (Jamar) and taking the average in both right and left arms. Time to complete 5 chair stands (seconds) and ability to stand from a chair without using arms (yes/no) were also measured.

Lifestyle factors include the self-report of smoking (current, past and never), alcohol intake (drinks/week), caffeine intake (mg/day), and time spent walking (hours/day). Overall physical activity was also measured by computing the physical activity summary scale for the elderly (PASE).(16) The modified Block Food Frequency Questionnaire was used to obtain dietary information related to calcium and vitamin D intake over the past year. (17)

Subjects were asked to report if they were ever told by a doctor, or health care provider, that they had certain medical conditions including hypertension, stroke, myocardial infarction (MI), chronic obstructive pulmonary disease (COPD), cancer, osteoporosis, osteoarthritis (OA), hyperthyroidism, hypothyroidism, Parkinson's disease, and kidney stones. Men were classified as having diabetes if they had glucose levels >126 mg/dl (after minimum of an 8 hour fast) at baseline, self-report of diabetes at baseline or visit 2, or reported taking insulin or hypoglycemic medications at baseline or visit 2. Men with diabetes were further categorized as having a history thiazolidinedione (TZD) versus non-TZD use. We are unable to distinguish type 1 and 2 diabetes. However, based on the older age of the participants and the shorter longevity

associated with type 1 diabetes for this birth cohort, it is likely that nearly all have type 2 diabetes.

Participants were asked to bring all current (within last 30 days) prescription and nonprescription medications with them to clinic. Interviewers completed a medication history for each participant, including name of medication and frequency of use. All prescription medications recorded by the clinics were stored in an electronic medications inventory database (San Francisco Coordinating Center, San Francisco, CA). Each medication was matched to its ingredient(s) based on the Iowa Drug Information Service (IDIS) Drug Vocabulary (College of Pharmacy, University of Iowa, Iowa City, IA).(18) Androgen use was defined as testosterone or dehydroepiandrosterone (DHEA) use. Anti-androgen use included use of flutamide or bicalutamide.

3.3.4 Statistical analysis

All statistical analyses were performed using the Statistical Analysis System (SAS, version 9.1; SAS Institute, Cary, NC). Analysis of variance (ANOVA) was used to compare the unadjusted skeletal site-specific vBMD across age groups with a test of trend and a Bonferroni adjustment for pairwise comparisons. Linear regression analyses for age and age and weight adjusted models were used to examine the association of each correlate with cortical and trabecular vBMD at the radius and tibia. The associations were expressed as a one unit increase for categorical variables and one standard deviation (SD) increase for continuous variables. The formula used to calculate the percent difference in vBMD per unit change of predictor variable was: (β coefficient*unit/mean vBMD)*100. The corresponding confidence intervals were calculated as: [(β coefficient ± 1.96*standard error)*(unit)/ (mean vBMD)*100]. Variables with a p-value less than 0.10 from the age and weight- adjusted models were entered into the multivariable models. We excluded men from the multivariable analysis who reported taking osteoporosis medication (n=42) or self-reported osteoporosis (n=41), since these men on average

had markedly lower vBMD compared with non-users. For the multivariable models, we used the backward elimination procedure at each skeletal site with age forced in the models. Stepwise selection procedure produced similar results. Multi-collinearity was assessed using the variance inflation factor (VIF). An additional analysis examined if correlates of vBMD differed after excluding non-white men in the multivariable analysis. To further understand if the association between diabetes and vBMD is independent or modified by hypoglycemic medication use, we performed secondary multivariable analyses with and without (diabetic versus non-diabetic) taking into account diabetic medications. For the diabetes medications parameter men were categorized as: non-users of hypoglycemic medication; insulin or hypoglycemic use without a history of TZD use; and any TZD use either with or without additional hypoglycemic medications.

3.4 RESULTS

The study population consisted of Caucasians (97.9%), African Americans (1.4%), Asians (0.2%), Hispanics (0.3%), and other (0.2%). The average age of the men was 77.2 ± 5.1 years and 61.9% had higher than a high school education. The mean and SD values for trabecular and cortical vBMD were 197 ± 46 mg/cm^3 and 1159 ± 34 mg/cm^3, respectively for the radius and were 231 ± 41 mg/cm^3 and 1136 ± 35 mg/cm^3, respectively for the tibia. Tables 3-1 and 3-2 show the age and age and weight adjusted models. These results were used to build the multivariable models.

3.4.1 Pairwise comparisons of trend for vBMD by age

Figure 3-1 and 3-2 show the unadjusted cross-sectional age-related patterns at each site. Trabecular vBMD at the radius did not differ by age (p for trend=0.483). The test of linear trend for tibial trabecular vBMD was statistically significant (p=0.002). Participants aged 69-74 had 4.1% and 5.7% greater trabecular vBMD at the tibia than those aged 80-84 (p=0.022) and 85+ (p=0.030), respectively. Cortical vBMD varied by age at both skeletal sites (p for trend <0.001). At the radius men aged 69-74 had 0.7% and 1.8% greater cortical vBMD than those aged 80-84 (p=0.008) and 85+ (p<0.001), respectively. Radial cortical vBMD was 1.5% greater among participants aged 75-79 than men aged 85+ (p<0.001). Also, men aged 80-84 had 1.0% greater radial cortical vBMD than those aged 85+ (p=0.021). At the tibia, men aged 69-74 had 0.9% and 1.1% greater cortical vBMD than those aged 80-84 (p=0.001) and 85+ (p=0.005), respectively. Finally, those aged 75-79 had 0.7% greater tibial cortical vBMD than men aged 80-84 (p=0.035).

3.4.2 Multivariable models

The results of the multivariable analyses are summarized in table 3-3. At the radius, multivariable models explained 4 and 9% of the overall variance for trabecular and cortical vBMD, respectively. The models for cortical and trabecular vBMD at the tibia site explained 8% and 10% of the overall variance. An increase of one SD (5.1 years) of age was significantly correlated with lower vBMD all sites with the exception of the trabecular vBMD at the radius. Having greater than a high school education was correlated with 3.0% greater trabecular vBMD of the tibia. Men from Monongahela Valley had -0.8% lower cortical vBMD than those from

Minneapolis. A one SD (13.5 kg) increase in weight was associated with 2.3% greater tibial trabecular vBMD. However, this increase in weight was correlated with lower cortical vBMD of the radius (-0.5%) and tibia (-0.3%). One SD (6.9 cm) increase in height was associated with lower trabecular vBMD at the tibia (-1.6%). One SD (259.3 mg/day) increase in caffeine was associated with lower cortical vBMD of the radius. Caffeine intake was also associated with lower trabecular and cortical vBMD of the tibia. A SD (564.9 mg/day) increase in total calcium was associated with greater cortical and trabecular vBMD at both skeletal sites. Past smokers had significantly lower (-0.4%) cortical vBMD of the radius than individuals who had never smoked. History of a non-trauma fracture was associated with lower trabecular and cortical vBMD at both sites. Men who had diabetes and used TZD had greater trabecular vBMD of the radius (9.3%) and tibia (8.0%) than those with no diabetes. Having hypertension was correlated with greater (4.9%) trabecular and cortical (0.6%) vBMD of the radius. RA was associated with a 5.3% greater trabecualar vBMD of the tibia. Androgen use was correlated with greater cortical vBMD at both sites. Men who used anti-androgens had lower (-24.9%) trabecular vBMD of the tibia. An increase of one SD (7.7 kgs) in grip strength was associated with greater (0.3%) cortical vBMD of the radius. Each SD (3.4 second) increase in time to complete five chair stands was associated with lower (-0.3%) cortical vBMD at the tibia. Men who need to use their arms to stand from a chair had lower (-7.2%) trabecular vBMD of the tibia.

3.4.3 Secondary Analyses

In the analysis that did not account for hypoglycemic medication use, men identified as having diabetes did not differ significantly by vBMD compared to men without DM. Insulin or

or hypoglycemic use in our study population of older men was not correlated with BMD of the radius or tibia. However, older men who used TZDs had significantly greater radial (8.9%) and tibial (9.6%) trabecular BMD than nonusers of anti-diabetic medication in MrOS. Also exclusion of non-whites from the multivariable analysis did not change correlates identified.

3.5 DISCUSSION

We chracterized demographic, anthropometric, lifestyle, and medical factors to understand their association with cortical and trabecular vBMD among older men. Unique features of this study include large sample size of men, comprehensive nature of the analyses and ascertainment of correlates and outcome. Our study results suggest that some correlates of trabecular vBMD may differ from those of cortical vBMD.

Age was not associated with lower radial trabecular (ultra-distal) vBMD, consistent with findings of a previous study by Dalzell et al.(19) but contrary to the findings of Riggs et al.(20;21) Reduction in trabecular vBMD has been shown to occur before mid-life (age 20-49) in men and women and continue unabated through life.(20-22) The mechanism for this early onset of trabecular vBMD loss remains unknown. A significant reduction in radial trabecular vBMD may have occurred at a younger age for this population. However, our results are cross-sectional comparisons across age groups and longitudinal studies are needed. Consistent with prior findings, increasing age was associated with lower cortical vBMD.(19-21;23) In women,

substantial cortical bone loss has been shown to occur after mid-life in association with menopause and estrogen deficiency.(24) However, cortical bone loss in men seems to decrease at a constant rate in young adulthood until accelerating late in life.(21)

This report showed a positive association between education and trabecular vBMD. This correlation has not been previously reported in men and may be a result of residual confounding. Higher education may lead to the adoption of a better lifestyle that could impact vBMD positively.

Body weight was associated with greater trabecular vBMD, consistent with two previous studies.(8;25) Several studies observed a positive association between weight and aBMD.(9;10;13) Larger skeletal frames and greater muscle and body fat in heavy individuals may increase the mechanical load on the skeleton promoting mineralization and structural adaptive responses that can strengthen bone.(26) However, body weight was associated with lower cortical vBMD similar to two previous studies.(8;27) It is also possible that mechanical factors or differences in bone geometry may explain these findings. Paradoxically, increased mechanical loads can lead to bone microdamage, increased bone turnover, and a transient reduction in cortical vBMD.(28) Also, overweight individuals may adapt by increasing periosteal bone diameter requiring lower cortical density to maintain the same strength.(29) Additional studies are needed to fully understand these relationships.

Similar to our findings, the Tobago Bone Health Study reported a negative association between height and trabecular vBMD, consistent with the negative association on femoral neck aBMD.(1;8) Taller individuals are known to experience greater height loss and have been shown to be at a higher risk of fracture than others.(30-32)

Several lifestyle and dietary variables were correlated with vBMD. Higher caffeine intake was associated with lower cortical and trabecular vBMD, similar to a previous study that examined calcaneus BMD in a young population of primarily Caucasian men.(25) However, some studies on aBMD in older Caucasian men (1;9;10) and another on vBMD in African men found no association.(8) Caffeine may reduce BMD by decreasing intestinal calcium absorption but it may also be a marker for lower calcium consumption in our population($r=-0.1$, $p=0.059$).(33) Our study found that past smokers had significantly lower cortical vBMD. A study on men of African descent found a negative correlation among smokers for trabecular and cortical vBMD(8) and another found a negative association among smokers for trochanter aBMD in a cohort of primarily elderly Caucasian men.(10) However, The Gothenburg Osteoporosis and Obesity Determinants (GOOD) failed to find a significant association between smoking and vBMD.(34) Smoking has been shown to have a negative effect on BMD by decreasing calcium absorption or by inhibiting the proliferation of osteoprogenitor cells.(35;36) Total calcium use was associated with greater trabecular and cortical vBMD which is consistent with studies on aBMD(1;12) and vBMD(25) in Caucasian men and another in African men(8). An adequate intake of calcium is needed to ameliorate the progressive loss of bone with age.(37)

History of fracture was one of the strongest variables related to lower trabecular and cortical vBMD at both skeletal sites and is consistent with studies of aBMD(1;13) and vBMD(23;38;39) in men. Diabetic men using TZD medication had greater trabecular vBMD at both skeletal sites. Greater weight among TZD users compared to diabetic men with no TZD use (95.3 kg vs. 88.5 kg, $p=0.013$) and non-diabetic men (95.3 kg vs. 82.9 kg, $p<0.001$) may have contributed to these findings. Prior research shows a negative association between TZD use and aBMD in diabetic men and women(40;41) consistent with the observation that TZDs increase

bone marrow adiposity resulting in a decrease in osteoblastogenesis, and possibly affect the aromatase pathway leading to a reduction in estrogen production(42;43). Our findings are surprising and need to be replicated in other studies. Hypertension was associated with higher trabecular and cortical vBMD comparable to previous studies for aBMD(1) in Caucasian men and vBMD in men of African heritage.(11) However, Orwoll et al. reported that hypertension was related to lower aBMD in a largely Caucasian population of men aged 60 and older.(13) A positive association may arise from confounding by the use of certain medications such as thiazide (TZ) diuretics that may increase BMD due to their ability to improve calcium retention.(9;44;45) However, excluding TZ diuretic users did not change the association, making it likely that other factors were involved. RA was unexpectedly associated with greater cortical vBMD of the radius and may have occurred by chance. An earlier report using the same cohort found no association between RA and aBMD(1) while another study found a negative correlation among older Caucasian men.(13) Anti-inflammatory medications such as non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to increase BMD.(1;46) Excluding men who used NSAIDs attenuated the association making it no longer significant.

Androgen replacement use was associated with higher cortical vBMD at both sites, consistent with past findings, that assessed trabecular vBMD (47), and aBMD(48), of the lumbar spine. High androgen levels may help maintain BMD by promoting osteoblast differentiation, and inhibiting osteoclast recruitment.(49) The very low prevalence (0.6%) of androgen users likely contributed to the null finding for trabecular vBMD. Anti-androgen use was independently related to lower trabecular vBMD of the tibia. The Tobago Bone Health Study also found that anti-androgen users had significantly lower cortical vBMD at the radius

and tibia.(8) Androgen deprivation therapy or surgery to treat prostate cancer may result in hypogonadism which is associated with lower BMD.(50;51)

The negative association between poor neuromuscular function and lower vBMD was documented in several studies.(1;23;52) Although the mechanism for this association is not entirely clear, it may be due to the lower skeletal loading that occurs as a result of having weaker muscles.(53) Lower grip strength was associated with lower cortical vBMD at the radius consistent with past reports on aBMD(1) and vBMD(23) in Japanese men. Our study also found a negative association between using arms to stand from a chair and lower trabecular vBMD, similar to a study in this cohort on aBMD.(1)

Our multivariable models explained up to 10% of the variance in trabecular vBMD and up to 9% of the variance in cortical vBMD. These estimates are slightly lower than findings on aBMD(1;9;13), but compare well to similar studies on vBMD(8;11;19;25) in men. A possible explanation for this observation may be that aBMD is a combination of density, size and geometry of both cortical and trabecular bone, while vBMD in our study reflects the density of the voxels in the cortical or trabecular bone. These results suggest that there are unknown factors, possibly genetic, related to vBMD in older men that we have yet to identify. Fewer correlates were identified for trabecular vBMD of the radius when compared to tibial trabecular vBMD. The tibia is a major weight bearing site compared to the radius so variables such as weight and physical function may exert a greater influence on vBMD.

This study had several potential limitations. Based on the cross-sectional nature of this study design, causality cannot be established because we are unable to determine temporal relationships between the variables. Many of the variables were collected through self-report so there was potential for recall bias resulting in misclassification. African Americans are known to

have higher aBMD(1;58) and central vBMD(59) than Caucasians. It is likely that the small number of African Americans (1.4%) in our sample reduced our potential to detect an association. Other findings may also have been impacted by lower power (e.g., current smokers (2.9%)). Despite statistical significance, the clinical significance of some of the findings is questionable due to low percentage differences in vBMD.

In summary, we determined that correlates of trabecular and cortical vBMD differed in our large cohort of older men. Bones vary in their composition with some primarily made of cortical bone and others that are almost entirely trabecular bone. An ascertainment of risk factors associated with trabecular and cortical vBMD may lead to better understanding and preventive efforts for osteoporosis in men. Our study also adds to the growing literature on the inverse association between body weight and cortical vBMD. Longitudinal studies are needed to better understand the mechanisms underlying these differential associations.

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3.7 TABLES AND FIGURES

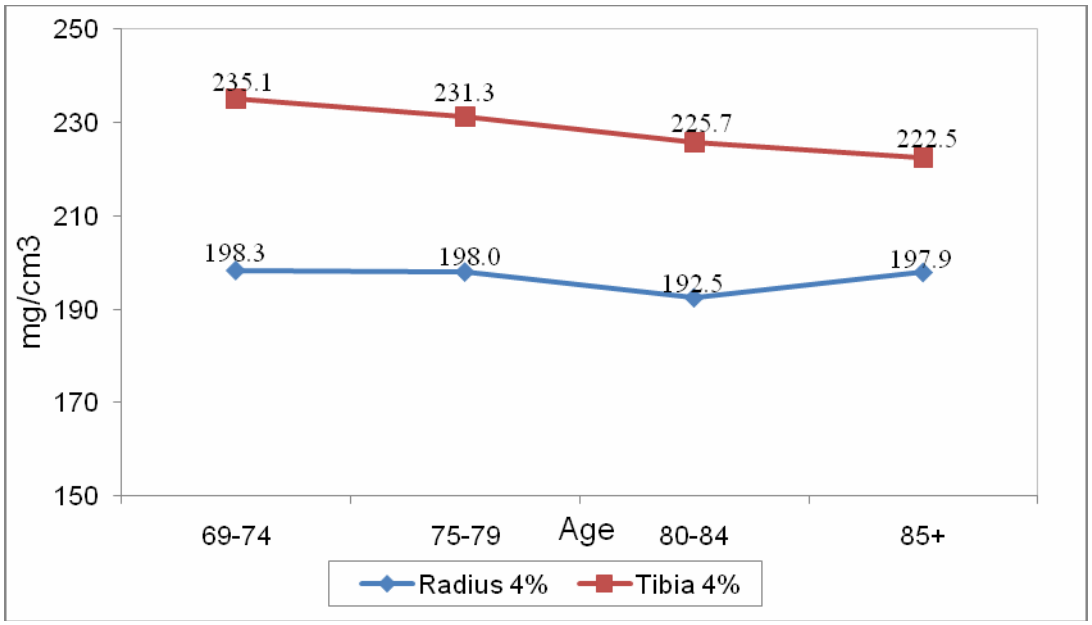

Figure 3-1 Trabecular vBMD by age

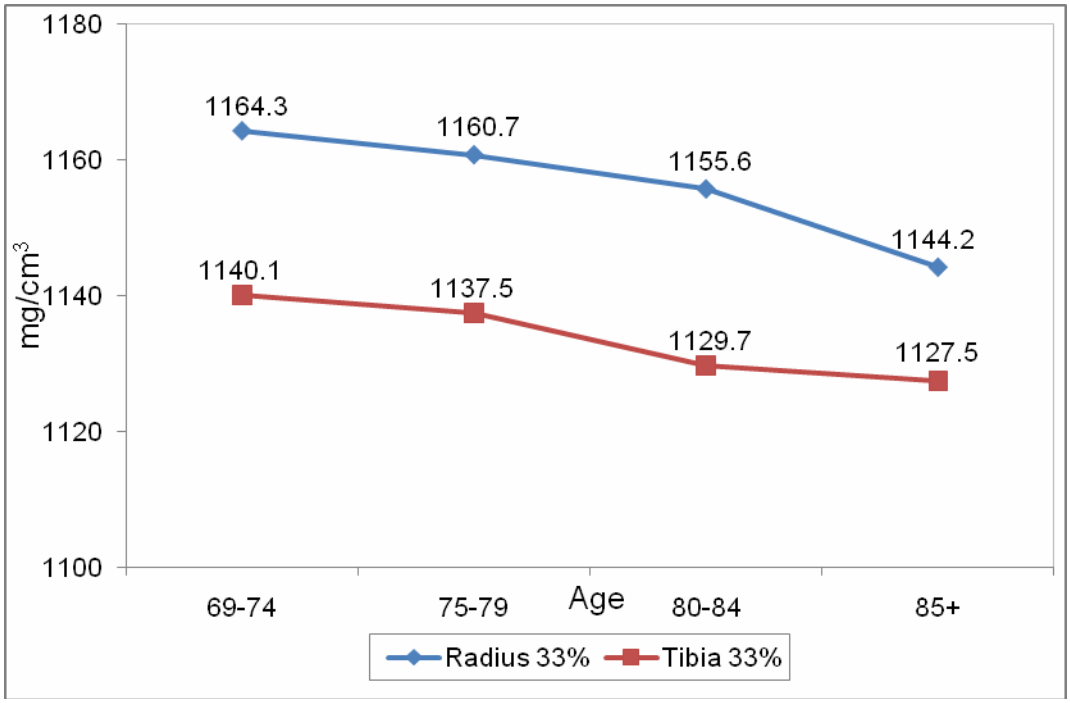

Figure 3-2 Cortical vBMD by age

Table 3-1 Percent difference in trabecular and cortical vBMD of the radius per unit change in potential correlates for age and age and weight adjusted models.

