

**DETERMINANTS OF SKELETAL HEALTH IN AFRO-CARIBBEAN MEN**

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Osteoporosis is a common senile condition with major public health impact in both genders of all races. Very little is known about the natural history and etiology of bone loss, and trabecular and cortical volumetric bone mineral density (vBMD) in men, especially in men of African heritage.

This research project was to evaluate age-related patterns and potential correlates for the rate of decline in areal BMD (aBMD) at the proximal femur, and vBMD at the radius and tibia in a cohort of Afro-Caribbean men aged 40 and above from the Tobago Bone Health Study. We also investigated the genetic associations of variants in a gene involved in the bone mineralization process, ectonucleotide pyrophosphatase/ phosphodiesterase 1 (ENPP1), with bone loss, aBMD and vBMD.

In longitudinal analyses, a significantly greater rate of bone loss was observed in men aged 40-45 than those aged 45-49 and 50-54. Thereafter, the rate of bone loss accelerated with advancing age. The rate of bone loss was also comparable with those observed in Caucasian men. Additionally, we identified low body mass index, weight loss, prostate cancer, and treatment for prostate cancer with androgen deprivation (ADT) as potential determinants for accelerated bone loss.

In cross-sectional analyses of vBMD, we observed an early decline of trabecular vBMD before age 50 and with a slower decline thereafter into 7<sup>th</sup> decade. Cortical vMBD, however,

appeared to decrease with advancing age in a linear fashion. Correlates of vBMD included weight, diabetes, prostate cancer, ADT, cigarette smoking and bone chewing.

In genetic association study, several variants in the *ENPP1* gene were strongly associated with bone loss, aBMD or vBMD. More associations were found with cortical vBMD than with the other phenotypes.

Our findings have important public health relevance as they increase our understanding of vBMD and age-related bone loss in an under-studied population. We have also identified a novel association of *ENPP1* gene variants with bone loss and BMD in this population of African heritage. Additional research is needed to better understand the factors related to BMD and bone loss in populations of African ancestry, especially the apparent early loss of bone mass.

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## **1.0 DISSERTATION OVERVIEW AND OBJECTIVES**

Osteoporosis, a disease defined by low bone mineral density (BMD), has been considered a condition that predominantly affects post-menopausal women. An abundance of research has been conducted to better understand the etiology and prevention of osteoporosis in women. However, men are also affected by osteoporosis and mortality associated with osteoporosis-related hip fractures appears to be higher among men than women (1). Although researchers have recognized the need to better understand skeletal health in men, non-white men have been under-represented in osteoporosis-related research.

Areal BMD (aBMD) measured by dual-energy x-ray absorptiometry (DXA) has been widely used to quantify bone density. Unlike cross-sectional studies, there are only a few longitudinal population-based studies that have examined the patterns and magnitude of aBMD changes with aging as well as factors associated with these changes in men (2-10). It is important to better understand the patterns and causes of aBMD changes to prevent osteoporosis, especially in the less-studied African populations.

DXA provides a two-dimensional measure of aBMD that is known to be confounded by differences in bone size. The use of quantitative computed tomography (QCT) provides a three-dimensional measure of true volumetric BMD (vBMD) and also enables a separate analysis of bone mass in the cortical and trabecular bone

compartments, which were known to have different metabolic characteristics. vBMD measures have not yet been well-characterized in the elderly or in subjects of African origin.

Genetic susceptibility also plays a major role in determining bone density. It has been estimated that genetic factors contribute to 50-80% of inter-individual variability in BMD (11-13). Several candidate genes for BMD and osteoporosis have been identified, but most of the research has been conducted in women and Caucasians and focused on aBMD. There is substantially less known about the genetics of bone loss and vBMD.

Therefore, the objectives of this dissertation project were to determine and better understand the potential environmental and genetic factors associated with rate of decline in aBMD and trabecular and cortical vBMD in men of African heritage.



## **2.0 INTRODUCTION**

### **2.1 EPIDEMIOLOGY OF OSTEOPOROSIS AND OSTEOPOROTIC FRACTURES**

#### **2.1.1 Prevalence of osteoporosis and osteoporotic fractures**

Osteoporosis is a common disorder characterized by compromised bone strength and is a strong predictor of fracture risk (14-17). According to the World Health Organization (WHO), osteoporosis is clinically defined by a bone mineral density (BMD) more than 2.5 standard deviations below the gender specific mean BMD of young adults (18). Osteoporosis affects 10 million people in the US, and is projected to affect approximately 14 million adults aged 50 and older by 2020 (19).

The third National Health and Nutrition Examination Survey (NHANES III, 1998-1994) also estimated that 13-18% or 4-6 million US non-Hispanic white women 50 years and older have osteoporosis and 37-50% or 13-17 million have osteopenia (BMD between 1 and 2.5 SD below the young adult mean) at the hip. In non-Hispanic white men, 1-2 million (3-6%) have osteoporosis and another 8-13 million (28-47%) have osteopenia at the hip. Despite the lower prevalence in men than women, the number of older men with low BMD is still substantial (20). Although the prevalence of

osteoporosis is less well-characterized in non-white populations, studies have shown that non-Hispanic whites have a higher prevalence of hip osteoporosis than non-Hispanic Blacks for both sexes (20). The prevalence among Mexican Americans is similar or slightly smaller than it is in non-Hispanic white women, but is lower than in Non-Hispanic Black men (20).

Osteoporosis or low BMD is one of the important risk factors for fractures. Osteoporotic fractures were estimated to affect 1.7 million of the world's population in 1990. This number is expected to rise to 6.3 million by year 2050 (21). Although the life time risk of fracture (including hip, vertebra, and forearm) is higher among women (40%) than in men (13%) aged 50 and older, the world wide incidence of hip fracture in men is projected to increase by 310%, compared to 240% in women by the year 2050 (21,22). Due to demographic changes throughout the world, the proportion of all hip fractures will increase dramatically in areas outside of North American and Europe. In Africa, with the increase in total population size, the number of hip fractures in men is expected to increase by 216% by 2025 and by 698% by 2050, compared to the number of hip fractures in 1990 (23). US Caucasians in general have a higher fracture incidence than any other ethnic group. The lower incidence of fractures among Blacks has generally been explained by their greater bone density (24).