Variable	Mean ± SD or Prevalence	Unit	Percent difference in vBMD per unit change (95% CI)			
			Age Adjusted Trabecular vBMD	Age and Weight Adjusted Trabecular vBMD	Age Adjusted Cortical vBMD	Age and Weight Adjusted Cortical vBMD
Demographics						
Age (years)	77.2 ± 5.1	5.1	-0.8 (-2.1, 0.6) ++	-0.3 (-1.8, 1.1)	-0.5 (-0.7, -0.3)*	-0.6 (-0.5, -0.1)*
Race						
Caucasian	97.9%					
African-American	1.4%		-8.3 (-20.3, 3.7)	-7.8 (-19.8, 4.1)	-0.9 (-2.4, 0.5)	-1.0 (-2.5, 0.4)
Asian	0.2%		-19.2 (-51.7, 13.3)	-16.7 (-49.3, 15.9)	-0.9 (-3.1, 5.0)	0.4 (-3.7, 4.4)
Hispanic	0.3%		-11.6 (-34.7, 11.4)	-12.2 (-35.2, 10.8)	-0.7 (-3.6, 2.1)	-0.6 (-3.5, 2.2)
Other	0.2%		18.1 (-14.5, 50.6)	18.0 (-14.5, 50.5)	3.9 (-1.2, 7.9)**	3.9 (-0.1, 7.9)**
>High School Education	61.9%		-0.3 (-3.1, 2.5)	-0.3 (-3.1, 2.6)	0.5 (0.1, 0.8)*	0.5 (0.1, 0.8)*
Site						
Monongahela Valley, PA	53.9%		-0.3 (-3.1, 2.4)	0.3 (-3.0, 2.5)	-0.3 (-0.1, 0.6)	-0.3 (-0.1. 0.6)
Anthropometry						
Weight (kg)	83.9 ± 13.5	13.5	1.6 (0.2, 3.0) +++,*	1.5 (0.1, 2.9)*	-0.2 (-0.3, 0.0) +++,**	-0.3 (-0.5, -0.1)*
Height (cm)	173.0 ± 6.9	6.9	-0.5 (-1.9, 0.9)	-1.4 (-3.0, 0.1)**	0.0 (-0.1, 0.2)	0.2 (0.0, 0.4)*
Lifestyle						
Smoking						
Never	36.2%					
Past	60.9 %		-3.4 (-6.3, -0.6)*	-3.8 (-6.7, -0.9)*	-0.6 (-1.0, -0.3)*	-0.6 (-0.9, -0.2)*
Current	2.9%		-7.5 (-15.7, 0.7)**	-7.4 (-15.6, 0.8)**	-0.7 (-1.7, 0.3)	-0.7 (-1.7, 0.3)
Caffeine intake (mg/day)	261.4 ± 259.3	259.3	-1.4 (-2.7, -0.0)*	-1.4 (-2.8, -0.0)*	-0.2 (-0.4, 0.0)*	-0.2 (-0.4, -0.0)*
Alcohol (drinks/week)	3.7 ± 6.8	6.8	-0.7 (-2.1, 0.6)	-0.8 (-2.2, 0.5)	-0.1 (-0.3, 0.1)	-0.1 (-0.3, 0.1)
Physical activity (PASE)	141.4 ± 65.9	65.9	0.0 (-1.4, 1.4)	0.2 (-1.2, 1.6)	0.2 (-0.0, 0.3)**	0.1 (-0.1, 0.3)
Walk for exercise (hrs/d)	1.8 ± 0.8	0.8	-0.9 (-2.3, 0.6)	-0.8 (-2.2, 0.7)	-0.0 (-0.2, 0.2)	-0.0 (-0.2, 0.2)
Supplemental calcium intake (mg/d)	278.3 ± 389.6	389.6	0.5 (-0.8, 1.9)	0.7 (-0.7, 2.0)	0.2 (0.0, 0.4)*	0.2 (0.0, 0.3)*
Total calcium (mg/d)	1126.7 ± 564.9	564.9	2.0 (0.6, 3.3)*	2.0 (0.7, 3.4)*	0.3 (0.1, 0.4)*	0.3 (0.1, 0.4)*
Daily vitamin intake (IU/d)	172.8 ± 123.2	123.2	1.2 (-0.2, 2.6)	1.2 (-0.2, 2.5)	0.0 (-0.1, 0.2)	0.1 (-0.1, 0.2)

Table 3-1 continued.

Medical History					
Non-Trauma fracture since age 50	15.3%	-7.1 (-10.9, -3.3)*	-7.2 (-10.9, -3.4)*	-1.0 (-1.4, -0.5)*	-0.9 (-1.4, -0.5)*
No diabetes	80.9%	-	-	-	-
Diabetes and non-TZD use	15.8%	3.0 (-0.8, 6.8)	3.9 (-1.3, 6.4)	-0.3 (-0.7, 0.2)	-0.1 (-0.6, 0.3)
Diabetes and TZD use	3.3%	10.8 (3.0, 18.4)*	9.8 (2.0, 17.6)*	-0.0 (-1.0, 0.9)	0.2 (-0.7, 1.2)
Hypertension	52.8%	5.1 (2.3, 7.8)*	4.8 (2.0, 7.5)*	0.3 (-0.0, 0.7)**	0.4 (0.1, 0.8)*
MI	17.1%	1.0 (-2.6, 4.7)	1.0 (-2.6, 4.7)	0.0 (-0.5, 0.5)	0.0 (-0.4, 0.5)
Stroke	6.9%	2.4 (-3.0, 7.9)	2.2 (-3.2, 7.7)	0.2 (-0.5, 0.9)	0.3 (-0.4, 1.0)
Cancer	30.3%	0.0 (-3.0, 3.0)	0.0 (-3.0, 3.0)	-0.2 (-0.5, 0.2)	-0.2 (-0.6, 0.2)
Osteoporosis	3.5%	-15.8 (-23.2, -8.5)*	-15.4 (-22.7, -8.0)*	-2.1 (-3.1, -1.2)*	-2.3 (-3.2, -1.4)*
Rheumatoid Arthritis	6.1%	1.6 (-4.3, 7.5)	1.2 (-4.7, 7.1)	-0.6 (-1.3, 0.1)	-0.5 (-1.2, 0.2)
Osteoarthritis	22.5%	1.1 (-2.2, 4.4)	1.1 (-2.2, 4.3)	0.1 (-0.3, 0.5)	0.1 (-0.3, 0.5)
Hyperthyroid	2.5%	1.3 (-7.3, 10.0)	1.2 (-7.5, 9.8)	0.1 (-1.0, 1.2)	0.1 (-0.9, 1.2)
Hypothyroid	6.4%	2.0 (-3.5, 7.5)	2.1 (-3.4, 7.6)	0.1 (-0.6, 0.8)	0.1 (-0.6, 0.8)
COPD	10.1%	-4.0 (-8.5, 0.6)**	-4.1 (-8.6, 0.5)**	-0.5 (-1.1, 0.1)**	-0.5 (-1.1, 0.1)**
Parkinson's	1.1%	-12.7 (-27., 1.9)**	-12.5 (-27.1, 2.1)**	-0.8 (-2.6, 1.0)	-0.8 (-2.6, 1.0)
Gastrectomy	5.5%	-2.0 (-8.0, 3.9)	-2.0 (-7.9, 4.0)**	-0.6 (-1.4, 0.1)**	-0.7 (-1.5, -0.1)*
Kidney stones	13.3%	-3.2 (-7.2, 0.8)	-3.4 (-7.4, 0.7)	-0.1 (-0.6, 0.4)	-0.1 (-0.6, 0.4)
Medications					
Androgens	0.6%	6.8 (-10.7, 24.2)	5.7 (-11.8, 23.1)	2.8 (0.7, 5.0)*	3.1 (0.9, 5.2)*
Testosterone injections	0.5%	7.1 (-11.8, 25.9)	6.2 (-12.6, 25.0)	1.4 (-1.0, 3.7)	1.6 (-0.8, 3.9)
Anti-androgen use	0.5%	-8.1 (-27.0, 10.7)	-8.1 (7.7, -23.9)	-3.2 (-5.5, -0.9)*	-3.2 (-5.5, -0.9)*
Cox-II inhibitors	2.0%	-4.9 (-14.6, 4.8)	-4.8 (-14.5, 4.9)	-0.1 (-1.3, 1.2)	-0.1 (-1.3, 1.1)
NSAID use	16.2%	-1.1 (-4.8, 2.6)	-1.5 (-5.2, 2.2)	-0.3 (-0.7, 0.2)	-0.2 (-0.6, 0.3)
Thiazide diuretic use	19.0%	3.3 (-0.2, 6.8)**	3.0 (-0.5, 6.5)**	0.3 (-0.1, 0.8)	0.4 (-0.0, 0.9)**
Non-thiazide diuretic use	11.8%	2.7 (-1.6, 7.0)	2.1 (-2.3, 6.4)	-0.1 (-0.6, 0.4)	0.0 (-0.5, 0.6)
Loop diuretics use	7.5%	2.5 (-2.8, 7.9)	1.7 (-3.7, 7.1)	-0.4 (-1.0, 0.3)	-0.2 (-0.8, 0.5)
Statins	45.2%	2.0 (-0.8, 4.8)	1.9 (-0.9, 4.6)	0.3 (-0.1, 0.6)	0.3 (-0.1, 0.6)
Nitrates	6.5%	2.3 (-3.3, 7.9)	2.2 (-3.4, 7.8)	0.6 (-0.1,1.3)	0.6 (-0.1, 1.3)
Beta blockers	33.3%	1.4 (-1.5, 4.3)	1.2 (-1.7, 4.1)	0.2 (-0.2,0.5)	0.2 (-0.1, 0.6)
Anti-convulsants	3.4%	1.6 (-6.0, 9.2)	1.6 (-6.0, 9.2)	-0.6 (-1.6, 0.3)	-0.6 (-1.6, 0.3)
SSRI use	4.3%	4.3 (-2.5, 11.1)	4.0 (-2.8, 10.8)	0.3 (-0.6, 1.1)	0.3 (-0.5, 1.2)
Tricyclic antidepressants	1.1%	1.0 (-11.8, 13.9)	0.1 (-12.7, 13.0)	1.1 (-0.5, 2.7)	1.3 (-0.3, 2.9)

Table 3-1 continued.

Thyroid hormone use	8.4%		1.3 (-3.6, 6.3)	1.3 (-3.6, 6.3)	0.3 (-0.3, 0.9)	0.3 (-0.3, 0.9)
Corticosteroids (any)	9.3%		-3.5 (-8.2, 1.3)	-3.4 (-8.2, 1.3)	-0.5 (-1.1, 0.1)**	-0.6 (-1.1, 0.0)**
Bisphosphonates	3.6%		-20.8 (-28.1, -13.5)*	-20.2 (-27.6, -12.9)*	-1.6 (-2.5, -0.7)*	-1.8 (-2.7, -0.9)*
General Health						
Health: good/excellent	85.9%		0.0 (-4.0, 4.0)	0.3 (-3.7, 4.3)	0.6 (0.1, 1.1)*	0.5 (0.0, 1.0)*
Neuromuscular						
Gait speed (m/s)	1.2 ± 0.2	0.2	-0.2 (-1.7, 1.3)	0.1 (-1.4, 1.6)	0.4 (0.2, 0.6)*	0.4 (0.2, 0.6)*
Chair stands (s)	11.6 ± 3.4	3.4	0.2 (-1.3, 1.7)	-0.1 (-1.6, 1.5)	-0.3 (-0.5, -0.1)*	-0.2 (-0.4, -0.1)*
Stands with arms	9.0%		-1.1 (-6.0, 3.8)	-2.1 (-7.0, 2.8)	-0.9 (-1.5, -0.3)*	-0.7 (-1.3, -0.1)*
Grip strength (kg)	37.5 ± 7.7	7.7	-0.5 (-2.0, 1.0)	-0.8 (-2.3, 0.7)	0.3 (0.1, 0.5)*	0.3 (0.1, 0.5)*

+For continuous variables the units approximate 1 SD; for dichotomous variables, the referent group does not have the characteristic.

$P \leq 0.05$*

$0.05 < P \leq 0.1$**

++Effect of age alone, +++Effect of weight alone

Table 3-2 Percent difference in trabecular and cortical vBMD of the tibia per unit change in potential correlates for age and age and weight adjusted models

Variable	Mean ± SD or Prevalence	Unit[+]	Percent difference in vBMD per unit change (95% CI)			
			Age Adjusted Trabecular vBMD	Age and Weight Adjusted Trabecular vBMD	Age Adjusted Cortical vBMD	Age and Weight Adjusted Cortical vBMD
Demographics						
Age (years)	77.2 ± 5.1	5.1	-1.8 (-2.8, -0.8) ++,*	-1.3 (-2.4, -0.3)	-0.4 (-0.6, -0.2) ++,*	-0.5 (-0.7, -0.3)*
Race						
Caucasian	97.9%		_	_	_	_
African-American	1.4%		-7.0 (-15.9, 2.0)	-6.3 (-15.3, 2.7)	0.3 (-1.3, 1.9)	0.2 (-1.4, 1.7)
Asian	0.2%		-15.0 (-39.4, 9.5)	- 12.1 (-36.6, 12.3)	2.8 (-1.4, 7.0)	2.3 (-1.9, 6.5)
Hispanic	0.3%		-7.1 (-24.4, 10.3)	-7.7 (-24.9, 9.6)	0.5 (-3.5, 2.5)	-0.4 (-3.3, 2.6)
Other	0.2%		7.6 (-16.8, 32.1)	7.5 (-16.9, 31.8)	4.4 (0.2, 8.6)*	4.5 (0.3, 8.7)*
>High School Education	61.9%		2.7 (0.5, 4.7)*	2.7 (0.6, 4.8)*	0.5 (0.1, 0.8)*	0.6 (0.3, 1.0)*
Site						
Monongahela Valley, PA	53.9%		-0.6 (-1.5, 2.6)	-0.7 (-1.4, 2.7)	-0.9 (-1.2, -0.5)*	-0.9 (-1.2, -0.5)*
Anthropometry						
Weight (kg)	83.9 ± 13.5	13.5	1.7 (0.6, 2.8) +++,*	1.7 (0.6, 2.8)*	-0.3 (-0.5, -0.1) +++,*	-0.3 (-0.5, -0.1)*
Height (cm)	173.0 ± 6.9	6.9	-0.2 (-1.3, 0.8)	-1.3 (-2.4, -0.1)*	0.0 (-0.6, 0.2)	0.2 (0.0, 0.4)*
Lifestyle						
Smoking						
Never	36.2%		_	_	_	_
Past	60.9%		-3.0 (-5.2, -0.9)*	-3.4 (-5.5, -1.2)*	-0.5 (-0.9, -0.1)*	-0.5 (-0.8, -0.1)*
Current	2.9%		-5.6 (-11.9, 0.4) **	-5.7 (-11.8, 0.4)**	-0.6 (-1.6, 0.5)	-0.6 (-1.7, 0.4)
Caffeine intake (mg/day)	261.4 ± 259.3	259.3	-1.1 (-2.2, -0.1)*	-1.2 (-2.2, -0.2)*	-0.2 (-0.3, 0.0)**	-0.2 (-0.3, 0.0)**
Alcohol (drinks/week)	3.7 ± 6.8	6.8	-0.4 (-1.4, 0.6)	-0.6 (-1.6, 0.5)	-0.2 (-0.3, 0.0)**	-0.1 (-0.3, 0.0)
Physical activity (PASE)	141.4 ± 65.9	65.9	0.7 (-0.4, 1.7)	0.9 (-0.2, 1.9)	0.1 (-0.1, 0.3)	0.1 (-0.1, 0.3)
Walk for exercise (hrs/d)	1.8 ± 0.8	0.8	-0.4 (-1.5, 0.7)	-0.3 (-1.4, 0.8)	-0.01 (-0.2, 0.1)	-0.1 (-0.3, 0.1)
Supplemental calcium intake (mg/d)	278.3 ± 389.6	389.6	-0.0 (-1.0, 1.0)	0.1 (-0.9, 1.2)	0.3 (0.1, 0.5)*	0.3 (0.1, 0.5)*
Total calcium (mg/d)	1126.7 ± 564.9	564.9	1.1 (0.1, 2.1)*	1.2 (0.1, 2.3)*	0.3 (0.1, 0.5)*	0.3 (0.1, 0.5)*
Daily vitamin intake (IU/d)	172.8 ± 123.2	123.2	0.9 (-0.1, 1.9)**	0.9 (-0.1, 1.9)**	-0.0 (-0.2, 0.2)	-0.0 (-0.2, 0.2)
Medical History						
Non-trauma fracture since age 50	15.3%		-8.2 (-11.0, -5.3)*	-8.2 (-11.0, -5.4)*	-0.9 (-1.4, -0.4)*	-0.9 (-1.4, -0.4)*

Table 3-2 continued.

No diabetes	80.9%	-	-	-	-
Diabetes and non-TZD use	15.8%	1.4 (-1.5, 4.2)	0.8 (-2.1, 3.6)	-3.8 (-0.8, 0.2)	-2.3 (0.2, -0.6)
Diabetes and TZD use	3.3%	9.2 (3.4, 15.0)*	7.9 (2.1, 13.8)*	-0.6 (-1.1, 0.9)	0.2 (-0.8, 1.2)
Hypertension	52.8%	2.7 (0.6, 4.7)*	2.4 (0.2, 4.3)*	0.1 (-0.2, 0.5)	0.2 (-0.2, 0.6)
MI	17.1%	0.2 (-2.5, 2.9)	0.3 (-2.5, 3.0)	-0.4 (-0.9, 0.1)**	-0.4 (-0.9, 0.1)**
Stroke	6.9%	-0.3 (-4.4, 3.7)	-0.5 (-4.6, 3.5)	0.2 (-0.5, 0.9)	0.3 (-0.4, 1.0)
Cancer	30.3%	0.3 (-0.2, 2.5)	0.4 (-0.5, 2.3)	0.1 (-0.3, 0.5)	0.1 (-0.3, 0.5)
Osteoporosis	3.5%	-14.0 (-19.5, -8.5)*	-13.5 (-19.0, -8.0)*	-2.0 (-3.0, -1.1)*	-2.3 (-3.2, -1.4)
Rheumatoid Arthritis	6.1%	4.1 (-0.2, 8.4)**	3.7 (-0.6, 8.0)**	-0.2 (-0.9, 0.6)	-0.1 (-0.8, 0.7)
Osteoarthritis	22.5%	1.7 (-0.8, 4.1)	1.7 (-0.8, 4.1)	-0.1 (-0.5, 0.4)	-0.1 (-0.5, 0.4)
Hyperthyroid	2.5%	1.7 (-5.8, 8.6)	1.7 (-5.2, 8.5)	0.5 (-0.7, 1.7)	0.5 (-0.7, 1.7)
Hypothyroid	6.4%	-1.6 (-5.8, 2.5)	-1.5 (-5.7, 2.6)	0.3 (-0.4, 1.1)	0.3 (-0.4, 1.0)
COPD	10.1%	-2.1 (-5.5, 1.3)	-2.2 (-5.6, 1.2)	-0.4 (-0.9, 0.2)**	-0.4 (-0.9, 0.2)**
Parkinson's	1.1%	-5.2 (-14.9, 4.4)	-5.1 (-14.7, 4.5)	0.6 (-1.1, 2.2)	0.5 (-1.1, 2.2)
Gastrectomy	5.5%	-4.6 (-9.1, -0.2)*	-4.6 (-9.1, -0.2)*	-0.2 (-0.9, 0.6)	-0.2 (-1.0, 0.6)
Kidney stones	13.3%	-2.7 (-5.7, 0.3)**	-2.8 (-5.8, 0.2)**	-0.0 (-0.6, 0.5)	-0.0 (-0.5, 0.5)
Medications					
Androgens	0.6%	11.2 (-1.9, 24.3)**	10.0 (-3.1, 23.1)	2.3 (0.1, 4.6)*	2.6 (0.3, 4.8)*
Testosterone injections	0.5%	8.0 (-6.2, 22.1)	7.0 (-7.1, 21.1)	0.6 (-1.8, 3.1)	0.8 (-1.6, 3.3)
Anti-androgen use	0.5%	-28.7 (-44.1, -13.3)*	-28.6 (-44.0, -13.3)*	-3.2 (-5.8, -0.5)*	-3.2 (-5.8, -0.5)*
Cox-II inhibitor use	2.0%	-4.0 (-11.4, 3.4)	-4.0 (-11.4, 3.4)	-0.4 (-1.7, 0.8)	-0.5 (-1.7, 0.8)
NSAID use	16.2%	0.8 (-2.0, 3.6)	0.4 (-2.4, 3.2)	-0.3 (-0.7, 0.2)	-0.2 (-0.7, 0.3)
Thiazide diuretic use	19.0%	2.6 (-0.1,5.2)**	2.1 (-0.5, 4.8)**	0.3 (-0.1, 0.8)**	0.4 (-0.1, 0.9)**
Non-thiazide diuretic use	11.8%	1.4 (-1.8, 4.6)	0.8 (-2.5, 4.0)	-0.5 (-1.0, 0.1)	-0.4 (-0.9, 0.2)
Loop diuretics use	7.5%	0.4 (-3.6, 4.4)	-0.4 (-4.5, 3.6)	-0.8 (-1.5, -0.2)*	-0.7 (-1.4, -0.0)*
Statins	45.2%	-0.7 (-2.7, 1.4)	-0.8 (-2.9, 1.3)	-0.2 (-0.5, 0.2)	-0.2 (-0.5, 0.2)
Nitrates	6.5%	0.3 (-3.9, 4.5)	0.2 (-3.9, 4.4)	-0.1 (-0.8, 0.7)	-0.1 (-0.8, 0.7)
Beta blockers	33.3%	0.3 (-1.9, 2.4)	-0.1 (-2.2, 2.1)	-0.0 (-0.4,0.3)	0.0 (-0.4, 0.4)
Anti-convulsants	3.4%	-1.2 (-6.7, 4.4)	-1.1 (-6.7, 4.4)	-0.1 (-1.0, 0.9)	-0.1 (-1.0, 0.9)
SSRI use	4.3%	3.5 (-1.6, 8.6)	3.2 (-1.9, 8.3)	0.3 (-0.6, 1.2)	0.4 (-0.5, 1.3)
Tricyclic antidepressants	1.1%	-1.8 (-12.2, 8.7)	-2.5 (-12.9, 8.0)	1.2 (-0.6, 3.0)	1.3 (-0.5, 3.1)
Thyroid hormone use	8.4%	-0.8 (-4.5, 2.9)	-0.8 (-4.7, 2.9)	0.4 (-0.3, 1.0)	0.3 (-0.3, 1.0)
Corticosteroid (any) use	9.3%	-2.4 (-5.9, 1.1)	-2.3 (-5.8, 1.2)	-0.3 (-0.9, 0.3)	-0.3 (-0.9, 0.3)

Table 3-2 continued.

Bisphosphonates	3.6%		-15.6 (-20.9, -10.2)*	-14.9 (-20.3, -9.5)*	-1.3 (-2.3, -0.4)*	-1.5 (-2.5, -0.6)*
General Health						
Health: good/excellent	85.9%		2.9 (-0.0, 5.9)**	3.3 (0.4, 6.3)*	0.9 (0.4, 1.5)*	0.9 (0.4, 1.4)*
Neuromuscular						
Gait speed (m/s)	1.2 ± 0.2	0.2	0.5 (-0.6, 1.6)	0.8 (-0.3, 2.0)	0.4 (0.8, 0.6)*	0.3 (0.1, 0.5)*
Chair stands (s)	11.6 ± 3.4	3.4	-0.2 (-1.2, 1.0)	-0.5 (-1.6, 1.0)	-0.3 (-0.5, -0.1)*	-0.3 (-0.5, -0.1)*
Stands with arms	9.0%		-5.8 (-9.5, -2.1)*	-6.9 (-10.6, -3.8)*	-0.9 (-1.5, -0.3)*	-0.8 (-1.4, -0.1)*
Grip strength (kg)	37.5 ± 7.7	7.7	-0.2 (-1.4, 0.9)	-0.5 (-1.7, 0.6)	0.2 (-0.0, 0.4)**	0.2 (0.0, 4.3)*

+For continuous variables the units approximate 1 SD; for dichotomous variables, the referent group does not have the characteristic.

P≤0.05*

0.05<P≤0.1**

++Effect of age alone.

+++Effect of weight alone

Table 3-3 Percent difference in vBMD per unit change of each correlate using the backward elimination procedure for multiple linear regression models

Variables	Unit	Percent change in vBMD per unit change (95%CI)			
		Radius Trabecular vBMD[1]	Tibia Trabecular vBMD[2]	Radius Cortical vBMD[3]	Tibia Cortical vBMD[4]
N		1058	1065	1048	990
Demographics					
Age (years)	5.1	-0.6 (-1.9, 0.8)	-1.2 (-2.3, -0.1)	-0.4 (-0.6,-0.2)	-0.4 (-0.6, -0.2)
>High school education		-	3.0 (0.9,5.2)	-	-
Site (Monongahela Valley, PA)		-	-	-	-0.8 (-1.2, -0.4)
Anthropometrics				-	
Weight (kg)	13.5	-	2.3 (1.1, 3.6)	-0.5 (-0.7,-0.3)	-0.3 (-0.5, -0.1)
Height (m)	6.9	-	-1.6 (-2.8, -0.4)	-	-
Lifestyle					
Caffeine intake (mg/d)	259.3	-	-1.2 (-2.2, -0.2)	-0.2 (-0.4, -0.1)	-0.2 (-0.4, -0.1)
Total calcium (mg/d)	564.9	2.5 (1.1, 3.9)	1.5 (0.5, 2.6)	0.2 (0.1, 0.4)	0.2 (0.1, 0.4)
Past smoker		-	-	-0.4 (-0.8, -0.1)	-
Medical History					
Non-Trauma fracture (any)		-7.2 (-11.1, -3.4)	-7.6 (-10.5, -4.8)	-0.8 (-1.3, -0.3)	-0.8 (-1.4, -0.3)
Hypertension		5.0 (2.2, 7.8)	-	0.6 (0.2, 0.9)	-
Diabetes and TZD use		9.3 (1.5, 17.0)	8.0 (2.2, 13.7)	-	-
RA		-	5.3 (0.9, 9.6)	-	-
Medications					
Androgens		-	-	3.0 (0.9, 5.0)	2.7 (0.4, 5.0)
Anti-androgen		-	-24.9 (-43.9, -5.9)	-	-
Neuromuscular Function					
Grip strength (kg)	7.7	-	-	0.3 (0.1, 0.5)	-
Chair stands (s)	3.4	-	-	-	-0.3 (-0.5, -0.1)
Stands with arms		-	-7.2 (-11.0, -3.4)	-	-
R^2		0.04	0.10	0.09	0.08

[1] Total calcium intake (mg/d), non-trauma fracture (any), hypertension, and diabetes and TZD use were significant in the model.

[2]Age, weight, height, education, caffeine intake (mg/d), total calcium intake (mg/d), non-trauma fracture (any), diabetes and TZD use, RA, anti-androgens, and stands with arms were significant in the model.

3Age, weight (kg), caffeine intake (mg/d), total calcium intake (mg/d), past smoker, non-trauma fracture (any), hypertension, androgens, grip strength (kg) were significant in the model.

[4]Age, weight (kg), site, caffeine intake (mg/d), total calcium intake (mg/d), non-trauma fracture (any), androgens, and time to complete 5 chair stands (s) were significant in the model.

4.0 THE ASS0CIATION OF SERUM 25-HYDROXYVITAMIN D AND BONE INDICES OF STRENGTH IN OLDER MEN

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4.1 ABSTRACT

There are limited data on serum 25-hydroxyvitamin D [25(OH)D] and bone density and geometry in older men of African heritage. To better understand if there are racial differences in vitamin D status and indices of bone strength we determined the association of 25(OH)D with peripheral quantitative computed tomography (pQCT) parameters including trabecular and cortical volumetric bone mineral density (vBMD), bone mineral content (BMC), bone geometry, and polar and axial strength strain index (SSIp and SSIx) among Caucasian (N=446) men living in the US and men of African (N=496) descent living in Tobago aged ≥65 years. Serum concentrations of total (D2 +D3) 25(OH)D were measured using liquid chromatography and tandem mass spectrometry and categorized as using clinical categories defined as sufficient (≥ 30 ng/ml), insufficient (20-<30 ng/ml) and deficient (<20 ng/ml). Men of African descent had higher 25(OH)D concentrations than Caucasians (34.7 vs. 27.6 ng/ml, p<0.001). In Caucasians, increasing 25(OH)D was associated with greater cortical vBMD (p trend=0.042), total BMC (p trend=0.007), cortical thickness (p trend=0.004), and SSIp (p trend=0.028) and SSIx (p trend =0.012) at the distal radius after adjusting for covariates including age, BMI, season of blood draw, and history of fracture. These associations were significant at the distal tibia, with the exception of cortical vBMD and SSI parameters. There was also greater total BMC among 25(OH)D sufficient versus deficient men at the distal radius and tibia. In contrast, in men of African descent, higher 25(OH)D levels were associated with lower total bone area (p trend=0.005) and SSIx (p trend=0.048). There was strong evidence that the association between 25OHD and pQCT parameters depended on race. Race modified the association between 25(OH)D and total BMC (p interaction=0.003), total bone area (p interaction=0.006), SSIp(p interaction=0.006), SSIx (p interaction=0.002), and trabecular vBMD (p interaction=0.015) of the radius. In Caucasians, there was evidence of a threshold effect on tibial total BMC and cortical thickness at 19 and 18 ng/ml of serum 25(OH)D, respectively. Beyond this 25(OH)D level, the effects of higher 25(OH)D levels on bone parameters appeared to be minimal. Prospective studies in diverse populations are needed to evaluate the association of 25(OH)D with trabecular and cortical bone loss in older men.

4.2 INTRODUCTION

Serum 25-hydroxyvitamin D (25(OH)D) is an indicator of vitamin D nutritional status(1) and decreases with advancing age.(2-4) The possible mechanisms for the age related loss in serum 25 (OH)D include lower vitamin D intake(5), decreased vitamin D absorption(6), limited sun exposure(2) and decreased cutaneous synthesis of vitamin D.(7) Low 25(OH)D levels have been linked to several comorbidities including Myocardial Infarction (MI), hypertension, osteoarthritis, and diabetes.(8-11) Vitamin D deficiency can also have a profound impact on bone growth and bone mineralization in young adults and on bone loss in older adults.(12)

Low bone mineral density(BMD) and osteoporosis is an increasing clinical and public health problem among older men.(13-15) Positive (16-24) and null(20;25;26) associations between serum 25(OH)D concentrations and areal bone mineral density (aBMD) have been reported in men. However, limitations of past studies include small sample sizes, failure to adjust for important confounders, reliance on dual X-ray absorptiometry (DXA) assessments of areal BMD(27), and the use of unreliable radioimmunoassay methods to measure serum 25(OH)D levels. Binkley et al. found markedly different serum 25(OH)D values after comparing radioimmunoassay (RIA) and competitive binding protein assays to high performance liquid chromatography (HPLC).(28) The use of RIA or competitive binding protein assays have been shown to overestimate 25(OH)D levels and have poorer reliability compared to HPLC and liquid chromatography tandem mass spectrometry (LC-MS/MS) methods.(29;30) Moreover, most studies have focused on Caucasian men; there are limited data on serum 25(OH)D concentrations and bone density and geometry in men of African ancestry residing in the Caribbean.

In this current study, we determined the associations between serum 25(OH)D levels measured using LC-MS/MS and bone mineral content (BMC), trabecular and cortical volumetric BMD, and bone structural geometry at the radius and tibia in men of Caucasian and African descent.

4.3 METHODS

4.3.1 Study population

The Osteoporotic Fractures in Men Study (MrOS) initially recruited 5995 men ages 65 and older from March 2000 through April 2002 as previously described.(31;32) Men with a history of bilateral hip replacement and men who were unable to walk without the assistance of another person were excluded from MrOS. 5229 men (95.5% of survivors) completed some portion of the second visit between March 21st, 2005 and May 3rd, 2006. For the current 25(OH)D sub-study, the Pittsburgh MrOS site was included for this analysis. Caucasian subjects (N=446) that had peripheral quantitative computed tomography (pQCT) measurements at visit 2 and visit 3 (March 2007-March 2009), were selected to have their serum assayed for 25(OH)D. However, the analysis was limited to pQCT measurements at visit 2.

Between 1997 and 2003, 3,170 men aged 40 years or older were recruited for the population based prostate cancer screening study on the Island of Tobago.(33) Eligible men had to be ambulatory, non-institutionalized and not terminally ill. Between 2004 and 2007, 2482 men (70% of survivors) completed a second clinic visit.(34) There were 618 Afro-Caribbean

men aged ≥65 years (with all 4 grandparents of African ancestry). A random sample of 496 of these men was chosen for 25(OH)D and pQCT measurements. The Institutional Review Board (IRB) for the University of Pittsburgh and the Tobago Division of Health and Social Services approved the study protocol and written informed consent was obtained from all participants.

4.3.2 Measurement of 25(OH)D

Measures for $25(OH)D_2$ (derived from ergocalciferol) and $25(OH)D_3$ (derived from cholecalciferol) in both cohorts were performed simultaneously at the Mayo Clinic by conventional LC-MS/MS as previously described in serum collected after an overnight fast and frozen until assay.(35) The minimum detectable limit for $25(OH)D_2$ was 4 ng/mL and for $25(OH)D_3$ was 2 ng/mL. The number of men with detectable $25(OH)D_2$ was 193 (20.5%), similar to a prior report.(17) Total 25(OH)D was quantified as the sum of $25(OH)D_2$ and $25(OH)D_3$. Reliability was assessed by measuring 46 duplicate $25(OH)D_3$ samples from the Caucasian population and the coefficient of variation (CV) was 5.8% for total 25(OH)D and 3.9% for $25(OH)D_3$.

4.3.3 Peripheral QCT Measurements

QCT examinations for visit 2 at both sites were performed on the Stratec XCT scanner series 2000. Trabecular bone properties were measured by taking an ultra distal slice at 4% of the length of the radius and tibia. Variables measured at this site included total bone area (mm^2), total BMC (mg/mm), trabecular vBMD (mg/cm^3), and the polar (SSIp) and axial (SSIx) strength

strain index (mm^3). Cortical bone properties were measured at a 33% slice of the radius and tibia. Measurements at this site included total bone area (mm^2), total BMC (mg/mm), cortical vBMD (mg/cm^3), cortical thickness (mm), and SSIp and SSIx (mm^4). The strength strain indices denote the density-weighted polar or axial section moduli and reflect the torsional and bending rigidity of the long bone shaft (mm^3).(36) SSI= $=\int d^2A*(vBMD_{vox}/vBMD_{max})/d_{max}$, where $vBMD_{vox}$=bone density of a voxel and $vBMD_{max}$=1200 mg/cm^3, maximal bone density, and d_{max}=maximum distance of a voxel from the center of mass.(37) A scoutview (an anatomic reference line for the relative location of subsequent scans) was obtained prior to the pQCT scan at the tibia and radius. High quality assessment of bone mineral properties by pQCT was assured by minimizing participant movement. A quality assurance (QA) phantom scan was scanned daily and used to monitor the stability of each pQCT scanner. QA scan checks that the density (attenuation) of the phantom does not change by more than +/- 0.5% which would be logged as a failure. No failures were noted for each scanning center. A cross-calibration check was also performed between the centers. The European Forearm Phantom (EFP) was scanned 3 times at each site at 200, 100 and 50 mg/cc, respectively. The same EFP was scanned at both centers and the densities compared to the densities certified for the EFP. The densities for all sections of the EFP were accurately determined by each scanning center within the machine precision (3-5 mg/cc). No corrections/adjustments were applied to the data from each of the scanning centers to account for any machine bias (none existed). Voxel size was 0.5 mm and the scan speed was 25 mm/s. Coefficients of variation (CV) were determined for pQCT scans by replicating measurements on 15 subjects (CV $\leq$ 2.1%).(38)

4.3.4 Potential Confounders

Questionnaires and clinical evaluations were administered by trained clinical staff to obtain demographic, anthropometric, medical and lifestyle information from the participants.(39) Demographic, lifestyle, and medical characteristics were obtained entirely through self-report with the exception of diabetes. Men were classified as having diabetes, if they self-reported diabetes, took insulin, or hypoglycemic medications (baseline or visit 2), or had glucose levels ≥126 mg/dl after a minimum of an 8 hour fast (at baseline).