### **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 (25). Fractures are also associated with an increased incidence of morbidity including back pain, kyphosis

and disability. Racial differences in mortality and morbidity following hip fractures are also observed. African Americans who sustain a hip fracture have increased morbidity and nearly twice the mortality as Caucasian Americans (26,27). Despite their lower risk of osteoporosis and fractures, men may have a higher mortality risk following a fracture than do women (1,27). Furthermore, black and white men have nearly identical mortality rates whereas black women may experience a higher mortality rate following a fracture than their white counterparts (27).

### **2.1.3 Economic Burden**

Osteoporotic fractures cost \$17-20 billion annually in the US (21,28). More than 20% of the expenditures for treatment of osteoporosis-related fractures were due to fractures in men (28,29). Men aged 65 and older accounted for 81% of the total medical cost among men. By race, approximately 80% of the costs among men are attributable to fractures in white men, who account for 80% of the fractures in men (28). The total cost for osteoporotic fractures is projected to approach \$25.3 billion by the year 2025 and \$50 billion by the year 2050 (28,30). These direct medical costs represent a greater burden than the projected annual cost of stroke, breast cancer, diabetes, or chronic lung disease (30). A recent study reported a race-specific increasing economic burden for osteoporotic fractures in the US (16). They projected that, by the year 2050, the total cost due to fractures will increase 37% in whites, 79% in blacks and 175% in Hispanics from the year 2005 (28). Worldwide, the economic burden of osteoporosis parallels that seen in the US.

## 2.2 BONE BIOLOGY

Normal bone consists of matrix (type I collagen and other noncollagenous proteins, called osteoid) and mineral (principally an amorphous calcium phosphate) organized into a mineralized structure. Bone is a dynamic organ, constantly being remodeled under the actions of osteoclasts and osteoblasts.

### 2.2.1 Osteoclasts and bone resorption

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-osteoclasts. 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).

### **2.2.2 Osteoblast and bone formation**

The osteoblast is the bone lining cell responsible for the production of the bone matrix constituents (collagen and ground substance). Osteoblasts originate from mesenchymal stem cells (or bone marrow stromal cells), which can differentiate into either adipocytes under the influence of peroxisome proliferator-activated receptors  $\gamma 2$  (PPAR $\gamma 2$ ) or pre-osteoblasts under the influence of the transcription factor runt-related transcription factor 2 (Runx2). The pre-osteoblasts then mature into osteoblasts. The active osteoblasts start the bone formation process by producing layers of osteoid and slowly refilling the cavity created by osteoclasts (31).

### **2.2.3 Bone remodeling**

Bone remodeling is a process involving the coupling of bone resorption and bone formation. Packets of bone that are resorbed by osteoclasts are replaced by osteoblasts during the formation period. This coupling process is believed to be mediated by humoral factors acting through osteoblasts and cells in the osteogenic lineage. There are many factors involved in the resorption and formation phases including various cytokines and hormones. The following table (Table 2-1) highlights the important factors in bone formation and bone resorption and their effects on bone remodeling.

**Table 2-1 Effects of hormones and cytokines on bone formation and resorption**

<b>Hormones/ Cytokines</b>	<b>Effects</b>
<b>RANKL</b> (Receptor Activator of Nuclear faktor kappa B ligand)	Binds to its receptor RANK to stimulate the differentiation of osteoclasts.
<b>RANK</b> (Receptor Activator of Nuclear faktor kappa B)	Binds to RANKL to stimulate the differentiation of osteoclasts.
<b>OPG</b> (Osteoprotegerin)	Acts as a nonsignaling decoy receptor for RANKL to inhibit bone resorption. It is a strong inhibitor of osteoclast formation and may also suppress the survival of osteoclasts.
<b>IL-1</b> (Interleukin 1)	A stimulator of osteoclast formation. Also a mediator of bone resorption and increased bone turnover in osteoporosis
<b>TNF-<math>\alpha</math></b> (Tumor necrosis factor- $\alpha$ )	Potent inhibitor of bone collagen synthesis and stimulator of osteoclastic bone resorption, the net effect of which is to cause bone loss
<b>M-CSF</b> (Macrophage Colony Stimulating Factor)	Required for the formation of osteoclasts. Works with RANKL and TGF- $\beta$ to cause the bone osteoclastic resorption.
<b>IL-6</b> (Interleukin 1)	A stimulator of bone formation. Stimulated and secreted by normal bone cells in response to PTH and 1,25(OH) $_2$ D $_3$
<b>TGF-<math>\beta</math></b> (Transforming Growth Factor- $\beta$ )	Secreted by both osteoblasts and osteoclasts. Its action in bone remodeling is complex and is thought to be responsible for coupling bone formation to bone resorption.
<b>PTH</b> (Parathyroid Hormone)	Stimulates both bone formation and bone resorption. Its effects on bone formation are stimulated by Cbfa1 which also regulates RANKL expression and osteoclast formation.
<b>1,25(OH)<math>_2</math>D<math>_3</math></b> (1,25 Dihydroxyvitamin D)	Indirectly stimulates the differentiation and fusion of osteoclast progenitors and also directly activates mature osteoclasts
<b>Calcitonin</b>	An inhibitor of osteoclastic bone resorption activity. However, osteoclasts eventually escape from this inhibitory effect after continued exposure of calcitonin.
<b>Leukotrienes</b>	Have been related to osteoclastic bone resorption
<b>Thyroid hormone</b>	Stimulates osteoclastic bone resorption. Increased bone loss has been observed in patients with hyperthyroidism.

#### 2.2.4 Bone compartments

The adult skeleton consists of cortical bone and trabecular bone with the proportion differing by anatomical regions. The proportion of cortical and trabecular bone does not change with age in the same way, which may explain the discrepancies in bone loss and fracture rates at different skeletal sites (32).

Cortical bone is dense or compact bone that comprises 85% of the total bone in the body and is the most abundant bone in the long bone shafts of the appendicular skeleton. The volume of cortical bone is regulated by the formation of bone on the periosteal surface and by resorption of bone on the endosteal surface. The periosteal formation increases the diameter of cortical bone and the endosteal resorption increases the thinning and porosity of cortical bone. In women, the loss of cortical bone occurs after the age of 40 and accelerates 5-10 years after menopause. This acceleration phase continues for 15 years and gradually slows down. Fragile cortical bone is a major predisposing factor for hip and wrist fractures (32).