All variables in MrOS with the exception of education (attended college versus never attended college), history of fracture, and alcohol intake (drinks/week), dietary calcium (mg/day), supplemental calcium (yes or no), and health status (Excellent/good vs. fair/poor/very poor) were obtained at visit 2.

Season of blood draw was categorized winter: January-March; spring: April-June; summer: July-September; fall: October-December. A calibrated balance beam scale was used to measure weight in kilograms (kg). Participants had to wear indoor clothing and remove their shoes when their weight was being measured. Height was measured in centimeters (cm) using a Harpenden Stadiometer (Dyfed, UK) or wall mounted height board. BMI was calculated using weight (kg)/height/(m^2). Other lifestyle factors included self-report of current smoking (yes or no), caffeine intake (mg/day), and average time walking per day in previous 7 days (≤1 hour, >1 hour). Dietary information on calcium and caffeine intake in Mr. OS was obtained using the modified Block Food Frequency.(40) Caffeine intake was assessed for Tobago men by assuming that one cup of caffeinated coffee, tea or soda contained 95, 55 and 45mg of caffeine, respectively. Dietary calcium intake in this population was determined by frequency of selected

food items including fish, bone chewing, green leafy vegetables, beans, milk, cheese, and cheese dishes that contain high dietary calcium and are frequently consumed in the local diet.(41) Subjects were asked to report if a doctor or health care provider informed them they had certain medical conditions including history of fracture, hypertension, MI, osteoporosis, osteoarthritis and rheumatoid arthritis (RA).

4.3.5 Statistical Analysis

All statistical analyses were performed using the Statistical Analysis System (SAS, version 9.1; SAS Institute, Cary, NC). Clinical cutoffs defined 25(OH)D "deficiency" as <20 ng/ml, "insufficiency" as 20-<30 ng/ml, and "sufficiency" as ≥30 ng/ml based on previous reports.(1) Chi-square tests for categorical variables and Analysis of Variance (ANOVA) for continuous variables were performed to test if baseline characteristics differed by 25(OH)D status. Fisher's exact test was used when the expected frequency was less than 5. The Kruskal-Wallis test was used if a continuous variable was not normally distributed. Race specific linear regression analysis was used to calculate least square means and examine associations between 25(OH)D and pQCT parameters. A Bonferroni correction was used to adjust for pairwise comparisons between 25(OH)D groups and pQCT parameters. Factors consistently associated with different bone parameters or those related to 25(OH)D level at $p<0.05$ were considered for inclusion in multivariable models. Interactions between race and vitamin D cutoffs were assessed to evaluate if the association between 25(OH)D and pQCT parameters differed by race. A test of linear trend across 25(OH)D clinical cutoffs was also performed based on the ordinal value for each category. To further explore these relationships, we performed spline analysis to test for possible

thresholds and linearity for all bone parameters. Restricted cubic spline linear regression was used with knots at the 5th, 25th, 75th and a reference group at the 95th percentile was set to create the spline plot. Predicted values from the regression were plotted against the 25(OH)D values. Threshold effects were evaluated by identifying potential inflection points on the spline and performing a test of equality to determine if the slopes above and below the cut point were equal. If more than one cutoff was identified ($p<0.05$) we chose the 25(OH)D value with the lowest p-value as the threshold. Spline plots were displayed if 25(OH)D thresholds were found. An additional analysis using 25(OH)D tertiles (based on the distribution of the entire population) was performed and the results were similar (not shown to avoid redundancy) to the analysis with clinical cutoffs. The purpose of this analysis was descriptive; therefore multiple outcomes are presented without adjusting for multiple comparisons.

4.4 RESULTS

Men of African descent had greater serum 25(OH)D (34.7 vs. 27.6 ng/ml, $p<0.001$) than Caucasians (Table 4-1). Caucasians were older and had greater BMI, caffeine intake, and dietary calcium intake than men of African descent. Caucasian men were also significantly more likely to have a college education, take calcium supplements, report good/excellent health, history of a fracture, and hypertension. Men of African descent had a greater prevalence of diabetes than Caucasians.

Table 4-2 shows baseline characteristics by cutpoints of 25(OH)D. Caucasian men who were 25(OH)D sufficient had lower BMI, higher probability of taking calcium supplements, less

likely to have RA and MI, and have their blood drawn in the summer than deficient men. Vitamin D sufficient men of African descent were less likely to have a history of a fracture than deficient men.

4.4.1 Association of 25(OH)D and pQCT parameters at the radius

At the distal radius, Caucasian men with sufficient 25(OH)D had greater total BMC (132.4 vs. 125.7 mg/mm, p=0.044), cortical thickness (3.32 vs. 3.11 mm, p=0.016) than deficient men (Table 4-3). Positive linear trends were observed for 25(OH)D level and cortical vBMD (p=0.042), total BMC content (p trend=0.007), cortical thickness (p trend=0.003) and SSIp (p trend=0.028) and SSIx (p trend=0.012). In men of African descent, 25(OH)D was negatively associated with total bone area (p for trend=0.005) and there was a borderline significant inverse association for SSIp (p for trend=0.060) and SSIx (p for trend=0.048). At the ultra distal radius, there was no association between 25(OH)D and any skeletal parameters among men of either Caucasian or African descent.

4.4.2 Association between 25(OH)D status and pQCT parameters at the tibia

Caucasians with sufficient 25(OH)D had greater total BMC (396.4 vs. 378.4 mm/mg, p=0.037) and cortical thickness (5.54 vs. 5.33 mm, p=0.017) than deficient and insufficient men (at distal radius), respectively (Table 4-3). There were positive linear trends at this skeletal site for total BMC (p=0.012) and cortical thickness (p for trend=0.009) in Caucasians. None of the tibial

measures were associated with 25(OH)D in men of African descent. Also, no significant associations were found at the ultra distal region for men of either race.

4.4.3 Effect of race on the association between 25(OH)D status and pQCT parameter

Significant interactions were observed between race and 25(OH)D primarily at the distal radius. Race modified the association between 25(OH)D levels and total BMC (p for interaction=0.003), total bone area (p for interaction=0.006), SSIp (p for interaction=0.005) and SSIx (p for interaction=0.002). The associations between 25(OH)D and total BMC, total bone area, and SSIp and SSIx were positive for Caucasians and null or negative for men of African descent leading to the significant interaction. Race also significantly (p=0.015) modified the association between 25(OH)D and trabecular vBMD at the ultra distal radius. Caucasians had lower radial trabecular vBMD in the sufficient (181.6 mg/cm^3) and insufficient (182.5 mg/cm^3) groups when compared to the deficient (194.0 mg/cm^3) group, p for trend=0.118. On the other hand men of African descent had greater trabecular vBMD with increasing 25(OH)D, but the trend was not significant.

4.4.4 Restricted Cubic Spline Analysis

There was evidence of a threshold effect on tibial total BMC (p for test of non-equal slopes=0.026) at 19 ng/ml of serum 25(OH)D among Caucasians (Figure 4-1). For tibial cortical thickness a threshold (p for test of non-equal slopes=0.011) was also identified for serum 25(OH)D at 18 ng/ml (Figure 4-2). These thresholds identified the approximate 25(OH)D levels

at which there is greater tibial total BMC and cortical thickness. In men of African descent, we observed no significant thresholds for any of the bone parameters.

4.5 DISCUSSION

In two cohorts of community dwelling men, we found that the association between serum 25(OH)D levels and pQCT measures differed by race. Among Caucasians residing in the US, there were significant associations between 25(OH)D and BMC, cortical thickness, SSIp and SSIx . However, in men of African descent residing in the Caribbean, the associations for 25(OH)D and pQCT parameters were primarily null.

Interactions found at the distal (BMC, area, SSIp, and SSI) and the ultra distal (trabecular vBMD) radius suggested that race modified the association between 25(OH)D and these skeletal parameters. In the Caucasian cohort, the associations were primarily positive between 25(OH)D and total BMC, total area, SSIp and SSIx at the distal radius, while in the African descent cohort these associations were either null or negative. However, for trabecular vBMD, Caucasian men with 25(OH)D sufficiency had lower vBMD than deficient men. On the other hand, men of African descent who were sufficient for 25(OH)D had greater trabecular vBMD than those who were deficient. A previous study failed to find a significant race by 25(OH)D interaction effect on hip, lumbar spine, and radial aBMD in a cohort of Caucasian, African American, and Hispanic men.(23) However, this cohort was significantly younger (mean age 48 years) and the negative association between 25(OH)D and aBMD in African Americans was smaller than in our study.(23)

The mean serum concentrations of 25(OH)D were substantially higher in the African ancestry compared with Caucasian men in our study. Several explanatory factors for this observation may include the higher exposure to sunlight, lower BMI (vitamin D is a lipid soluble molecule), and lower smoking and alcohol consumption among the men of African descent. Serum levels of 25(OH)D are generally lower among blacks compared with Caucasian individuals in the US(4) African Americans typically have lower levels of serum 25(OH)D than Caucasians across all age groups and both genders due at least in part to differences in skin pigmentation and dietary vitamin D intake (4;42-45). However, mean 25(OH)D concentrations in the African continent are relatively high, but vary considerably according to geography, climate, and other factors.(46) There are limited data in other African ancestry populations, particularly in tropical climates where there is high sun exposure such as on the Caribbean island of Tobago where our subjects were recruited.

Although several studies have examined the cross-sectional association between 25(OH)D and different bone measures in men(16;18;19;21-23;47-49), most inadequately controlled for confounders, only included Caucasians, used unreliable assay methods, estimated aBMD instead of vBMD, and were unable to distinguish cortical and trabecular bone. Direct comparison of our 25(OH)D concentrations with other studies is difficult because different cut points have been used to define deficiency/insufficiency, and values were derived using different assay methods. The serum 25(OH)D levels in this study (despite being relatively high in men of African descent) were lower than studies that have used RIA or protein binding assays (reported means ≥40 ng/ml), but compare well to HPLC and studies that have used LC-MS/MS.(28-30) Similar to our findings, the MINOS study found that 25(OH)D levels (measured with RIA) were positively associated with cortical thickness ($p<0.05$) and BMC ($p<0.01$) at the femoral neck in

older (ages 56-85) Caucasian men after adjusting for age, body weight, testosterone concentration, and season.(18) Similarly, the Rancho Bernardo study reported that 25(OH)D (measured with a competitive binding protein assay) was positively associated with aBMD at the hip (p=0.01) and spine (p=0.001) among 414 Caucasian men (mean age 74 years) in multivariable models.(19) Conversely, another study that included 372 men aged 65-96, reported that 25(OH)D (measured with RIA) was not associated with cortical vBMD in Caucasian men.(48)

Few past studies have found the association of serum 25(OH)D with skeletal parameters in ethnic minorities.(16;23) The National Health and Nutritional Examination Survey (NHANES III) which included 13,432 participants of Caucasian, Mexican and African heritage found a positive association between race specific 25(OH)D (measured by RIA) quintiles and total hip aBMD in men aged 50 years and older for all ethnic groups. However, the mean difference in hip aBMD between the highest and lowest quintile was larger in Caucasians (0.040 g/cm^2, $p<0.001$) than men of African descent (0.024 g/cm^2, $p=0.03$) after adjustment for potential confounders.(16) On the other hand, Hannan et al. found no association between 25(OH)D and hip, spine, and radial aBMD among 331 black men in Massachusetts.(23) We found no associations between 25(OH)D and vBMD in men of African descent. However, men of African descent had lower bone area, SSIp and SSIx with greater 25(OH)D concentration. There is no clear explanation for why men of African descent would have lower bone size and strength with higher 25(OH)D. Intermittent and chronic PTH regimens have been shown to stimulate osteoblastic bone formation at the periosteal surface and postpone osteoblast apoptosis resulting in greater bone area and cortical thickness and thus bone strength(50;51) Also, men of African descent have also been shown to be resistant to the resorptive effects of

PTH on bone and markers of bone resorption.(52) Other potential factors identified that may be related to these disparate ethnic differences are the polymorphisms in the vitamin D receptor (VDR) gene.(53) The absence of the BsmI restriction site and the FokI polymorphism for the VDR receptor occur at a higher frequency in whites than blacks and were found to be associated with lower aBMD.(54-56)

The associations of serum 25(OH)D and bone parameters were only observed at the distal (cortical) bone regions of the radius and tibia. Consistent with our findings, Lauretani et al. found that 25(OH)D was positively associated with tibial cortical vBMD, but not trabecular vBMD in women.(48) The mechanisms responsible for the differential associations of 25(OH)D with cortical versus trabecular bone are unclear. PTH excess has been shown to have primarily catabolic effects on cortical bone and maintain or even have anabolic effects on trabecular bone.(57) Several studies have shown that PTH administration and elevated serum PTH levels were associated with preserved trabecular bone structure.(58-62) In cortical bone, excess levels of PTH can result in increased endocortical resorption, cortical thinning and cortical porosity.(63;64)

Serum 25(OH)D thresholds were identified for total BMC and cortical thickness at the tibia in Caucasians. The optimal serum 25(OH)D concentration needed to maintain adequate bone density and geometry has not been established.(65) Optimal concentrations have been defined as those for which PTH levels plateau in the normal range.(66) However, this definition has led to the reporting of a wide range of 25(OH)D thresholds: from 8 to 46 ng/ml. A previous cross-sectional study based on aBMD levels found a threshold at 31 ng/ml with a target of 37 to 41 ng/ml for 25(OH)D for men and women aged ≥20 years of Caucasian, Mexican and African American descent.(16) Our results suggest a 25(OH)D threshold among older Caucasian men at

19 and 18 ng/ml for total BMC and cortical thickness at the tibia, respectively. The association of 25(OH)D with tibial total BMC and tibial cortical thickness appears to be positive and linear at values less than the threshold and null above these values. Recent cohort studies in older men and women found that hip fracture risk was reduced among participants with 25(OH)D levels >20 ng/ml.(65;67). In addition, men with 25(OH)D levels below 20 ng/ml experienced greater hip aBMD loss while rates were similar at higher levels.(17) All of this evidence suggests a lower 25(OH)D threshold may exist for bone density and geometry than previously recognized. Interestingly, 95% of the African ancestry men had 25(OH)D concentrations above these thresholds in Caucasians. This may explain the absence of associations between 25(OH)D with bone measures among the African ancestry men in the current study.

There are no known reports on the prospective impact of baseline pQCT measurements on fracture risk. However, a recent report using the MrOS cohort found that the strongest predictor of non-vertebral fracture was SSIx (HR=2.2 per SD decrease).(68) There was also a 70% increase in risk of fracture per SD decrease in total BMC of the radius. The difference between 25(OH)D sufficiency and deficiency (Caucasians) for total BMC and SSIx in our study was approximately 0.4 SDs. Among the elderly, differences of 0.5 SD in BMD can greatly increase the risk of an osteoporotic fracture.(69)

This report had several potential limitations. Comparisons of these findings to other studies is limited primarily due to differences in 25(OH)D assay methods. Also, the two cohorts in our study varied considerably. Men of African descent had markedly lower calcium intake, caffeine intake, physical activity, education, and prevalence of fracture and MI, compared to Caucasians. Therefore, we were unable to infer whether race modified the association between 25(OH)D and skeletal parameters based on strictly biological differences, lifestyle disparities, or

a combination of both factors. Also, participants selected from the MrOS cohort may have been potentially healthier than other members of the cohort based on exclusion of subjects that were unable to participate in follow-up pQCT scans. However, after comparing characteristics of selected MrOS subjects to those that were excluded they only differed significantly by age (76.9 years vs. 78.0 years, respectively). Finally, by having multiple outcomes some of findings may reach significance by chance alone. However, our study also has several notable strengths including the use of a LC-MS/MS assay to measure serum 25(OH)D, adjustment for many potential confounders, a large sample of men of African descent, and the use of pQCT to measure vBMD, geometry, indices of bone strength, and distinguish cortical and trabecular bone.

In summary, men of Caucasian and African descent differed in the association of serum 25(OH)D levels to several skeletal parameters. Levels of serum 25(OH)D were more likely to be associated with bone measures in Caucasians than men of African descent. Thresholds for 25(OH)D were identified for Caucasians at approximately 20 ng/ml indicating that above this level there is minimal relationship with bone measures. The association between 25(OH)D and bone measures was more evident at the regions that primarily consist of cortical bone. Prospective studies that analyze the association of serum 25(OH)D with trabecular and cortical bone loss in men of different ethnicities are needed.