Trabecular bone comprises only 15% of the adult skeleton. Declines in trabecular bone may occur as early as 30 years of age, before the loss of cortical bone and is a major contributor to spine osteoporosis due to the high proportion of trabecular bone in vertebral bodies (32). The loss of trabecular bone results from not only thinning of the bone plates, but also by the complete perforation and fragmentation of trabeculae. Moreover, men and women appear to lose trabecular bone in different manners. In women, the loss of trabecular bone occurs by increased resorption with loss of trabecular numbers and connectivity, whereas it occurs by trabecular thinning in men (33).

### **2.3 PATHOGENESIS OF OSTEOPOROSIS**

Osteoporosis results from a failure to achieve optimal peak bone mass in early adulthood and/or from a high rate of bone loss with aging.

### 2.3.1 Peak bone mass

Peak bone mass is the maximum bone density achieved at the end of the skeletal maturity. Peak bone mass is an important predictor of postmenopausal osteoporosis (34,35). Although peak bone mass is known to be achieved in early adulthood, both cross-sectional and longitudinal studies suggest that peak bone mass is achieved as early as late adolescence(36,37).

Peak bone mass accrual during adolescence or early adulthood reflects an increase in bone size, which produces an increase in mineralized bone within the periosteal envelop (38). Bone size is determined mostly during the pubertal growth period. Growth velocity is high immediately after birth and it slows rapidly thereafter. At 12 months of age, the rate of bone growth starts to accelerate due to appendicular bone growth. Appendicular growth remains more rapid than axial growth velocity in the pre-pubertal years. At puberty, long bone growth slows down, but axial bone growth accelerates. Therefore, with the later onset of puberty, men appear to have longer bones than women. Sex differences in bone width are also established during puberty. Cortical width increases by periosteal bone formation in men, and by less periosteal bone formation but more endocortical apposition in women. Thus, compared with women, men have larger bones (longer and wider), which results in higher bone mineral content and aBMD, but not vBMD, compared with women. Trabecular vBMD increases by thickening of the trabecular plates. This increase seems to be similar in young men and women of the same ethnic origin. But men and women of different ethnicities do not have similar trabecular thickness. For example, African Americans have a higher trabecular volumetric BMD



due to their greater trabecular thickness. The trabecular thickness increases similarly by gender but differently by race during puberty (38).

Peak bone density is highly heritable with heritability estimated as high as 80%(39). Besides genetic contribution, there are many environmental factors that can affect bone mass accrual. The most important factors for peak bone mass accrual in adolescent and young adults include physical activity, nutrition (mostly calcium intake) and hormonal status.

### **2.3.2 Age-related bone loss**

Age-related bone loss is a result of an imbalance in bone remodeling where bone formation does not replace all of the bone that is resorbed away by osteoclasts. Factors contributing to bone loss can be broadly categorized into hormonal, anthropometric, lifestyle and genetic factors.

#### **2.3.2.1 Hormonal factors**

Menopause affects bone mass in women due to the loss of estrogen. Estrogen reduces bone resorption by inhibiting osteoclast function. Estrogen loss after menopause results in more bone resorption than bone formation and thus leads to rapid bone loss. Recent studies also suggest an important role of endogenous estrogen and bone mass in men (40,41). For example, mutations in the gene encoding the aromatase gene, the enzyme responsible for converting androgens to estrogens, has been linked to low BMD in men (42). Androgens are also an important predisposing factor for osteoporosis in men. Men treated with androgen antagonists or gonadotropin releasing hormone agonists for

prostate cancer metastases experience rapid bone loss(43). Age-related hypogonadism is also thought to contribute to decreased BMD in men (44).

Age-related changes in the growth hormone (GH)/insulin-like growth factor (IGF-1) axis may also contribute to age-related bone loss (45). IGF-1 enhances the function of mature osteoblasts, increases bone matrix synthesis and prevents osteoblast apoptosis (45).

Parathyroid hormone (PTH) and 1,25-dihydroxy vitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) are the principle regulators of calcium homeostasis. PTH stimulates the release of calcium and phosphate in bone and stimulates the reabsorption of calcium and inhibits the reabsorption of phosphate in kidney. Furthermore, PTH also enhances the synthesis of 1,25(OH)<sub>2</sub>D<sub>3</sub>, which increases the intestinal absorption of calcium and phosphate. The interactions between PTH and 1,25(OH)<sub>2</sub>D<sub>3</sub> maintain the blood calcium concentration. For example, a rise in blood calcium concentration decreases the secretion of PTH, while a decrease in blood calcium concentration increases the secretion of PTH. In addition, 1,25(OH)<sub>2</sub>D<sub>3</sub> and low phosphate increase blood calcium concentration thus inhibits PTH secretion. This regulation interaction is essential for the structural integrity of the skeleton (46).

Interlukin-6 (IL-6) produced by osteoblasts, monocytes and T cells promotes osteoclast differentiation and activation and therefore plays a prominent role in post-menopausal bone loss (47). Aging is associated with increasing levels of IL-6 and tumor necrosis factor-alpha (TNF- $\alpha$ ). In bone, genetic variants in the IL-6 gene have been associated with IL-6 gene expression and bone resorption (48,49). TNF- $\alpha$  is a cytokine that enhances osteoclast formation by upregulating the stromal cell production of

RANKL and M-CSF (50,51). TNF- $\alpha$  not only stimulates osteoclast activity but also inhibits osteoblastogenesis (52). Therefore, the effect of TNF- $\alpha$  drives an imbalance between bone formation and bone resorption which may result in bone loss.

### **2.3.2.2 Lifestyle and anthropometric factors**

Many lifestyle and anthropometric factors have been associated with bone density. It is important to thoroughly understand the roles of these factors in order to prevent or lower the risk of osteoporosis. The established risk factors for low BMD or osteoporosis include: low body weight, physical inactivity, cigarette smoking, excessive alcohol drinking, vitamin D deficiency, low calcium intake, use of glucocorticoids and certain medical conditions. The most common medical conditions contributing to low BMD are cystic fibrosis, muscular dystrophy, conditions that lower estrogen levels (eating disorders, excessive physical activity, ovarian failure of any cause, and menopause), endocrine disorders (diabetes, hyperparathyroidism, and Cushing's syndrome), gastrointestinal diseases (celiac disease, intestinal malabsorption, primary biliary cirrhosis, and Crohn's disease), blood disorders (hereditary anemias and multiple myeloma, leukemia), rheumatoid arthritis, lupus, and depression (53).

### **2.3.2.3 Genetic factors**

Genetic factors also play an important role in the development of osteoporosis. Twin and family studies indicate that the heritability of aBMD ranges from 50% to 85% and that differences in heritability exist by gender and anatomical regions examined (11-13). Although most studies have examined the heritability of aBMD in Caucasians, men and women of Asian (54,55) and African (56) heritage also show high heritability of aBMD.

In contrast to the cross-sectional measures of aBMD, heritability for longitudinal aBMD change is less well defined. A twin study with 12 monozygotic (MZ) and 19 dizygotic(DZ) twins showed that the intra-class correlation for lumbar spine aBMD change was 0.93 among MZ twins and 0.51 among DZ twins, while another study (25 MZ and 21 DZ) found that the heritability of aBMD change at the radius was 15% (57-59). A more recent study of 388 premenopausal Caucasian women found a 38% and 35% heritability for change in area and aBMD at the femoral neck (60). Significant heritability of aBMD change was also reported in Mexican American families with heritability of 39%, 46% and 45% at the hip, spine and radius, respectively (61). Moreover, studies have also suggested a strong genetic effect on the concentration of bone turnover markers (62-64). These findings also indirectly suggest that bone loss with aging is a heritable phenotype.

Although population-based epidemiological studies suggest that family history of fractures is a risk factor for fractures and its association is independent of aBMD (65,66), results from heritability studies seem to be inconsistent. Some studies reported that susceptibility to fractures was not influenced by genetic factors (67), whereas others suggested that about 50% of the variation of fractures at the hip and wrist may be explained by genetic factors (68,69). Environmental factors also play an important role in the occurrence of fractures and were often not considered in these studies.

We have recently reviewed the genetic epidemiology of osteoporosis (70). The most widely studied candidate genes for aBMD and related phenotypes have been the vitamin D receptor (VDR), estrogen receptor alpha (ESR1), type I collagen (COL1A1), transforming growth factor- $\beta$  (TGF- $\beta$ ), and the low-density-lipoprotein receptor related

protein-5 (LRP5) genes(70). The majority of these genetic association studies have focused on women and Caucasians.

## **2.4 AREAL BMD IN MEN**

### **2.4.1 Assessment of aBMD**

Dual-energy X-ray absorptiometry (DXA) has been the most frequently used technique for assessing bone density and diagnosing osteoporosis in clinical and in research settings. DXA is a non-invasive technique with a very small radiation dose and excellent precision. The underlying concept of the DXA technology is that photon attenuation *in vivo* is a function of tissue composition. Rectilinear scanning of the supine body is performed and divides the body into a series of pixels, within each of which the photon attenuation is measured at two different energies. The DXA body composition measurement assumes that the body consists of three components that are distinguishable by X-ray attenuation properties: fat, bone mineral and fat-free or “lean” soft tissue (71). DXA is typically used to measure aBMD, bone mineral content (BMC) and cross-sectional area (CSA) at the lumbar spine, whole body, forearm and hip in a two-dimensional plane. The hip measurement is further subdivided into several regions such as femoral neck, trochanter, intertrochanter, and ward’s triangle.

### **2.4.2 Cross-sectional studies**

There are a few large-scale epidemiologic studies of aBMD and osteoporosis in men and most of them were conducted in Caucasians from different continents, such as Study of Osteoporotic Risk in Men (STORM, USA) (72), Rancho Bernardo Study (USA) (73), Osteoporotic Fractures in Men Study (MrOS, USA) (74), Rotterdam Study (Netherlands) (2), TROST (Tromsø Osteoporosis Study, Norway) (75), DOES (Dubbo Osteoporosis Epidemiology Study, Australia) (76) and EVOS (European Vertebral Osteoporosis Study, Germany) (77). The mean aBMD value among Caucasian men aged 55 and older is between 0.99 to 1.10 g/cm<sup>2</sup> at the lumbar spine, 0.73 to 0.83 g/cm<sup>2</sup> at the femoral neck and 0.88 to 0.99 g/cm<sup>2</sup> at the total hip (78-81). In contrast to Caucasian men, men of other ethnicities have not been well represented in these osteoporosis-related studies. Studies of racial differences in aBMD have shown that African American men have 6-10% and 15-21% higher unadjusted and adjusted aBMD at the lumbar spine and femoral their Caucasian counterparts, respectively (82-84). We also demonstrated a higher hip aBMD in men of African descent compared to Caucasian men in the Tobago Bone Health Study (85,86).

### **2.4.3 Longitudinal studies**

Although men have higher aBMD at all sites compared with women, men also experience significant aBMD decline with aging. In the Framingham study, Caucasian men aged 67-90 had a significantly lower rate of decline in femoral neck aBMD but a similar rate of decline in forearm and lumbar spine aBMD, compared with women (3). In the Dubbo and

Rancho Bernardo studies, Caucasian men and women aged 60 or above had a similar rate of decline in hip aBMD (10,73). Table 2 summarizes the main results from several longitudinal studies of aBMD change with aging in older men. The mean annualized rates of decline in aBMD vary across different skeletal sites and studies. These discrepancies are likely due to, in part, different study designs, follow-up duration, assessments of aBMD, sample sizes, geographic areas, age distributions and other characteristics of the populations studied. However, the patterns of decline in aBMD with age are mostly consistent. Lumbar spine aBMD remained stable or increased with aging, whereas proximal femur aBMD decreased with aging in most studies listed in Table 2-2, with a larger decline observed at the femoral neck than the total hip. At the radius, especially at the shaft region, the rate of decline in aBMD appears to be much higher than at any other skeletal site. The higher rates of aBMD decline at the femoral neck and distal radius than lumbar spine may reflect differences in bone composition and/or falsely elevated lumbar spine aBMD due to spinal degenerative disease and osteophytosis which is known to interfere with DXA BMD measurements. Only one of these published studies described the magnitude of bone loss in men of African descent (87). This study found that African American men aged  $\geq 65$  had a lower rate of aBMD decline at the femoral neck and total hip than Caucasian men. However, our preliminary results comparing annualized aBMD decline rates between older Afro-Caribbean men from the Tobago Bone Health study and Caucasian and African American men from MrOS suggested that Afro-Caribbean men may experience a similar rate of aBMD decline compared with Caucasian and African American men (88). Moreover, no difference in aBMD decline rate was observed between Caucasian and African American men in the MrOS.