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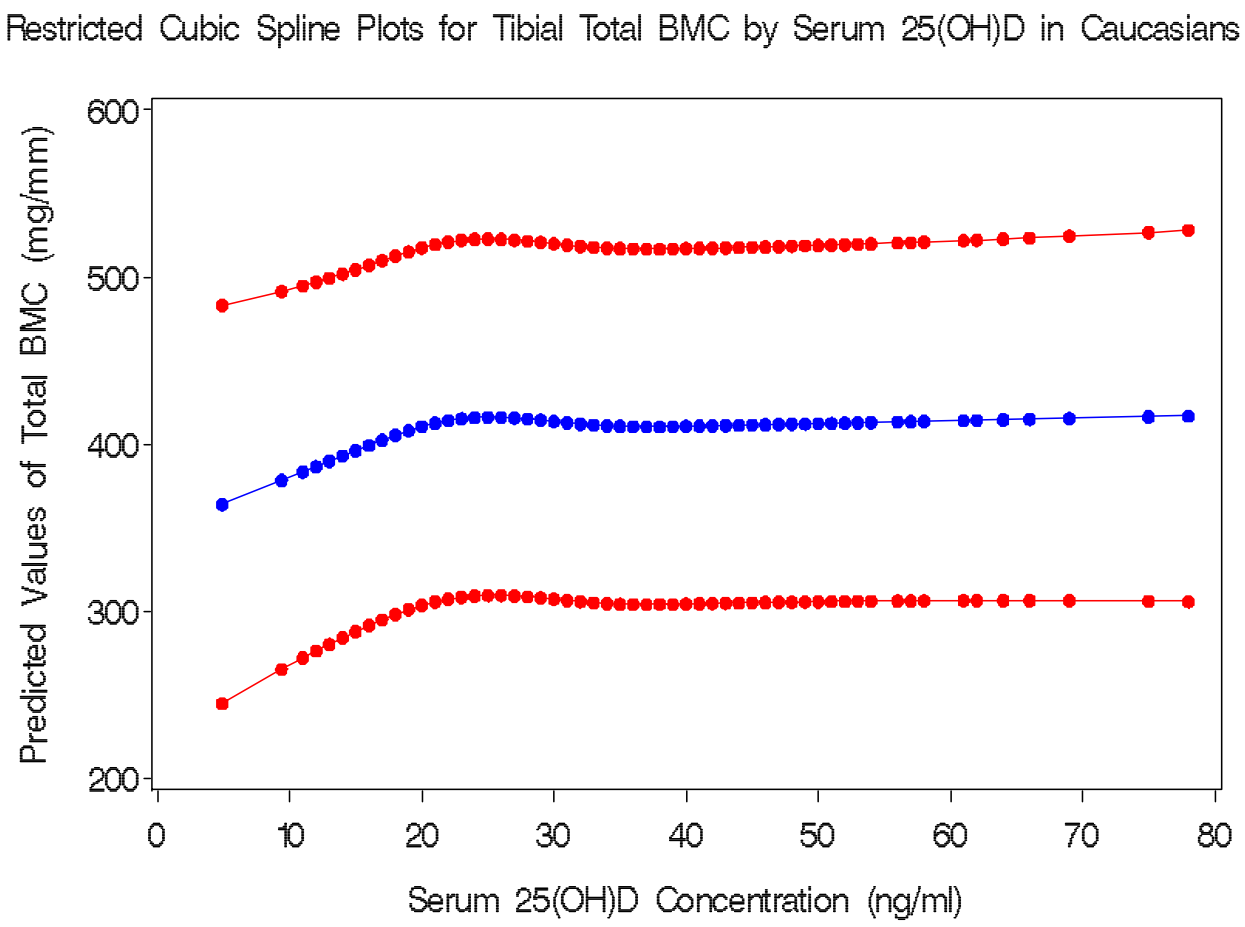

Figure 4-1 Restricted Cubic Spline Plot for Tibial Total BMC by Serum 25(OH)D in Caucasians

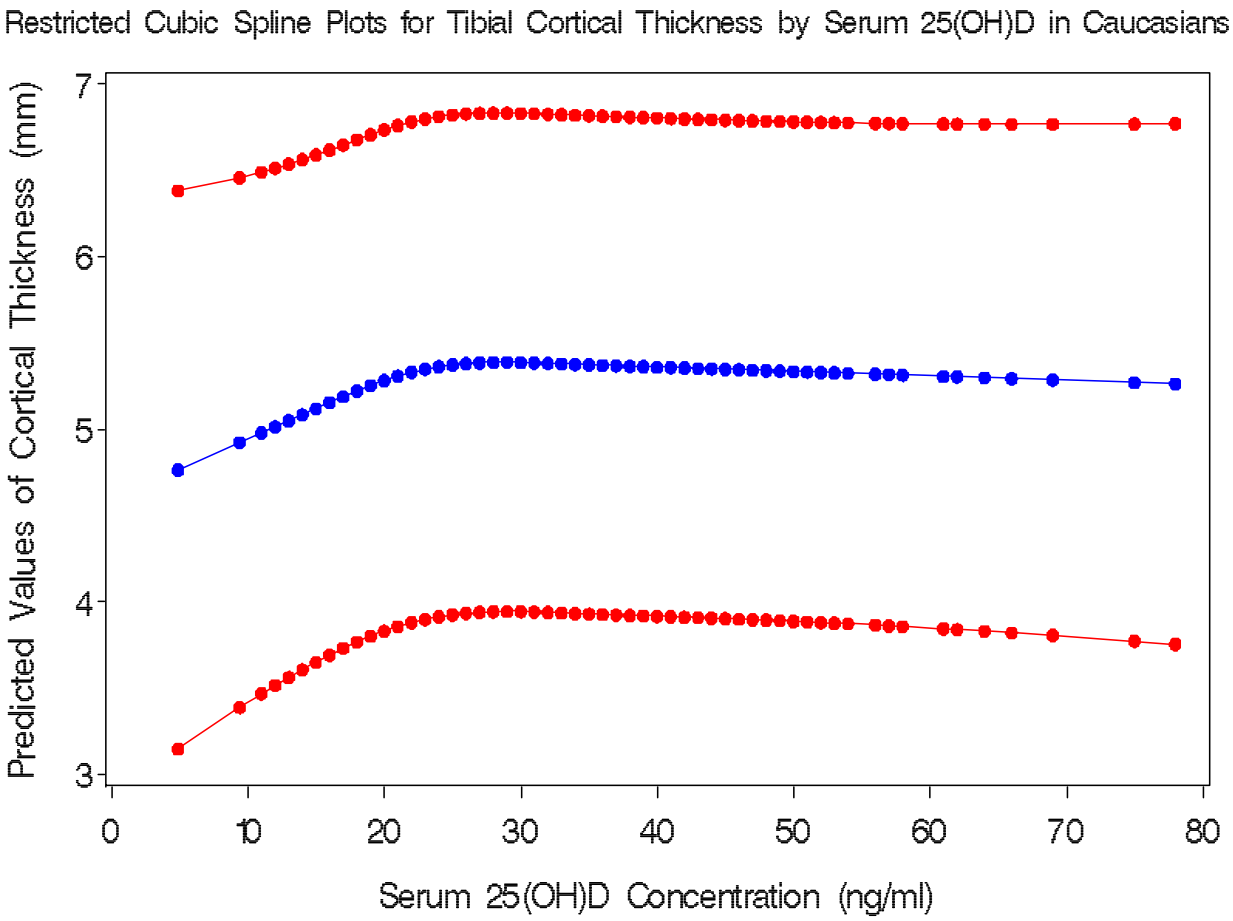

Figure 4-2 Restricted Cubic Spline Plot for Tibial Cortical Thickness by Serum 25(OH)D in Caucasians

Table 4-1 Baseline characteristics by race

	Caucasians (N=446)	African Descent (N=496)	P-value
Serum 25(OH)D level, mean (SD), ng/ml	27.6 ± 8.3	34.7 ± 9.7	<0.001
Age, mean (SD),yrs	76.9 ± 4.9	71.6 ± 5.9	<0.001
BMI, mean (SD), kg/m^2	28.5 ± 4.2	27.2 ± 9.1	0.009
Season of blood draw, %			0.006
Winter	24.2	31.6	
Spring	26.7	20.0	
Summer	24.9	20.4	
Fall	24.2	28.0	
Attended college, %	46.9	3.8	<0.001
Average walk time per day,%			
≤1 hour	45.7	92.7	
>1 hour	53.3	7.3	
Caffeine intake, median (IQR), mg/day	184.0 (0.0-408.0)	100.0 (45.0-150.0)	<0.001
Alcohol, median (IQR), drinks/week	0.5 (0.0-6.0)	0.0 (0.0-1.0)	<0.001
Smoking currently,%	3.6	6.1	0.080
Dietary calcium intake, mean (SD), mg/day	793.5 ± 356	439.0 ± 209.8	<0.001
Supplemental calcium, %	64.6	28.8	<0.001
Health good or excellent, %	89.2	82.0	0.002
History of any fracture, %	54.7	13.0	<0.001
Diabetes, %	19.7	34.1	<0.001
Hypertension, %	52.9	46.2	0.041
Myocardial Infarction, %	16.1	1.4	<0.001
Osteoporosis, %	3.6	3.1	0.716
Osteoarthritis, %	26.7	5.6	<0.001
Rheumatoid arthritis, %	6.7	3.7	0.040

Table 4-2 Baseline characteristics by clinical cutoffs of total serum 25(OH)D (ng/ml)

	Caucasians				African Descent			
	Deficient (<20) N=70	Insufficient (20-<30) N=214	Sufficient (≥30) N=162	p-value	Deficient (<20) N=22	Insufficient (20-<30) N=122	Sufficient (≥30) N=352	p-value
Age, mean (SD),yrs	77.5 ± 5.1	76.9 ± 4.8	76.7 ± 4.8	0.581	72.8 ± 7.5	72.1 ± 6.4	71.4 ± 5.7	0.339
BMI, mean (SD), kg/m^2	29.0 ± 4.6	28.8 ± 4.1	27.8 ± 4.0	0.022	27.1 ± 4.5	28.3 ± 4.9	26.9 ± 10.3	0.347
Season of blood draw, %				0.001			29.5	0.599
Winter	38.6	25.2	16.7		40.9	36.1	20.2	
Spring	21.4	31.3	22.8		22.7	18.9	20.2	
Summer	14.3	23.4	31.5		13.7	22.1	30.1	
Fall	25.7	20.1	29.0		22.7	22.9		
Attended college, %	48.6	50.9	40.7	0.139	4.6	5.7	3.1	0.346
Average walk time per day,%				0.606				0.603
≤1 hour	55.7	43.9	43.8		95.5	82.0	85.0	
>1 hour	44.3	56.1	56.2		4.5	18.0	15.0	
Caffeine intake, median (IQR), mg/day	272.0 (136.0-408.0)	192.0 (0.0-368.0)	140.0 (0.0-408.0)	0.164	75.0 (45.0-150.0)	100.0 (45.0-150.0)	100.0 (45.0-150.0)	0.682
Alcohol, median (IQR), drinks/week	0.5 (0.0-4.0)	0.6 (0.0-6.0)	0.5 (0.0-6.0)	0.706	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.289
Smoking currently,%	5.7	2.3	4.3	0.326	9.1	4.9	6.3	0.609
Dietary calcium, mean (SD), mg/day	727.0 ± 333.5	792.6 ± 360.7	823.4 ± 357.3	0.167	389.2 ± 198.3	443.1 ± 201.2	440.7 ± 213.5	0.520
Supplemental calcium, %	44.3	65.0	72.8	0.002	23.8	31.3	28.3	0.730
Health good or excellent, %	84.3	89.7	90.7	0.330	81.8	82.0	82.1	0.999
History of any fracture, %	55.7	51.4	58.6	0.371	27.3	8.4	13.7	0.042
Diabetes, %	24.3	19.6	17.9	0.532	31.8	36.1	33.5	0.855
Hypertension, %	61.4	51.9	50.6	0.290	47.3	42.2	47.3	0.525
Myocardial Infarction, %	15.7	21.0	9.9	0.014	4.6	0.8	1.4	0.329
Osteoporosis, %	4.3	4.2	2.5	0.619	4.6	3.3	2.9	0.892
Osteoarthritis, %	27.1	25.7	27.8	0.899	18.2	4.2	5.3	0.051
Rheumatoid arthritis, %	14.3	5.1	5.6	0.023	4.6	5.8	2.9	0.204

Table 4-3 The distribution of measures of density, geometry, and bone strength by clinical cutoffs for total serum 25(OH)D (ng/ml)

	Caucasians				African Descent				
	Deficient (<20) N=70 Mean (SE)	Insufficient (20-<30) N=214 Mean (SE)	Sufficient (≥30) N=162 Mean (SE)	P for trend	Deficient (<20) N=22 Mean (SE)	Insufficient (20-<30) N=122 Mean (SE)	Sufficient (≥30) N=352 Mean (SE)	p for trend	P for interaction between race and serum 25 (OH)D
Distal radius (33%)									
Cortical vBMD (mg/cm^3)	1149.3 (6.7)	1151.6 (6.1)	1158.3 (6.3)	0.042	1200.7 (6.3)	1199.6 (2.8)	1203.9 (1.6)	0.229	0.359
Total bone mineral content (mg/mm)	125.7 (3.6)	128.0 (3.3)	132.4 (3.4)*	0.007	149.4 (4.1)	147.2 (1.8)	144.5 (1.1)	0.107	0.003
Cortical thickness (mm)	3.11 (0.10)	3.19 (0.09)	3.32 (0.09)*	0.003	3.52 (0.09)	3.52 (0.04)	3.54 (0.02)	0.670	0.054
Total area (mm^2)	143.2 (4.1)	143.4 (3.8)	145.9 (3.9)	0.291	161.6 (5.0)	155.7 (2.2)	150.5 (1.3)	0.005	0.006
SSIp (mm^3)	354.0 (14.4)	363.3 (13.3)	376.5 (13.7)	0.028	436.4 (18.2)	426.3 (8.1)	412.1 (4.7)	0.060	0.005
SSIx (mm^3)	193.5 (7.9)	200.0 (7.3)	207.8 (7.5)	0.012	243.2 (10.4)	239.9 (4.6)	230.4 (2.7)	0.048	0.002
Ultra distal radius (4%)									
Trabecular vBMD (mg/cm^3)	194.0 (9.0)	182.5 (8.3)	181.6 (8.5)	0.118	187.5 (11.9)	201.2 (5.3)	204.9 (3.0)	0.170	0.015
Total bone mineral content (mg/mm)	126.1 (4.8)	122.6 (4.5)	127.9 (4.6)	0.310	139.7 (5.4)	142.1 (2.4)	140.6 (1.4)	0.792	0.480
Total area (mm^2)	361.9 (13.6)	362.4 (12.6)	371.8 (13.0)	0.244	349.3 (13.0)	354.3 (5.8)	344.6 (3.3)	0.217	0.109
SSIp (mm^3)	493.8 (27.0)	484.6 (25.0)	516.6 (25.8)	0.118	596.1 (30.2)	589.3 (13.4)	576.2 (7.7)	0.327	0.079
SSIx (mm^3)	267.9 (14.8)	258.6 (13.7)	275.1 (14.1)	0.252	316.6 (16.6)	320.9 (7.4)	316.9 (4.3)	0.754	0.328
Distal tibia (33%)									
Cortical vBMD (mg/cm^3)	1122.0 (6.6)	1124.6 (6.1)	1129.8 (6.2)	0.090	1173.3 (7.5)	1166.4 (3.2)	1168.6 (1.8)	0.975	0.232
Total bone mineral content (mg/mm)	378.4 (9.4)	388.9 (8.7)	396.4 (8.8)*	0.012	403.9 (12.6)	415.7 (5.4)	413.4 (3.1)	0.840	0.465
Cortical thickness (mm)	5.31 (0.14)	5.33 (0.13)	5.54 (0.13)**	0.009	5.19 (0.18)	5.37 (0.08)	5.36 (0.04)	0.595	0.314
Total area (mm^2)	461.8 (11.2)	475.3 (10.3)	474.4 (10.5)	0.241	506.1 (17.7)	515.9 (7.6)	508.0 (4.3)	0.559	0.525
SSIp (mm^3)	2046.7 (71.7)	2126.3 (66.3)	2158.5 (67.3)	0.053	2384.6 (107.8)	2510.7 (46.3)	2458.8 (26.3)	0.800	0.500
SSIx (mm^3)	1278.5 (46.3)	1333.7 (42.8)	1350.9 (43.5)	0.060	1463.0 (73.4)	1549.2 (31.5)	1513.8 (17.9)	0.783	0.382
Ultra distal tibia (4%)									
Trabecular vBMD (mg/cm^3)	224.5 (7.8)	222.3 (7.1)	228.7 (7.2)	0.282	224.0 (10.4)	233.4 (4.5)	226.3 (2.6)	0.475	0.383
Total bone mineral content (mg/mm)	359.1 (12.2)	359.9 (11.2)	369.3 (11.3)	0.173	374.0 (14.0)	380.5 (6.0)	370.4 (3.4)	0.277	0.215
Total area (mm^2)	1252.8 (34.7)	1253.5 (31.9)	1260.2 (32.3)	0.731	1260.6 (40.3)	1214.1 (17.2)	1204.1 (9.9)	0.225	0.298
SSIp (mm^3)	2215.6(140.6)	2273.6(129.3)	2375.3(131.1)	0.099	2536.4 (154.2)	2606.1 (65.9)	2526.7 (37.7)	0.481	0.231
SSIx (mm^3)	1189.7 (77.0)	1193.3 (70.9)	1242.0 (71.8)	0.267	1264.3 (82.1)	1322.8 (35.1)	1270.1 (20.1)	0.429	0.397

*p ≤0.050 (Sufficient vs. deficient 25(OH)D, Bonferroni-adjusted)

**p ≤0.050 (Sufficient vs. insufficient 25(OH)D, Bonferroni-adjusted)

All models adjusted for age, BMI, season of blood draw, walk time, caffeine intake, alcohol, smoking, dietary calcium, supplementary calcium, health status, history of fracture, diabetes, myocardial infarction, and rheumatoid arthritis

5.0 ADIPONECTIN NOT LEPTIN PREDICTS BONE LOSS AND INCIDENT FRACTURES IN OLDER WOMEN AND MEN

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5.1 ABSTRACT

Leptin and adiponectin are hormones secreted by adipose cells that may impact bone mineral density (BMD) and modulate bone formation. Few studies have examined the longitudinal association of leptin and adiponectin to rates of BMD loss and fracture. To test the hypothesis that low leptin and high adiponectin levels are associated with a higher rate of BMD loss and fracture we studied 3,046 women and men, aged 70-79, from the Health Aging and Body Composition (ABC) study. Areal BMD (aBMD) of the hip was measured 5 times using dual energy x-ray absorptiometry (DXA) over a 10 year period. Random slope and intercept models accounted for within person correlation due to repeated hip aBMD measurements. Incident fractures were assessed every 6 months by self-report and validated by radiology reports over an average of 6.5 years. Cox regression was used to estimate the hazard ratios for fracture. Among women, higher adiponectin (p trend=0.019) levels predicted greater aBMD loss, but not fracture, after adjusting for covariates including age, race, BMI, and weight change. Conversely, in multivariable models, adiponectin was associated with incident fractures in men, but not bone loss. Men with the highest adiponectin level (tertile 3) had a 207% higher risk of fracture (HR=2.07; 95% CI 1.30, 3.30) compared to the lowest tertile (tertile 1), p for trend=0.002. Leptin did not predict fracture risk in men (p trend=0.869) or women (p=0.873) and was largely attenuated by BMI. Results suggest that adiponectin not leptin is a risk factor for bone loss and fractures. Associations for adiponectin also differed by sex with bone loss in women only and incident fractures in men. Future studies are needed to evaluate and explain these sex differences of adiponectin with bone loss and fracture.