**Table 2-2 Longitudinal studies of DXA aBMD changes with aging among older men**

Study/ Author	Annualized rate of aBMD change	Skeletal Site	Age	Population description
Melton LJ, et al. (4)	0.4% 0.12% 0.19% -0.19% 0.03% -0.29% -1.69% -2.26% -0.48% -0.97%	Spine Femoral Neck Total Hip Ultradistal Radius Midradius	$\geq 50$ $\geq 70$ $\geq 50$ $\geq 70$ $\geq 50$ $\geq 70$ $\geq 50$ $\geq 70$	Sample size: 348 Follow-up duration: 4 years Race/country: white, USA
Rancho Bernardo (6)	0.3% 0.08% -0.17% -0.66% -0.39% -0.77%	Spine Femoral Neck Total Hip	65-74 $\geq 75$ 65-74 $\geq 75$ 65-74 $\geq 75$	Sample size: 297 Follow-up duration: 4 years Race/country: white, USA
MOST study (87)	White: -2.1% Black: -1.12% White: -0.8% Black: 0.05%	Femoral Neck Total Hip	$\geq 65$	Sample size: 349(white)/ 119(Black) Follow-up duration: 1.5 years Race/country: white/black, USA
Framingham Study (3)	-0.09% -0.38% -0.77% -0.90%	Spine Femoral Neck Ultradistal Radius Radial Shaft	67-90	Sample size: 278 Follow-up duration: 4 years Race/country: white, USA
MrOS(88)	White: -0.35% Black: -0.40% White: -0.37% Black: -0.44%	Femoral Neck Total Hip	$\geq 65$	Sample size: 3585(white)/ 133(Black) Follow-up duration: 4.6 years Race/country: white/black, USA
Warming L et al. (81)	0.4% (over 2 yrs) -0.7% -0.6%	Spine Femoral Neck Total Hip	$\geq 50$	Sample size: 96 Follow-up duration: 2 years Race/country: white, Denmark
TROST study (9)	-0.48% -0.39%	Distal Forearm Ultradistal Forearm	45-84	Sample size: 2197 Follow-up duration: 5 years Race/country: white, Norway
EPIC study (5)	-0.17%	Total Hip	65-74	Sample size: 470 Follow-up duration: 3 years Race/country: white, UK
Dennison et.al. (8)	0.19% 0.64% 0.22% -0.31% -0.30% -0.06% -0.31% -0.10% 0.23%	Spine Femoral Neck Total Hip	60-64 65-69 70-74 60-64 65-69 70-74 60-64 65-69 70-74	Sample size: 173 Follow-up duration: 4 years Race/country: white, UK
DOES study (10)	0.56% -0.85%	Spine Femoral Neck	$\geq 60$	Sample size: 241 Follow-up duration: 2.5 years Race/country: white, Australia
Rotterdam Study (2)	-0.28 %	Femoral Neck	$\geq 55$	Sample size: 1856 Follow-up duration: 2 years Race/country: white, Netherland
Tobago Bone Health Study (88)	-0.62% -0.53%	Femoral Neck Total Hip	$\geq 65$	Sample size: 318 Follow-up duration: 4.2 years Race/country: black, Tobago



#### **2.4.4 Areal BMD and Fracture**

aBMD has been shown to be a strong predictor of fracture risk in women (14). The age-adjusted relative risk of fractures increases by 1.5 to 2.5 fold with each standard deviation decrease (SD) in aBMD (89,90). Although there are relatively fewer studies relating aBMD to fractures in men, the findings still support a strong inverse association between aBMD and risk of fracture. For example, in the Rotterdam study, the age-adjusted relative risk of hip fractures increased by 3-fold with every 1 SD decrease in femoral neck BMD among Caucasian men aged 55 years and older (91). In addition, the risk of fractures decreased from 30% to 65% at the hip, ankle, vertebrae and rib with every 1 SD increase in femoral neck aBMD (76). These results suggest that low BMD is an important determinant of osteoporotic fractures risk in men. However, the relationship between aBMD and fracture risk in men of African descent is less well defined.

#### **2.4.5 Correlates of areal BMD and accelerated bone loss**

Identifying risk factors for low aBMD or accelerated aBMD loss is important for the prevention of osteoporosis and osteoporotic fractures. Advanced age and low body weight have been well-established as risk factors in male osteoporosis; however, other factors such as smoking and physical inactivity have yielded inconsistent results. The conflicting findings may, in part, reflect the different methods used to collect information on lifestyle factors or different sample sizes.

#### **2.4.5.1 Age**

Advanced age is one of the most important risk factors associated with low aBMD in men. Age is known to be inversely correlated with aBMD at most skeletal sites. The correlation between age and spine aBMD is less consistent (83,92,93). Unlike other skeletal sites, spine aBMD seems to increase with age. This association most likely reflects the confounding effect of osteoarthritis and calcification of the aorta (94). In the third NHANES survey, by age 80-85, Caucasian men lost approximately 25% of their femoral neck aBMD measured in the 20s (20). In the MrOS, every 5 year increase in age was associated with a 2-3% decrease in hip BMD and 0.7-1.6% increase in spine aBMD (79,80). In the longitudinal studies, the rate of decline in hip aBMD is significant in men (2-4,6,10,76) and is slower in men than in women (2-4,8,9,81). Although the relationship between age and longitudinal aBMD change in elder men and women of African heritage is less well defined, one study showed that the rate of hip aBMD decline accelerated with age among African American and Caucasian women after the age of 75 (African Americans: from -0.16% to -0.70% per year (Caucasians: from -0.43% to -0.87% per year) (95).

#### **2.4.5.2 Body weight and weight change**

Body weight is one of the strongest correlates of aBMD in men and women. A positive association between body weight (or body mass index, BMI) and aBMD among middle-aged and elderly men has been well documented for both Caucasians and African Americans in cross-sectional studies (72,78,83,92,93,96). The variation in body weight may account for 10% to 17% of the total variation in aBMD at the lumbar spine and

femoral neck (10). Body fat and lean mass, the major components of body weight, are also known to be related to aBMD in Caucasian and Afro-Caribbean men (78,97,98).

Weight loss with aging is common among the elderly and has an important impact on skeletal health. In the Rancho Bernardo study, weight change was an important correlate of bone loss. For example, men who lost 2kg or more of their body weight had a significantly lower hip aBMD than those who gained more than 2kg of their weight (92). In addition, weight losers were twice as likely as others to lose at least 1% of their aBMD per year (73). Lower initial BMI was also significantly associated with greater bone loss than higher BMI (2,6,8). In the Framingham, Rancho Bernardo, MrOS and EPIC studies, men who lost 5% of their baseline weight had a significantly greater rate of aBMD decline at the lumbar spine, hip and radius than those who gained 5% of their baseline weight (3,5-7). However, the relationship between weight loss and aBMD change has not yet been established in men of African heritage.

#### **2.4.5.3 Physical activity and muscle strength**

The skeleton responds to weight-bearing activity by increasing new bone formation and altering the distribution of bone mass to accommodate the mechanical forces applied to it (99). The forces applied to bone from daily activities and exercise are therefore important for normal development and maintenance of bone mass and strength (100). The effect of exercise on aBMD or bone loss in men is not well defined. A greater current or historical physical activity level has been associated with higher aBMD in middle-aged and elderly men (72,80,92). Men engaged in regular exercise also had a higher aBMD at the spine, total hip and femoral neck (6,80). However, some studies found no significant association between exercise and aBMD in men (79). The positive effects of exercise on aBMD are

likely due to the beneficial effects of exercise on muscle strength (10). Muscle strength, often defined by grip strength, has been associated with higher aBMD in several studies of male skeletal health (72,76,80). The impact of physical activity on age-related aBMD decline in men is not well characterized. Men engaged in intense activity had a slower aBMD decline rate than those that did not engage in intense activity (59). Disability is sometimes used as an indirect surrogate of physical inactivity. In the Rotterdam study, men with lower limb disability had a greater decline in aBMD than those without disability (2). Association between physical activity and bone loss in men has been primarily examined in Caucasians only.

#### **2.4.5.4 Cigarette Smoking**

Smoking is an established risk factor for osteoporosis. Animal studies suggest that exposure to nicotine impairs bone formation (101). Nicotine produces a dose- and time-dependent reduction in DNA synthesis and cellular proliferation of osteoblast-like cells *in vitro*, suggesting that nicotine may have direct toxic effects on bone cells (102). It has also been proposed that cigarette smoking may contribute to increased bone loss and low aBMD by accelerating normal age-related declines in androgenic hormone levels (103). Current smokers had a lower aBMD compared with non-smokers (92,104-106), although some studies showed no association between smoking and aBMD (72,79,80). In a meta-analysis, male smokers had 0.3 SD lower femoral aBMD compared with nonsmokers and the effect size was similar to that observed among women (107). In the limited publications of longitudinal studies of aBMD changes in men, all in Caucasian men, smokers consistently had a greater aBMD decline rate at the hip compared to nonsmokers (2,3,6,73).

#### **2.4.5.5 Alcohol drinking**

The mechanisms for alcohol-induced osteoporosis are not known, but evidence suggests that impaired osteoblastic activity with normal osteoclastic activity may contribute to reduced bone mass in alcoholic patients (108). Moderate alcohol consumption has been associated with a higher bone density in several (92,93,109), but not all (72,76) studies of middle-aged and elderly Caucasian men. However, the effect of moderate alcohol drinking on aBMD may also be mediated through other confounding factors such as age and weight (110). There have been few studies of alcohol consumption and rates of bone loss in men. Several of them found a positive but non-significant association between moderate alcohol consumption and the aBMD change in older Caucasian men (2,3,6,73,76). No longitudinal study of aBMD changes has examined the relationship between BMD changes and alcohol consumption in black men.

#### **2.4.5.6 Calcium intake**

Calcium is the major mineral component of bone. Calcium deficient diet has been linked to decreased bone calcium content. Adequate vitamin D is essential for optimal calcium absorption. An inadequate intake of either calcium or vitamin D results in a reduced calcium absorption and a slightly low blood concentration of ionized calcium. In response to this, blood PTH concentration increases and stimulates bone turnover (111). Calcium deficiency is the most common cause of increased bone resorption in older individuals likely due to low dietary intake, lack of sun exposure, malabsorption and anorexia (112). Population studies have not consistently linked higher dietary calcium intakes to higher bone density or reduced bone loss with aging in men. Positive association between aBMD and calcium intake was observed in some (2,72,79,92), but

not all studies (76,80). However, the effect of calcium on bone density might be site-specific. In the Rancho Bernardo study, older men who consumed 800mg calcium per day had a greater aBMD at the lumbar spine, but not hip, compared to those whose calcium intake was under 800mg/day (92). Dietary calcium intake or calcium supplement use was not significantly correlated with aBMD change in older men in most of the longitudinal studies (5,6,73,76). The impact of calcium intake and vitamin D on aBMD and aBMD change with aging in men of African descent is unknown. However, studies have shown that African American women had significantly lower calcium intake and lower serum vitamin D levels compared to their white counterparts but still maintained a high level of aBMD (113).