5.2 INTRODUCTION

Leptin is secreted by adipose cells and has been shown to be highly correlated with body fat mass(1), involved in fat metabolism, and appetite control.(2) At high levels it may increase the likelihood of insulin resistance, or metabolic syndrome(3). Leptin may also have an important impact on bone mineral density (BMD),(4) and the modulation of bone formation in humans by enhancing differentiation of bone marrow stroma into osteoblasts and inhibiting its differentiation into osteoclasts and adipocytes.(5;6) Some population based cross-sectional studies reported that leptin is positively(7-13) associated with BMD, while other results were null(14-16) or negative.(17-22) A 12-month longitudinal study in Estonia among 35 older women reported that higher leptin predicted lower loss of whole body BMD.(11) However, this study failed to adjust for measures of adiposity that may have confounded this association. Another prospective study (follow-up period 4 years) using the a cohort from Rancho Bernardo found that leptin did not predict BMD loss among older women and men.(7)

In vivo studies of mice have supported some of these findings by showing an increase in BMD after leptin administration.(23-25) Positive effects on BMD were also observed after administration of leptin prevented the fall in plasma osteocalcin observed during a 24-hour and 5-day starvation study in male mice.(24) Hemrick et al. found that leptin treatment induced loss of bone marrow adipocytes and increased bone formation in leptin-deficient ob/ob mice.(25) In addition, several in vitro studies have shown that higher leptin levels can have a positive effect on BMD.(26-28)

Adiponectin, another adipocyte derived hormone regulates insulin sensitivity and has anti-inflammatory properties.(29;30) At low levels it has been shown to be associated with

various comorbidities including diabetes, myocardial infarction (MI), and atherosclerosis.(31-33) Opposite leptin, adiponectin levels are lower among obese individuals.(34) Adiponectin and its receptors are expressed in human osteoblasts, suggesting that adiponectin may be a hormone linking bone and fat metabolism.(35) However, adiponectin may have negative effects on bone by stimulating the receptor activator of nuclear factor-κB ligand (RANKL) pathway and inhibiting the production of the decoy receptor for RANKL, osteoprotegerin.(36)

Several Epidemiological studies reported that lower levels of adiponectin have been linked with higher BMD(8;11;37-39) while others have failed to find an association between adiponectin and BMD.(16;18;20;20;29;40-42) Three prior studies in the US, Sweden and Estonia, evaluated the impact of adiponectin on BMD longitudinally.(11;39;43) However, the study in Sweden(39) only measured BMD once (after an average of 12 years of follow-up) and thus did not assess change in BMD over time. Recently, an additional study in Rancho Bernardo failed to find an association between adiponectin and bone loss in older women and men after a follow-up period of 4 years.(43)

Few studies have determined the association of leptin(13;44) and adiponectin(39;43;45) to fracture with conflicting results for both. The aim of this report is to test the hypothesis that lower baseline serum leptin levels and higher adiponectin levels will be associated with greater hip aBMD loss and a higher risk of a fracture, in older men and women.

5.3 METHODS

5.3.1 Study population

This study population includes 3,046 women and men (who had serum leptin or adiponectin measured) enrolled in the Health Aging and Body Composition (ABC) study, all aged 70-79, at baseline from two field centers, Pittsburgh, PA and Memphis, TN. Among women and men enrolled, 46% and 37% were blacks, respectively. To be eligible to participate in Health ABC, subjects had to report no difficulty walking at least 1/4 mile and or climbing a flight of stairs. 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 and Memphis. Exclusion criteria included reported difficulty performing basic activities of daily living, obvious cognitive impairment, inability to communicate with the interviewer, intention of moving within 3 years, or participation in a trial involving a lifestyle intervention. The institutional review board (IRB) at each center approved the study protocol, and written informed consent was obtained from all the participants. Baseline data were collected from 1997 to 1998.

5.3.2 Hip aBMD

Areal BMD (aBMD (g/cm^2) of the hip (total and subregions) was measured using DXA (QDR 4500A; software version 9.03; Hologic, Bedford, MA, USA). DXA quality assurance procedures were conducted at both study sites and monitored by the study Coordinating Center, ensuring scanner reliability and identical scan protocols. An anthropometric spine phantom was

scanned daily, and a hip phantom was scanned once per week to assess longitudinal performance of the scanners. These measurements were taken at baseline, year 3, year 5/6, year 8, and year 10.

5.3.3 Fractures

Incident fractures were assessed every 6 months by self-report and validated by radiology reports. Adjudication of fractures was complete through June 30, 2007 for the Pittsburgh clinic, and through December 31, 2005 for the Memphis clinic. The average follow-up time was 6.5 years. Nontraumatic and traumatic fractures were included since both have been linked to low BMD.(46) Pathological fractures and fractures of unknown etiology were excluded.

5.3.4 Leptin and adiponectin

Specimens were obtained by venipuncture in the morning after an overnight fast, processed, aliquoted into cryovials, frozen at –70°C, and subsequently shipped to the Health ABC Core Laboratory at the University of Vermont. Baseline leptin (N=3020) concentrations were measured in duplicate using the Sensitive Human Leptin radioimmunoassy (RIA) Kit (product number SHL-81K) from Linco Research, Inc. (St. Charles, MO). The assay is a competitive RIA in which the concentration of leptin is determined by competition with 125I-Human Leptin, with a maximum detectable leptin level of 50 ng/ml. The intra-assay CV is 3.7–7.5% and the inter-assay CV is 3.2–8.9%. Adiponectin (N=3044) was assayed from baseline serum specimens

Total circulating levels of adiponectin were measured in duplicate by RIA (Linco Research, St. Charles, MO) with an intra-assay coefficient of variation of 1.8–3.6%.

5.3.5 Potential confounders

Demographic variables included self-report of age, race, (black or white), sex, site, and education (<HS, HS graduate, or post secondary). Whole body DXA was also used to measure total lean body mass (kg) and body fat (kg). Weight was measured on a standard balance beam scale to the nearest 0.1 kg, and height was measured by a stadiometer to the nearest 0.1 cm. BMI (kg/m^2) was calculated by using the formula weight (kg) /$height^2$(m^2). Annual weight change (through visit 10) was estimated as the weight difference from baseline to the most recent re-assessment divided by the respective time. Lifestyle factors included self-report of smoking (never, current, or former), and alcohol consumption (no consumption in last year, <1 drink per week, 1-7 drinks per week, or >1 drink per day). To assess supplementary intake for vitamin D and calcium, and non-steroidal anti-inflammatory drug (NSAID) use, participants were asked to bring all prescription and over the counter medications, which were coded based on the Iowa Drug Information System.(47) To estimate dietary intake of calcium and vitamin D, participants completed a 108-item interviewer-administered FFQ (Block Dietary Data Systems, Berkeley, CA). Physical activity (kcal/week) was determined using the caloric expenditure in the past week for self-reported walking, climbing stairs, and exercise.(48) Diabetes was defined using fasting glucose (≥126 mg/dl), self-report, or hypoglycemic medication use. Similarly, subjects were classified as having hypertension through measurement of blood pressure (systolic ≥140 or diastolic ≥90), self-report or antihypertensive medication use. Other medical conditions were

determined by asking respondents if they have ever been told by a doctor that they had a specific diagnosis of MI and history of fracture after age 45. Handgrip strength (kg) was measured using a hand-held dynamometer (Jamar; TEC, Clifton, NJ). The dynamometer was adjusted for hand size for each participant, and two trials were performed on each hand with the maximum value recorded as the observation.

5.3.6 Statistical analysis

All statistical analyses were performed using the Statistical Analysis System (SAS, version 9.1; SAS Institute, Cary, NC). Two-sample t-tests (Wilcoxon rank-sum test for non-parametric measures) and chi-square tests of independence were used to evaluate mean and proportion differences by sex. Leptin and adiponectin means differed in women and men and could not be explained by BMI. Therefore, sex-specific tertiles were used in the analysis (Table 2). For normally distributed variables a test of linear trend was performed by treating leptin or adiponectin tertiles as continuous. The Cochran-Armitage test for trend was used for dichotomous variables. For non-parametric variables with more than 2 groups the Jonckheere-Terpstra test of trend was performed.

Linear mixed-effects models used repeated measures to determine the temporal association between leptin, adiponectin and hip aBMD. These models included a random intercept for each subject and a random slope for time to account for within person correlation. Participants were excluded if they lacked a baseline hip aBMD measurement or had only one measurement during the study. We estimated the average change in hip aBMD annually by

leptin and adiponectin tertiles. Regression coefficients were estimated for the interaction between time and all the independent variables in the model.

Cox proportional hazards models were used to compare the time to fracture by tertiles of leptin and adiponectin and estimate the hazards ratio while controlling for potential confounders. Schoenfeld residuals were used to the test the assumption of proportionality.

To further explore the dose response relationship between adiponectin and fractures we performed spline analysis to test for possible inflection points. Restricted cubic spline linear regression was used with knots for adiponectin at the 5th, 25th, 75th and a reference group at the 95th percentile was set to create the spline plot. Threshold effects were evaluated by identifying potential inflection points on the spline and performing a test of equality to determine if the slopes above and below the cut point are equal.

Backward elimination procedure was used with the variables age, race, BMI and the exposure of interest forced in all the multivariable models. Weight change was added in the final model to determine if this factor better explained our associations. Multi-collinearity was assessed using the variance inflation factor (VIF). Interactions between sex and leptin/adiponectin were evaluated.

5.4 RESULTS

Table 5-1 shows baseline characteristics by sex. Women had significantly greater serum leptin (21.4 vs. 7.9 ng/ml) and adiponectin levels (13.3 vs. 9.5 μg/ml) than men, $p<0.001$. Women were also younger, had higher BMI, and total body fat, lower weight change and total lean mass,

more likely to never smoke or consume alcohol, had lower dietary vitamin D or calcium intake, lower prevalence of diabetes and MI, and lower grip strength than men. Men (0.97 g/cm^2) had significantly ($p<0.001$) higher hip aBMD than women (0.91 g/cm^2) at baseline.

Baseline characteristics by leptin and adiponectin sex-specific tertiles were summarized in table 5-2. Greater serum leptin levels were significantly (p trend<0.05) associated with higher BMI, no consumption of alcohol in past year, diabetes, hypertension, and higher aBMD in both men and women. Among women only, increasing leptin tertiles were significantly associated with lower age, higher weight change, <HS education, supplementary calcium intake, lower dietary and supplementary vitamin D intake, lower physical activity, lower history of fracture prevalence, and higher NSAID and grip strength. The direction of these associations was reversed for adiponectin with the exception of NSAID use in women.

5.4.1 Multivariable Analysis for Hip aBMD Loss

Table 5-3 summarizes the repeated measures longitudinal association of leptin and adiponectin with hip aBMD loss. Lower levels of adiponectin (p trend=0.023) were associated with lower hip aBMD loss in women after adjusting for age, race, BMI, weight change, diabetes and baseline hip aBMD. However, after adjusting for weight change, the association between leptin and aBMD loss was no longer significant (p trend=0.134). Among men, the association of leptin and adiponectin with hip aBMD loss was not significant in the unadjusted and adjusted regression models.

5.4.2 Fractures

A total of 406 fractures occurred over an average follow-up of 6.4 years. For women and men the fracture rates per 1000 person-years were: 27.5 and 14.0, respectively. Based on the unadjusted model, greater leptin levels were significantly (p trend=0.009) associated with lower fracture rates, and lower adiponectin concentration predicted (p trend=0.003) higher fracture risk in women, table 5-4. In men, only adiponectin was a significant predictor (p trend=0.001) of fracture risk in the unadjusted models. However, the association of leptin and adiponectin with fracture in women was attenuated after adjusting for age, race, and BMI p trend=0.794 and p trend=0.658, respectively. In the full multivariable model, higher adiponectin was associated with higher fracture rates in men [(HR=2.07 (95% CI=1.30-3.30), p trend=0.002] after adjusting for age, race, BMI, and education. Sex modified (p for interaction=0.017) the association between adiponectin tertiles and fracture risk in the full multivariable model. However, there was no evidence of a threshold for serum adiponectin and risk of a fracture in men (p for test of threshold>0.05), figure 5-1. The association between adiponectin and fracture (in men only) was fairly linear, and tests of threshold did not approach significance at all adiponectin values.

5.5 DISCUSSION

This is the largest and most comprehensive study to evaluate the association of leptin and adiponectin to BMD loss and fracture. Higher adiponectin levels were associated with a higher risk of fracture independent of potential confounders including BMI and weight change in men,

but not women. Conversely, lower adiponectin levels predicted lower hip aBMD loss in women only. The impact of leptin on fractures and bone loss was largely explained by BMI and weight change in both groups.

Sex modified the association between adiponectin and fracture. Men in the highest adiponectin tertile had a greater than two fold risk of having fracture than men in lowest tertile. This association was independent of BMI, weight change and other covariates of interest. In contrast, adiponectin levels were unrelated to fracture risk in women. BMI and weight change were associated with fracture among women, but not in men. Our results were similar to Rancho Bernardo Study which reported an association between adiponectin and vertebral fractures [adjusted OR:1.13; 95% CI:1.08-1.23, p=0.009] in men not women.(43) Participants of the Rancho Bernardo Study were similar to our subjects in age and smoking prevalence, but had lower BMI, fat mass, adiponectin and prevalence of diabetes. Also, consistent with our findings, a cross-sectional study in Japan among 231 men and 170 post-menopausal Japanese women, reported that one standard deviation difference in adiponectin was associated with higher odds of vertebral fracture in men, but not women.(45) In contrast, a recent prospective study (15 year follow-up) in Sweden, did not find an association adiponectin levels and fracture, among 507 men aged 70 and older.(39) The study population in Sweden had similar characteristics related to age, BMI, and serum adiponectin levels. The results of the spline analysis among men, suggested that this association was very linear with no evidence of an adiponectin threshold for fracture risk. In the recent Swedish study (which found no association), the authors did not report tests for thresholds. However, based on their spline plot there appears to be no major changes in trajectory of the curve indicating a lack of a potential threshold effect.(39)

A recent study reported that recombinant adiponectin induced RANKL and inhibited OPG mRNA expression in human osteoblasts in a dose and time dependent manner leading to osteoclast formation.(36) The risk of bone loss and fracture was higher with greater levels of adiponectin perhaps reflecting greater osteoclast activation and bone resorption. Population based longitudinal studies evaluating the association of adiponectin and markers of bone turnover are needed.

Leptin did not predict incident fractures, in men or women, and the association was largely attenuated by BMI. A ten year cohort study among 250 Italian women and men with comparatively lower leptin levels reported that subjects in the highest leptin tertile had a significantly reduced risk of a non-traumatic fracture when compared to the lowest tertile group after adjusting for weight and other confounders.(44) However, this study included relatively younger participants (mean age 58.5 years), and a small number of cases (N=31). Higher leptin levels were also associated with a lower prevalence of vertebral fractures independent of percent fat, among 139 Japanese postmenopausal women, aged 48-78.(13) The two previous studies have relatively younger cohorts and older age is associated with a greater number of comorbidities. Therefore, age and other covariates (BMI, weight change, grip strength) in our study were able to explain fracture risk better than leptin levels.

After adjusting for weight change, the association between leptin and hip BMD loss, was no longer significant. Past cross-sectional associations reports have shown that leptin is positively associated with BMD(8-10;12;13). Few prior studies have assessed the impact of leptin on BMD longitudinally.(7;11) A twelve month prospective study in Estonia among 35 women mean age 69.7 years, reported that higher leptin was correlated with lower loss of whole body aBMD.(11) However, this study did not adjust for measures of adiposity. The Rancho

Bernardo study found that leptin (measured on average 4 years before BMD) predicted higher femoral neck, total hip, lumbar spine, and radial aBMD in women but men. Consistent with our findings, they failed to find an association between leptin and hip aBMD loss (2 BMD measurements over 4 years).

Higher adiponectin levels were associated with greater hip aBMD loss, in women, but not men. There have been several cross-sectional studies that have reported that adiponectin is inversely associated with BMD.(8;11;37;38). Three prospective studies found an inverse association between adiponectin and BMD.(11;39;43) The study in Estonia (did not control for measures of adiposity) reported that higher adiponectin was associated with higher aBMD loss of the lumbar spine.(11) The Swedish longitudinal study reported that higher adiponectin (measured on average 12 years before BMD) was associated with lower aBMD of lumbar spine, proximal femur and whole body in older men and women.(39) However, this study did not adjust for BMI or body composition measures and failed to assess longitudinal change in BMD over time. Finally the Rancho Bernardo study found that adiponectin (measured on average 4 years before BMD) predicted lower femoral neck, total hip, and radial aBMD in women and men.(7) However, adiponectin was not associated with bone loss in the Rancho Bernardo Study at any of these sites in men or women. Prior studies have shown that the most rapid bone loss occurs between the ages of 40 and 55, therefore 4 years of follow-up and a mean age of 75 years may explain their null finding.(49;50)

It is uncertain why there are sex differences for the association of adiponectin with bone loss and incident fractures. Adiponectin was 40% higher in women compared to men. However, it is uncertain if sex differences in adiponectin account for these differential associations. BMI and weight change explained fracture risk in women; however in men these variables did not

predict fractures. Consistent with our findings, a previous study in mice showed that there was a stronger inverse correlation for adiponectin and bone mass in females compared to males.(51) Prior studies suggest that the association between adiponectin and bone may be influenced by sex hormones.(43) Future prospective studies that control for sex hormones are needed.

Study had several potential limitations. We measured serum leptin and adiponectin at baseline only. In addition, survivors may have greater BMD than non-survivors(52), and study participants are likely to be healthier than the general population. Finally, we adjusted for many covariates, but residual confounding remains a potential limitation. Strengths of this study include a large sample size, reliable ascertainment of exposure (leptin, adiponectin) and outcome (fracture, hip aBMD) data, adjustment for many potential confounders, long follow-up period, and multiple time points for assessment of hip aBMD data.

In summary, in a large cohort of men and women, greater adiponectin levels were associated with a higher fracture risk in men, but not women, after controlling for multiple confounders including measures of adiposity. Conversely, adiponectin was associated with bone loss in women only. This study provides evidence that adiponectin, though not leptin, may clinically impact BMD loss and fracture rates. Future studies are needed to evaluate and explain these sex differences of adiponectin with bone loss and fracture.

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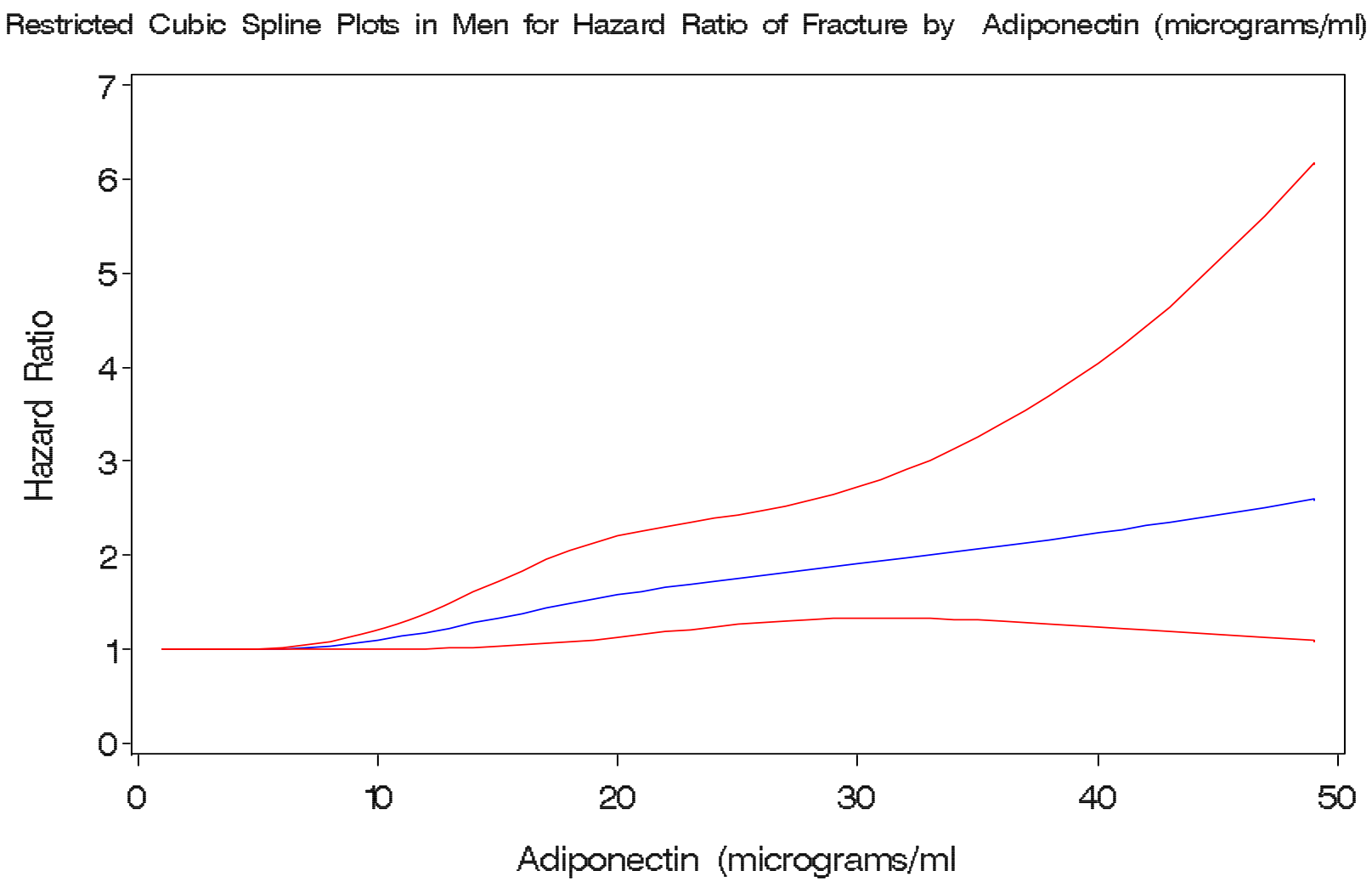

Figure 5-1 Restricted cubic spline plots in men for hazard ratio of fracture by adiponectin (µg/ml)

Table 5-1 Characteristics among older women and men

	Women (N=1478)	Men (N=1568)	P-value
Leptin (ng/ml), mean (SD)	21.4 ± 14.7	7.9 ± 6.9	<0.001
Adiponectin (μg/ml), mean (SD)	13.3 ± 7.4	9.5 ± 5.6	<0.001
Age (years), mean (SD)	73.5 ± 2.9	73.8 ± 2.9	0.013
BMI (kg/m^2), mean (SD)	27.7 ± 5.5	27.1 ± 4.0	<0.001
Weight change (kg), mean (SD)	-0.37 ± 1.74	-0.40 ± 1.94	<0.001
Total fat mass (kg), mean (SD)	29.2 ± 9.3	24.2 ± 7.2	<0.001
Total lean mass (kg), mean (SD)	41.3 ± 6.2	57.1 ± 7.3	<0.001
Race, %			<0.001
Caucasians	53.9	63.2	
African Americans	46.1	36.8	
Education, %			<0.001
<HS	23.2	27.3	
HS graduate	39.5	25.5	
Post secondary	37.3	47.2	
Site, %			0.885
Memphis	50.1	50.4	
Pittsburgh	49.9	49.6	
Smoking, %			<0.001
Never	57.2	29.4	
Current	9.9	10.8	
Former	32.9	59.8	
Alcohol, %			<0.001
No consumption in last year	57.7	42.6	
< 1 per week	21.9	19.6	
1-7 per week	16.9	26.2	
>1 per day	3.5	11.6	
Dietary calcium (mg/day), mean (SD)	753.2 ± 363.6	817.3 ± 428.9	<0.001
Supplementary calcium, %	28.4	7.2	<0.001
Dietary vitamin D (IU/day), mean (SD)	192.6 ± 131.1	225.9 ± 147.5	<0.001
Supplementary vitamin D, %	13.4	3.4	<0.001
Physical activity (kcal/week), median (IQR)	298.5 (26.7-920.4)	688.0 (165.1-1832.1)	<0.001
History of fracture after age 45, %	27.4	16.1	<0.001
Diabetes, %	16.3	22.0	<0.001
Hypertension, %	77.6	74.0	0.020
MI, %	17.9	22.4	0.051
NSAID use, %	25.2	18.4	<0.001
Grip strength (kg), mean (SD)	25.1 ± 6.3	40.8 ± 8.6	<0.001
Hip aBMD (g/cm^2)	0.81 ± 0.15	0.97 ± 0.15	<0.001

Table 5-2 Characteristics by leptin and adiponectin tertiles in older women and men

	Leptin				Adiponectin			
	Low	Middle	High	P Trend	Low	Middle	High	P Trend
Women	N=518	N=517	N=517		N=562	N=501	N=505	
Leptin (ng/ml)	0.3-12.4	12.5-24.1	24.2-99.3	<0.001	-	-	-	
Adiponectin (μg/ml)	-	-	-		1.0-9.0	10.0-15.0	16.0-49.0	<0.001
Age (years), mean (SD)	73.8 ± 3.0	73.5 ± 2.8	73.2 ± 2.8	0.001	73.1 ± 2.8	73.4 ± 2.8	74.1 ± 2.9	<0.001
BMI (kg/m^2), mean (SD)	23.7 ± 3.9	27.4 ± 4.0	31.8 ± 5.0	<0.001	30.1 ± 5.4	27.5 ± 5.1	25.3 ± 4.8	<0.001
Weight change (kg), mean (SD)	-0.22 ± 1.39	-0.30 ± 1.67	-0.56 ± 2.07	0.002	-0.40 ± 1.50	-0.31 ± 1.35	-0.38 ± 2.25	0.780
Total fat mass (kg), mean (SD)	22.1 ± 6.7	28.6 ± 6.1	36.7 ± 8.4	<0.001	32.5 ± 9.3	29.3 ± 8.8	25.4 ± 8.3	<0.001
Total lean mass (kg), mean (SD)	38.3 ± 5.0	40.9 ± 5.7	44.6 ± 6.0	<0.001	44.1 ± 6.3	40.8 ± 5.8	38.7 ± 5.1	<0.001
Race, %				<0.001				<0.001
Caucasians	68.0	55.3	39.1		29.9	61.9	72.9	
African Americans	32.0	44.7	60.9		70.1	38.1	27.1	
Education, %				<0.001				<0.001
<HS	18.0	21.8	29.6		33.1	17.7	17.8	
HS graduate	40.1	42.2	37.4		37.2	42.8	38.8	
Post secondary	41.9	37.0	33.0		29.7	39.6	43.4	
Site, %				0.007				0.117
Memphis	47.7	46.8	56.1		48.0	49.7	52.9	
Pittsburgh	52.3	53.2	43.9		52.0	50.3	47.1	
Smoking, %				0.136				0.007
Never	58.7	54.9	57.7		53.0	58.0	61.1	
Current	13.5	11.0	5.2		10.9	8.8	9.9	
Former	27.8	34.0	37.1		36.1	33.2	29.0	
Alcohol, %				0.002				<0.001
No consumption in last year	54.3	55.9	62.6		65.1	54.2	52.8	
< 1 per week	21.9	22.8	21.1		20.8	22.8	22.2	
1-7 per week	19.7	16.6	14.5		11.2	20.0	20.2	
>1 per day	4.1	4.7	1.8		2.9	3.0	4.8	
Dietary calcium (mg/day), mean (SD)	788.1 ± 375.0	732.7 ± 357.2	743.9 ± 358.7	0.065	764.5 ± 388.6	729.2 ± 345.2	764.5 ± 352.9	0.970
Supplementary calcium, %	37.7	26.4	21.1	<0.001	20.4	30.5	35.1	<0.001
Dietary vitamin D (IU/day), mean (SD)	212.8 ± 140.2	178.3 ± 123.5	186.7 ± 127.4	0.002	192.9 ± 134.4	184.9 ± 122.7	199.7 ± 135.2	0.450
Supplementary vitamin D, %	18.7	12.0	8.7	<0.001	9.8	12.6	17.3	<0.001
Physical activity (kcal/week), median (IQR)	425 (55-1040)	289 (47-895)	213 (5-788)	<0.001	207 (8-793)	343 (42-1025)	371 (70-898)	0.001
History of fracture after age 45, %	30.9	27.3	24.0	0.013	20.7	27.7	34.5	<0.001
Diabetes, %	10.7	16.0	21.9	<0.001	30.1	10.7	6.3	<0.001
Hypertension, %	72.0	78.1	82.2	<0.001	84.3	76.5	71.3	<0.001
MI, %	8.3	8.3	6.6	0.299	7.8	9.0	6.7	0.529
NSAID use, %	21.0	26.0	27.9	0.011	23.6	24.5	27.6	0.140
Grip strength (kg), mean (SD)	24.4 ± 6.3	25.2 ± 6.1	25.5 ± 6.4	0.003	26.2 ± 5.7	24.9 ± 6.3	24.0 ± 6.7	<0.001

Table 5-2 continued.

Hip aBMD (g/cm^2)	0.75 ± 0.14	0.80 ± 0.13	0.88 ± 0.14	<0.001	0.87 ± 0.14	0.80 ± 0.14	0.75 ± 0.14	<0.001
Men	N=488	N=491	N=489		N=519	N=452	N=505	
Leptin (ng/ml)	0.1-4.3	4.4-8.4	8.5-60.3	<0.001	-	-	-	
Adiponectin (µg/ml)	-	-	-		1.0-6.0	7.0-10.0	11.0-53.0	<0.001
Age (years), mean (SD)	73.8 ± 2.9	73.7 ± 2.8	73.7 ± 2.9	0.307	73.4 ± 2.8	73.7 ± 2.9	74.1 ± 2.9	<0.001
BMI (kg/m^2), mean (SD)	24.2 ± 2.8	27.1 ± 3.0	29.9 ± 3.8	<0.001	28.1 ± 3.7	27.1 ± 3.9	26.0 ± 4.0	<0.001
Weight change (kg), mean (SD)	-0.33 ± 2.18	-0.36 ± 1.67	-0.51 ± 1.94	0.163	-0.43 ± 1.81	-0.38 ± 2.18	-0.37 ± 1.84	0.615
Total fat mass (kg), mean (SD)	18.2 ± 4.4	24.4 ± 4.8	29.8 ± 6.9	<0.001	25.2 ± 6.8	24.6 ± 7.0	22.7 ± 7.5	<0.001
Total lean mass (kg), mean (SD)	54.2 ± 6.3	57.3 ± 6.9	59.7 ± 7.7	<0.001	58.8 ± 7.4	57.3 ± 7.2	55.1 ± 6.9	<0.001
Race, %				0.078				<0.001
Caucasians	63.3	68.0	57.9		49.3	64.8	76.2	
African Americans	36.7	32.0	42.1		50.7	35.2	23.8	
Education, %				0.248				0.033
<HS	30.0	26.1	26.0		30.1	24.6	26.7	
HS graduate	24.6	25.9	26.0		27.8	24.8	24.0	
Post secondary	45.4	48.0	48.0		42.1	50.6	49.3	
Site, %				0.049				0.117
Memphis	46.5	52.3	52.8		48.9	48.0	53.9	
Pittsburgh	53.5	47.7	47.2		51.1	52.0	46.1	
Smoking, %				0.023				0.174
Never	31.1	27.8	29.9		29.7	33.0	25.8	
Current	16.0	8.6	7.5		10.4	11.7	10.3	
Former	52.9	63.6	62.6		58.9	55.3	63.8	
Alcohol, %				0.051				<0.001
No consumption in last year	39.0	43.4	45.4		47.1	41.1	39.3	
< 1 per week	19.7	20.1	19.2		21.7	20.7	16.4	
1-7 per week	29.1	25.8	23.5		22.3	25.3	30.9	
>1 per day	12.2	10.7	11.9		8.9	12.9	13.4	
Dietary calcium (mg/day), mean (SD)	824.4 ± 431.3	807.5 ± 416.9	820.0 ± 440.5	0.878	802.1 ± 441.3	822.4 ± 445.1	828.0 ± 401.8	0.355
Supplementary calcium, %	7.6	6.7	7.4	0.887	5.6	7.8	8.3	0.089
Dietary vitamin D (IU/day), mean (SD)	225.8 ± 151.6	221.7 ± 138.7	230.8 ± 152.9	0.630	222.7 ± 153.4	221.6 ± 146.2	232.2 ± 142.2	0.330
Supplementary vitamin D, %	2.5	3.3	4.5	0.080	2.5	3.6	4.2	0.142
Physical activity (kcal/week), median (IQR)	766 (189-1977)	657 (121-1840)	647 (178-1715)	0.279	579 (148-1539)	794 (172-2003)	761 (178-1944)	0.070
History of fracture after age 45, %	17.2	14.9	16.0	0.591	16.6	14.9	16.6	0.983
Diabetes, %	15.2	22.0	28.9	<0.001	35.0	19.4	11.2	<0.001
Hypertension, %	72.3	70.5	78.9	0.019	76.5	75.0	70.9	0.041
MI, %	16.8	12.5	17.6	0.715	16.6	16.6	13.7	0.211
NSAID use, %	17.7	18.3	19.1	0.583	16.7	17.8	20.8	0.094
Grip strength (kg), mean (SD)	40.9 ± 9.1	40.9 ± 8.4	40.6 ± 8.4	0.595	41.9 ± 8.7	41.0 ± 8.9	39.5 ± 8.2	<0.001
Hip aBMD (g/cm^2)	0.93 ± 0.15	0.97 ± 0.14	1.02 ± 0.15	<0.001	1.02 ± 0.15	0.98 ± 0.14	0.92 ± 0.15	<0.001

Table 5-3 The association between sex-specific tertiles of leptin/□diponectin and hip aBMD loss[a] using linear mixed-effects regression models

	Leptin				Adiponectin			
	Low	Middle	High	P Trend	Low	Middle	High	P Trend
Women								
Unadjusted	-0.52 (-0.60, -0.44)	-0.43 (-0.51, -0.34)	-0.60 (0.69, -0.51)	0.983	-0.50 (-0.57, -0.42)	-0.47 (-0.56, -0.39)	-0.57 (-0.66, -0.48)	0.886
Age adjusted	-0.51 (-0.59, -0.44)	-0.43 (-0.51, -0.47)	-0.62 (-0.71, -0.53)	0.758	-0.51 (-0.59,-0.44)	-0.49 (-0.57, -0.49)	-0.55 (-0.65, -0.46)	0.518
Age and race adjusted	-0.57 (-0.65, -0.49)	-0.44 (-0.53, -0.36)	-0.57 (-0.66, -0.48)	0.178	-0.43 (-0.51, -0.36)	-0.55 (-0.61, -0.44)	-0.65 (-0.74, -0.55)	0.043
Age race and BMI adjusted	-0.61 (-0.70, -0.52)	-0.45 (-0.53, -0.36)	-0.52 (-0.63, -0.42)	0.021	-0.42 (-0.50,-0.34)	-0.53 (-0.61, -0.44)	-0.66 (-0.76, -0.57)	0.015
Base model†	-0.62 (-0.71, -0.53)	-0.45 (0.53, -0.36)	-0.53 (-0.64, -0.43)	0.023	-0.41 (-0.50, -0.33)	-0.53 (-0.62, -0.44)	-0.70 (-0.80, -0.59)	0.005
Base model† + weight change	-0.60 (-0.70, -0.51)	-0.46 (-0.55, -0.37)	-0.57 (-0.68, -0.46)	0.134	-0.43 (-0.51, -0.35)	-0.56 (-0.64, -0.47)	-0.67 (-0.77, -0.58)	0.019
Men								
Unadjusted	-0.61 (-0.68, -0.53)	-0.54 (-0.62, -0.46)	-0.56 (0.65, -0.48)	0.089	-0.55 (-0.62, -0.48)	-0.51 (-0.59, -0.43)	-0.66 (-0.74, -0.57)	0.439
Age adjusted	-0.61 (-0.68, -0.53)	-0.55 (-0.62, -0.47)	-0.57 (-0.65, -0.49)	0.101	-0.56 (-0.64, -0.49)	-0.51 (-0.59, -0.43)	-0.65 (-0.73, -0.56)	0.234
Age and race adjusted	-0.61 (-0.69, -0.54)	-0.56 (-0.63, -0.48)	-0.56 (-0.65, -0.48)	0.064	-0.54 (-0.61, -0.46)	-0.53 (-0.61, -0.45)	-0.68 (-0.77, -0.60)	0.149
Age race and BMI adjusted	-0.59 (-0.67, -0.50)	-0.56 (-0.63, -0.48)	-0.59 (-0.68, -0.50)	0.440	-0.54 (-0.62, -0.47)	-0.53 (-0.61, -0.45)	-0.67 (-0.76, -0.58)	0.278
Base model‡	-0.56 (-0.65, -0.46)	-0.58 (-0.66, -0.49)	-0.67 (-0.78-0.57)	0.490	-0.55 (-0.63, -0.47)	-0.55 (-0.64, -0.47)	-0.71 (-0.80, -0.62)	0.147
Base model‡ + weight change	-0.60 (-0.69, -0.51)	-0.58 (-0.66, -0.50)	-0.60 (-0.70-0.51)	0.427	-0.55 (-0.63, -0.47)	-0.54 (-0.63, -0.47)	-0.70 (-0.79, -0.61)	0.148

a Mean annualized % change in aBMD (95% CI)

†Adjusted for age, age*time, race, race*time, BMI, BMI*time, diabetes*time, and baseline hip aBMD

‡ Adjusted for age, age*time, race, race*time, BMI, BMI*time, physical activity, lean mass*time, total fat*time, and baseline hip aBMD

Table 5-4 Cox proportional hazards model[a] according to sex-specific tertiles of leptin (ng/ml) and adiponectin (µg/ml)