#### **2.4.5.7 Medication**

Chronic use of several medications may reduce bone density and increase the risk of fractures. The use of *glucocorticoids*, *thiazide diuretics*, and *thyroid hormone* are among the most common and well-studied medications that have been known to be associated with low aBMD, aBMD decline and osteoporotic fractures. The effect of medication use on osteoporosis risk has not been confirmed in all population studies, most likely due to inadequate statistical power from the low frequency of use of these medications. *Glucocorticoids*, an anti-inflammatory and immunosuppressive medication, decrease bone mass by directly inhibiting osteoblastic function and formation, decreasing intestinal calcium absorption, and increasing urinary calcium excretion. Chronic use of corticosteroid is linked to secondary osteoporosis among men (114). However, studies found inconsistent effects of corticosteroids on aBMD. No significant association between corticosteroids use and hip aBMD was observed among men in the STORM

study (72), but a reversed association was observed in MrOS (80). *Thiazide diuretics* or thiazide-like diuretics, a common hypertension medication, decrease urinary calcium excretion and have been associated with increased aBMD in men in the cross-sectional studies (72,80,115,116). This effect also seemed to be site-specific (115). However, a longitudinal analysis from the same study showed no association between thiazide diuretics use and aBMD change (6). *Thyroid hormone* is used chronically to treat hypothyroidism. Use of thyroid hormone has been associated with reduced aBMD in women (117). Due to the less common use of thyroid hormone among men, information regarding the impact of thyroid hormone use on aBMD in men is limited. One study found that men who reported an average of 15.5 years use of thyroid hormone had similar levels of aBMD at multiple skeletal regions as nonusers (118).

Androgen deprivation therapy (ADT) is used to prevent the recurrence of prostate cancer after radical prostatectomy or radiation therapy. While it suppresses tumor growth, ADT also decreases aBMD (43). Androgens help to maintain the balance between bone synthesis and degradation, but decrease bone resorption via the aromatization of testosterone to estrogen. ADT disrupts this normal hormonal balance required for bone health (119). In the MrOS study, prostate cancer was associated with low spine but not hip BMD (80), whereas a higher hip aBMD was observed among men with prostate cancer in Afro-Caribbean population from the Tobago prostate cancer survey study (120). Another study also showed that men with prostate cancer may experience significant bone loss due to the nature of the disease even before undergoing ADT (121). To our knowledge, no study has reported the relationship between aBMD and use of glucocorticoids, thiazide diuretics, thyroid hormone and ADT in men of African descent.

Although the Tobago study has reported the relationship between aBMD and prostate cancer, the effect of prostate cancer treatment was not evaluated (120).

#### **2.4.5.8 Medical conditions**

Several medical conditions have been associated with aBMD in men. Secondary causes of osteoporosis include excessive use of glucocorticoids or other immunosuppressive drugs, hypogonadism, chronic obstructive pulmonary disease and asthma, cystic fibrosis, gastrointestinal disease, hypercalciuria and hyperparathyroidism, and immobilization (122). Besides these secondary causes of osteoporosis, type II diabetes has also been related to increased aBMD independent of body weight in some (123-125), but not all (126,127) studies. Recent longitudinal studies have shown an increased risk of hip fractures associated with type II diabetes (128). In the Health, Aging, Body Composition (Health ABC) study, both Caucasian and African American men and women with type II diabetes had a significantly higher aBMD at the femoral neck and total hip compared with those with normal glucose homeostasis. However, association between diabetes and bone loss was only observed in women, but not in men (129).

## **2.5 VOLUMETRIC BMD IN MEN**

### **2.5.1 Assessment of vBMD**

Quantitative computed tomography (QCT) is an established technique for measuring BMD in the axial spine and peripheral skeleton. In contrast to DXA measures of aBMD,



QCT provides a three-dimensional measure of volumetric that is not confounded by differences in bone size. In addition, QCT has the ability to distinguish trabecular and cortical bone. Compared with DXA, QCT is more sensitive to detecting bone loss because it selectively measures changes in the more metabolically active trabecular bone compartment.

Peripheral QCT (pQCT) scanners have been employed for measuring the bone mineral content and volumetric density of the appendicular skeleton in pediatrics because of the lower radiation exposure than QCT. It also provides different skeletal quality measures besides density for trabecular and cortical bone. The ultra-distal and shaft regions of the radius and tibia are the common scanning sites for pQCT due to the different amounts of trabecular and cortical bone in these regions. The ultra-distal scanning site contains up to 80% of trabecular bone and is used for the examination of the trabecular compartment. The shaft region contains approximately 90% of cortical bone. The bone mineral content remains fairly constant between 10% to 90% of the radius length, making this site preferable for measuring cortical properties (130).

### **2.5.2 Results from cross-sectional and longitudinal studies**

Population studies of vBMD have been predominantly conducted in women. There are few studies in men and most of them have focused on young males. There are also limited data on the epidemiology of vBMD in men of African heritage.

### **2.5.2.1 Cortical vBMD**

Greater cortical bone density in Caucasian men compared with women is observed in several cross-sectional studies (131-133). However, Riggs et al. have recently found that young women aged 20 to 29 had about 10% higher cortical vBMD at the femoral neck, radius and tibia than men at the same age (134). This association was reversed when comparing men and women aged 70 to 97 years old (135). A study by Sigurdsson et al. also reported similar cortical vBMD between elderly men and women (136).

Age-related decrease in cortical vBMD occurs in both genders. In Caucasian men, cortical vBMD at the femoral neck, radius and tibia decreased with age (131,134). Studies reported that cortical vBMD decreased slowly over the entire life span in Italian men (131), but decreased significantly greater after middle age in American men (134). Very few studies have measured vBMD by QCT or pQCT among men of African descent. Taaffe et al. compared vBMD at the femoral shaft, predominantly cortical bone, and found no significant differences in vBMD between Caucasian and African American men aged 70-79 (133).

### **2.5.2.2 Trabecular vBMD**

Studies have shown a greater trabecular vBMD in Caucasian men than women at the tibia and radius (131,132,134) and hip(136). However, gender differences in trabecular vBMD at the lumbar spine have not been consistent (134,136,137). Therefore, the gender differences in trabecular vBMD may be site-dependent. The decrease in trabecular vBMD seems to occur in a linear fashion starting as early as 30 years of age for both men and women (131,134,138,139). There is a more profound decrease in trabecular vBMD with age at the central (50%) than peripheral (25%) skeletal sites (134).