	Leptin				Adiponectin			
	Low	Middle	High	P Trend	Low	Middle	High	P Trend
Women								
Unadjusted	(Ref)	0.74 (0.56-0.98)	0.68 (0.51-0.92)	0.009	(Ref)	1.55 (1.14-2.11)	1.61 (1.19-2.19)	0.003
Age adjusted	(Ref)	0.75 (0.56-0.99)	0.70 (0.52-0.94)	0.015	(Ref)	1.52 (1.12-2.08)	1.56 (1.14-2.12)	0.006
Age and race adjusted	(Ref)	0.80 (0.60-1.06)	0.84 (0.62-1.44)	0.217	(Ref)	1.24 (0.90-1.72)	1.19 (0.86-1.66)	0.369
Age race and BMI adjusted	(Ref)	0.86 (0.63-1.16)	0.98 (0.67-1.41)	0.794	(Ref)	1.19 (0.86-1.66)	1.11 (0.79-1.56)	0.658
Base model†	(Ref)	0.86 (0.63-1.16)	0.96 (0.66-1.39)	0.732	(Ref)	1.17 (0.84-1.64)	1.08 (0.76-1.53)	0.796
Base model† + weight change	(Ref)	0.85 (0.62-1.17)	0.99 (0.68-1.45)	0.873	(Ref)	1.12 (0.79-1.57)	0.96 (0.70-1.42)	0.869
Men								
Unadjusted	(Ref)	0.89 (0.59-1.33)	0.70 (0.45-1.09)	0.118	(Ref)	1.44 (0.88-2.35)	2.30 (1.48-3.58)	<0.001
Age adjusted	(Ref)	0.90 (0.60-1.35)	0.72 (0.46-1.12)	0.146	(Ref)	1.41 (0.86-2.31)	2.22 (1.43-3.47)	<0.001
Age and race adjusted	(Ref)	0.90 (0.60-1.35)	0.75 (0.49-1.17)	0.209	(Ref)	1.35 (0.82-2.21)	2.00 (1.27-3.14)	0.002
Age race and BMI adjusted	(Ref)	0.94 (0.61-1.46)	0.82 (0.48-1.39)	0.467	(Ref)	1.34 (0.82-2.19)	1.95 (1.23-3.08)	0.004
Base model‡	(Ref)	0.87 (0.56-1.36)	0.75 (0.44-1.28)	0.731	(Ref)	1.35 (0.82-2.21)	2.04 (1.28-3.23)	0.002
Base model‡ + weight change	Ref)	0.85 (0.54-1.33)	0.72 (0.42-1.25)	0.242	(Ref)	1.29 (0.78-2.13)	2.07 (1.30-3.30)	0.002

aValues are hazard ratios (95% CI);
†Adjusted for age, race, BMI, education, history of fracture, and grip strength
‡ Adjusted for age, race, BMI, and education

6.0 GENERAL DISCUSSION

6.1 SUMMARY

This dissertation identified factors associated with fracture, bone mineral density, bone geometry and bone strength in older adults. The aims of this dissertation project were: 1) examine correlates of trabecular and cortical vBMD of the radius and tibia in primarily older community dwelling Caucasian men and test the hypothesis that the correlates differ; 2) Test the hypothesis that higher levels of serum 25(OH)D will be positively associated with vBMD, bone geometry, and bone strength measures in men of Caucasian and African heritage; 3) and test the hypothesis that higher baseline serum leptin levels and lower adiponectin levels will be associated with a lower risk of fracture and slower rates of bone loss in women and men.

In the first study, our multivariable models explained up to 10% of the variance in trabecular vBMD and up to 9% of the variance in cortical vBMD. These results suggest that there are unknown factors, possibly genetic, related to vBMD in older men that we have yet to identify. Also, fewer correlates were identified for trabecular vBMD of the radius when compared to tibial trabecular vBMD. The tibia is a major weight bearing site compared to the radius, so variables such as weight and physical function may exert a greater influence on vBMD.

Correlates of trabecular and cortical vBMD differed. Higher body weight was associated with greater trabecular vBMD and lower cortical vBMD. Increased mechanical loads have been shown to lead to bone microdamage, increased bone turnover, which may explain the reduction in cortical vBMD. Height was negatively associated with lower trabecular vBMD and previous studies show that taller individuals may experience greater height loss than others. A positive correlation for education and trabecular vBMD was reported which may be a result of residual confounding. Smoking was significantly associated with lower cortical vBMD. Previous reports hypothesize that smoking can possibly lower BMD by decreasing calcium absorption or inhibiting the proliferation of osteoprogenitor cells, and influence estrogen metabolism. Surprisingly, diabetic men using TZD medication had greater trabecular vBMD at both skeletal sites. Greater weight among TZD users compared to diabetic men with and non-diabetic men (95.3 kg vs. 82.9 kg, $p<0.001$) may have contributed to this findings. RA was unexpectedly associated with greater cortical vBMD of the radius. Androgen replacement therapy was associated with higher cortical vBMD at both skeletal sites and anti-androgen use was independently correlated lower trabecular vBMD of the tibia. Finally, lower grip strength was associated with lower cortical vBMD at the radius and using arms to stand from a chair was associated with lower trabecular vBMD.

Several variables were correlated with both cortical and trabecular vBMD. Age was significantly associated with lower vBMD at all sites with the exception of the trabecular site at the radius. A substantial loss in trabecular vBMD of the radius and tibia has been shown to occur before mid-life (age 20-49). Caffeine intake was associated with lower vBMD. Caffeine may reduce BMD by decreasing intestinal calcium absorption, but it may be a marker for lower calcium consumption. Total calcium intake was positively correlated with vBMD. An adequate

intake of calcium can ameliorate the progressive loss of bone with age. History of non-trauma fracture was strongly associated with lower vBMD at all sites. Hypertension was positively correlated with vBMD. A positive association may have arisen from confounding by the use of certain medications such as thiazide (TZ) diuretics that may increase BMD due to their ability to improve calcium retention.

Some factors were not associated with cortical or trabecular vBMD that have been shown to be correlated to BMD in past studies. Most medication variables were not correlated with vBMD. The prevalence of medication use (example SSRI) was relatively low therefore power was a limitation for some covariates. Other correlated identified in past studies that were null include alcohol use, physical activity and hyperthyroidism (very low prevalence).

In the second study, serum 25(OH)D was significantly ($p<0.001$) higher in men of African descent (34.7 ng/ml) versus Caucasians (27.6 ng/ml). African Americans typically have lower levels of serum 25(OH)D than Caucasians across all age groups and both genders. However, mean 25(OH)D concentrations in the tropical regions of the African continent have been shown to compare well to the Tobago and MrOS populations high. Men of Caucasian and African descent differed in the association of serum 25(OH)D levels to indices of bone strength. At the distal radius greater 25(OH)D had a positive impact on bone measures (cortical vBMD, total BMC, cortical thickness, and strength strain index) in Caucasians and a negative or null effect on these parameters in men of African descent. However, at the ultra distal radius, and specifically for trabecular vBMD, 25(OH)D sufficiency was associated with lower vBMD in Caucasians and greater vBMD in men of African descent. In men of African descent no associations were found with the exception of total bone area at the distal radius, and axial strength strain index, which were significantly lower in 25(OH)D sufficient subjects. It is

currently unknown why men of African descent would show lower bone size and strength with higher 25(OH)D. Blacks have been shown to be resistant to the anti-resorptive effects of PTH which were found to be lower in men of African descent. Also, PTH has been shown to stimulate osteoblastic bone formation at the periosteal surface and postpone osteoblast apoptosis resulting in greater bone area and cortical thickness and thus bone strength. Serum 25(OH)D thresholds were identified among Caucasians, at approximately 20 ng/ml, for tibial total BMC and tibial cortical thickness, respectively. Indicating that after this level the effects on bone are minimal. The validity of older 25(OH)D values has been questioned due to the use of insensitive and unreliable radioimmunoassay-based methods which were fraught with inaccuracies due to protein binding artifacts. Thus, direct comparison of our 25(OH)D concentrations with other studies is difficult because different cut points have been used to define deficiency/insufficiency, and values were derived using different assay methods.

In the third study, the adiponectin hormone, not leptin, was a strong independent (HR:2.0) determinant of fracture. After stratifying by gender, this association was confined to older men, not women. The hazard of a fracture in the high adiponectin tertile was approximetley 200% higher than the lowest tertile in men. There did not appear to be any threshold effect of adiponectin on fracture in men. The associations between adiponectin and BMD loss were only significant among women. The impact of leptin on fractures and BMD was largely attenuated by BMI (leptin was strongly correlated with BMI) and weight change. Introduction of recombinant adiponectin to human osteoblasts has been shown to induce osteoblast formation, as well as stimulate the osteoclast RANKL pathway while inhibiting its decoy receptor, OPG in a dose and time dependent manner leading to osteoclast formation. It's possible that subjects in the highest tertile for adiponectin had greater osteoclast activation and bone resorption.

6.2 FUTURE RESEARCH DIRECTIONS

Despite the larger number of potential correlates of vBMD that we examined in chapter 3, our regression models explained an estimated 10% of the variation in trabecular and cortical vBMD. The heritability of BMD was shown to be rather high, at 59% to 73%, for trabecular and 17% to 42%, for cortical vBMD.(4-6) Thus it is likely that a larger portion of the unexplained variance reflects genetic factors that were not accounted for in the analysis. Comprehensive genetic studies of trabecular and cortical vBMD are needed and are underway. In MrOS, a large genetic wide association study (GWAS) has recently been funded and will include analyses of over one million single nucleotide polymorphisms (SNPS) that is intended to identify important genes that contribute to vBMD. Yerges at al. recently reported that trabecular vBMD and cortical vBMD are under different genetic control.(7) Consistent with this observation we found that demographic, anthropometric, medical, and lifestyle correlates also differ for trabecular and cortical vBMD. In addition to the lack of genetic studies for trabecular and cortical vBMD, there is a dearth of studies that included sex hormones, markers of bone formation or resorption, and other biochemical markers including 25(OH)D, and PTH.

One of the more interesting observations was that weight was associated with greater trabecular vBMD, but lower cortical vBMD. Similar observations have been shown in two prior studies using QCT measurements in men.(8;9) Increased mechanical load due to having greater fat and lean mass may paradoxically lead to bone microdamage, increased bone turnover, and a transient reduction in cortical vBMD.(10) Also, individuals who are obese may adapt by increasing periosteal bone diameter requiring lower cortical density to maintain the same strength.(11) In women, a meta-analysis determined the association of weight and hip fractures

and concluded that lower weight increased the risk of hip fracture.(12) In men, the association for weight and hip fractures is not well understood. A previous study using older white men from NHANES I showed that BMI at baseline did not differ among individuals with and without a hip fracture after 14 years of follow-up(13) Conversely, a study among 730 men from 14 centers in Southern Europe did find that lower BMI was a risk factor for hip fracture.(14) Future research could investigate the longitudinal impact of weight on cortical bone loss (various sites including distal radius, distal tibia, and femoral neck), and fractures of the hip (including femoral neck, femoral head, intertrochanteric and subtrochanteric fractures). We hypothesize that an increase of one standard deviation in weight will be associated with an increase in cortical vBMD loss and hip fractures in men.

Past studies(15;16) indicated that TZD use was associated with increased BMD loss; we found a positive correlation for TZD use and trabecular vBMD at the radius and tibia. Yaturu et al. in a cohort of 160 men found that TZD users had significantly greater femoral neck, and total hip aBMD loss than non-TZD men who had diabetes.(16) However, a major limitation of this study is that it did not adjust for any potential confounders. Shwartz et al. found that TZD users had higher lumbar spine aBMD loss than non-users among diabetics, in women, but not men.(15) The former report may suggest that gender may modify this association. A case-control study of diabetics found that the odds of nonvertebral (hip, wrist and humorous) fracture was significantly higher among TZD uses compared with non-users. However, they found no association for vertebral fractures.(17) Also, the previous study only identified 59 vertebral fractures; therefore a cohort with a larger incidence of vertebral fracture is needed. TZD users in our study had significantly greater weight than non-diabetics, and non-TZD users who were diabetics although we adjusted for weight. Future studies should evaluate if this finding reflects

a physiological mechanism that is unique to trabecular vBMD. We propose a prospective analysis among diabetic men that follows TZD users and compares them to non-TZD users. We hypothesize that TZD users will have a lower rate of trabecular vBMD loss and vertebral fractures after adjusting for measures related to body composition.

In chapter 4 we examined the association of serum 25(OH)D and indices of bone strength in older men of Caucasian and African descent. Race cohort modified the association between 25(OH)D and indices of bone strength. Among Caucasian men, serum 25(OH)D was positively associated with pQCT measures at the distal (cortical regions), but not ultra distal (trabecular regions) radius and tibia. In men of African descent, 25(OH)D was negatively correlated with total bone area and axial strength index at the distal radius.

This raises the possibility of differential associations between 25(OH)D and indices of bone strength by race. The association of 25(OH)D with indices of bone strength is still not well understood, and has been understudied(18;19) in individuals of African descent. The proportion of European admixture in US African American men is much higher than in Afro-Caribbean men we studied, which may be influencing the findings. The men in our study are genetically more homogeneous (have 4 grandparents of African ancestry) than most other cohorts that include individuals of African descent. However, there were many lifestyle and cultural differences in addition to race between the US and Afro-Caribbean men. Therefore, we were unable to determine if these differential associations are primarily a result of genetic or environmental factors. To better understand the genetic component involved in these disparate associations a new study using genetic admixture as a variable is needed. We propose to conduct a cross-sectional study examining the association of 25(OH)D with indices of bone strength among older African American men from a single community (more homogenous cultural background).

After analyzing the DNA, men with ≥50% African admixture will be in one group and the others will be in a separate group. We hypothesize that the correlation between 25(OH)D and indices of bone strength will be more likely to be null or negative in men with ≥50% African admixture, compared to the other subjects.

In addition to men of African descent, Hispanics, and Asian men have been understudied in this area. A previous study that included Hispanics in the US found a positive association between 25(OH)D and BMD in younger and older adults.(18) However, Hispanics in the US, like African American men, are likely to be 25(OH)D deficient. A methodological issue in studying Hispanics is their extremely diverse genetic pool which includes African, Caucasian, Asian, and indigenous heredity. Future research could potentially expand this study design to only include Hispanic men (excluding Afro-Caribbean, and individuals with known European or Asian ancestry) from a specific Caribbean nation, perhaps Dominican Republic. We hypothesize that 25(OH)D values will be higher among Caribbean Hispanics versus US Hispanics, due to greater exposure to UV light. This association is not well understood among Asian men and there are no US studies to our knowledge. A study in China reported a null finding for 25(OH)D and hip aBMD among 407 men aged 50 or older.(20) Future research could expand this design to include Asian American men. We hypothesize that 25(OH)D will be positively associated with vBMD and other indices of bone strength in Caribbean Hispanics and Asian American men.

Adiponectin not leptin was associated with bone loss and incident fractures. Among women, but not men, adiponectin was associated with lower rates of bone loss. Gender modified the association between adiponectin and fracture. In men, the risk of a fracture was 2.1 (p trend=0.002) times more likely if they were in the highest adiponectin tertile versus the lowest independent of potential covariates including, but not limited to age, race, BMI, and weight loss.

In women this association was not statistically significant. The association of leptin with bone loss and fractures was largely attenuated by BMI and weight loss in both groups.

The underlying mechanism that contributes to bone loss and incident fractures among individuals with high serum adiponectin needs further investigation. Based on an *in vivo* study, adiponectin may have a negative impact on bone by stimulating the RANKL pathway and inhibiting the production of the decoy receptor for RANKL, OPG.(21) Studies that examine the impact of adiponectin on bone resorption markers are needed. A recent cross-sectional study in China evaluated the association of adiponectin with markers of bone resorption in women.(22) They found a positive correlation (among postmenopausal women) between adiponectin and N-terminal type I collagen telopeptide (NTX) after adjusting for age and fat mass. We propose a study design which evaluates the cross-sectional and longitudinal association of adiponectin and markers of bone resorption including NTX and carboxyterminal cross-linking telopeptide of bone collagen (CTX) in older adults. We hypothesize that adiponectin will be positively correlated with bone resorption markers at baseline. Also, we conjecture that higher serum adiponectin at baseline will be associated with a greater increase in NTX and CTX overtime.

Prior studies have only reported an association of adiponectin and fractures in men. In our study it was lower BMI, and greater weight loss, that increased the fracture risk in women. To better understand why this association is only significant in men we need to measure the sex hormone level in older women and men. Specifically, we need to assess the correlation between adiponectin and bioavailable estradiol and testosterone in women and men. Past literature shows that bioavailable estradiol and testosterone are negatively correlated with adiponectin.(23) We hypothesize that the correlation of adiponectin with bioavailable estradiol will be stronger and

more likely to explain fracture in women than men. We also conjecture that the association between bioavailable testosterone and adiponectin will be similar in women and men and less correlated when compared to estradiol in both populations. Based on these observations we would be able to better understand how adiponectin in men not women, acts independently of BMI, lean mass, fat and other potential confounders (related to sex hormones) to predict fracture.

This information will ultimately help us to better comprehend the natural history and etiology of osteoporosis and osteoporotic fractures as well as the underlying factors for gender and racial differences in skeletal health.

6.3 PUBLIC HEALTH SIGNIFICANCE

Osteoporosis is a growing major public health problem that leads to disability and an increased risk of mortality after a fracture. Much less is known about determinates of osteoporosis and fracture in men compared to women and in minority groups. An estimated 13% of men over the age of 50, will have an osteoporotic related fracture, and lose an estimated 1% of their BMD every year.(1) In addition to this public health burden, annual mortality rate after a hip fracture is higher in men compared to women.(2) As life expectancy increases the prevalence of osteoporosis and its associated fractures is expected to increase in the general population and among various demographic groups. Also, medical costs for osteoporosis and osteoporotic fractures are projected to increase over the next few decades, especially in men.(3)

Few studies have comprehensively assessed the risk factors associated with cortical and trabecular bone compartments. The study in the MrOS cohort of older men is the largest most

comprehensive study on correlates of vBMD. Past studies used DXA to estimate BMD however this procedure is dependent on bone size (e.g., bone size differs in men compared to women), and therefore provides an assessment of areal BMD not volume and does not estimate true density. DXA also fails to distinguish trabecular and cortical bone. Bones differ in their composition of trabecular and cortical bone. For example, the spine contains an estimated 65% trabecular bone and the femoral neck consists of approximately 75% cortical bone. Identifying correlates of trabecular bone can lead to a better understanding of what factors might be associated with spine fracture. On the hand understanding correlates of cortical bone may improve our knowledge of the etiology of femoral neck hip fracture. Also, identifying these correlates may facilitate osteoporosis prevention strategies

Vitamin D deficiency can have a profound impact on bone growth and bone mineralization in young adults and on bone loss in older adults. In addition, our study uses a population of men of African descent with relatively low European admixture (4 grandparents of African ancestry required) relative to other black cohorts. This more homogenous population allows us to understand these associations better in a population of black men with little or no European admixture. The association of 25(OH)D and bone is not well understood in men of African descent. Blacks have lower fracture rates, greater BMD, but lower 25(OH)D than Caucasians. This paradox is also not understood. This study attempted to identify hypotheses for these disparate associations and to also better comprehend what serum 25(OH)D threshold is required to maintain adequate bone strength among to two distinct racial cohorts by using tests of threshold.

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