### **2.5.3 Volumetric BMD and fracture**

Few studies have examined pQCT or QCT measures of vBMD and its relationship with fractures. For both men and women, those with vertebral fracture had a lower total, cortical and trabecular vBMD compared to controls (132). A study by Jamal et al. showed that every one SD decrease in cortical, but not trabecular, vBMD at the radius was associated with a 16-fold risk of fractures among 52 hemodialysis patients after adjusting for age, weight and sex (140). The relationship between vBMD and fractures in men of African descent has not been established.

#### **2.5.3.1 Correlates of volumetric BMD**

Unlike aBMD, there are very few studies on the correlates of vBMD in men. In some studies, a higher level of exercise was not associated with cortical vBMD (141-143). On the other hand, exercise seemed to be associated with higher trabecular vBMD (142). Several studies showed that physically active men have a higher trabecular vBMD than sedentary men (141,142,144). Grip strength was also found to be positively correlated to cortical and trabecular vBMD (132,145). Smoking is known to have a negative effect on aBMD; however, what we knew about effect of smoking on vBMD was limited. In the GOOD study, there was no difference in cortical vBMD between male smokers and nonsmokers(106), where Kaji et al. also found similar cortical and trabecular vBMD in male smokers compared with non-smokers (132).

## **2.6 GENETICS OF OSTEOPOROSIS**

### **2.6.1 Importance of genetics in osteoporosis**

We have known for several decades that genetic factors and their interaction with environmental factors have a major impact on bone mineral density and the risk of developing osteoporosis in men and women. Understanding the genetic effects on bone density may have a major impact on the diagnosis, treatment, and prevention of osteoporosis. Research has clearly confirmed a strong genetic contribution to BMD. However, the genes and allelic variants conferring susceptibility to this condition have only more recently begun to be identified.

### **2.6.2 Candidate gene studies of osteoporosis**

Several strategies are used to identify the genetic factors contributing to osteoporosis related phenotypes such as genome-wide linkage mapping in families, genome-wide linkage disequilibrium (LD) mapping, and candidate gene association studies. In brief, genome-wide linkage mapping follows the segregation of chromosomal regions marked by genetic variants in families in the search for regions of the genome that co-segregate with the disease or phenotype. This approach has great potential to identify genes or protein products with previously unknown function. For example, low density lipoprotein receptor-related protein 5 (LRP5), a co-receptor for the wntless-type family of growth factor, was shown to segregate with a high aBMD phenotype and its function on bone metabolism was unknown until its discovery using the genome-wide linkage mapping

approach. Genome-wide LD mapping with single nucleotide polymorphisms (SNPs) in unrelated individuals has also been proposed to localize osteoporosis susceptibility genes. This approach tests for differences in allele frequencies between cases and controls or tests for differences in mean values of bone related phenotypes (e.g., BMD) across genotypes on a genome-wide basis. Candidate gene association studies rely on traditional epidemiologic study designs to identify the relationship between alleles and/or genotypes and phenotype. Using this approach, candidate genes are identified first according to their established biological role in bone metabolism. Then, epidemiologic methods are used to test whether the genotype(s) is/are associated with phenotypes. Although several candidate genes for osteoporosis have been investigated to date, studies often yielded inconsistent or inconclusive results due to differences in the populations being studied or study design. The most widely studied candidate genes have been the vitamin D receptor (VDR), estrogen receptor alpha (ESR1), type I collagen (COL1A1), and Transforming Growth Factor- $\beta$  (TFG-  $\beta$ ) genes (70).

Most of the genetic studies of osteoporosis in humans have examined DXA measures of aBMD. However, studies have shown that vBMD measured by QCT and pQCT also has a high heritability. Lenchik et al. found that the heritability for vBMD at the lumbar spine was 73% (146). In the Tobago Family Study, the estimated heritability of cortical vBMD was 29% at the radius and 42% at the tibia, whereas the heritability of trabecular vBMD was 70% at both the tibia and radius. In this study, the heritability of trabecular vBMD was also higher than the heritability observed in aBMD at the whole body, lumbar spine, total hip and femoral neck(56).

Some recent candidate gene association studies have examined vBMD by pQCT as the phenotype. For example, Lorentzon and colleagues examined the association of polymorphisms in aromatase (CYP19), the key enzyme in the conversion of testosterone to estradiol, with both aBMD and vBMD in young adult males aged 18 to 20. They reported that a CYP19 polymorphism was independently associated with aBMD of the radius, lumbar spine, and total body. Trabecular vBMD was not associated with *CYP19* polymorphisms at the radius and tibia, whereas cortical vBMD was significantly associated with CYP19 polymorphisms at the radius only (42). The same research team also investigated the association of a functional polymorphism in the catechol-O-methyltransferase (COMT) gene, involved in the degradation of estrogens, and vBMD in young adult males (147). This polymorphism was found to associated with trabecular and cortical vBMD at the radius(147).

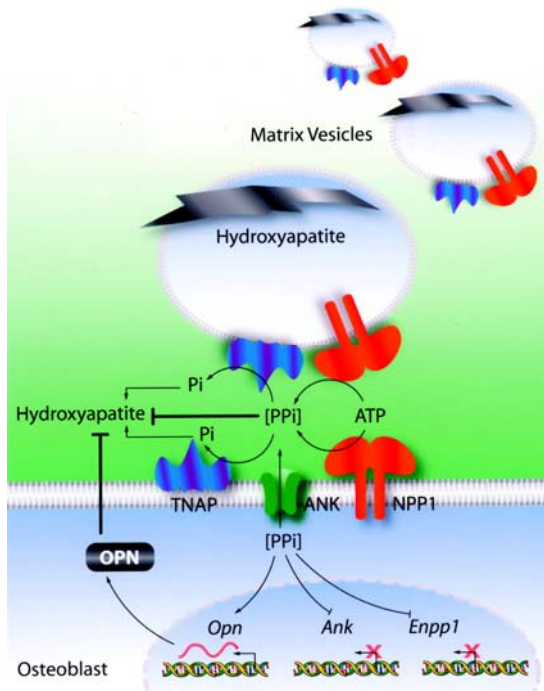
There have been few other candidate gene association studies of QCT measures of vBMD and very limited data on the genetic association of vBMD in men or women of African heritage. In the proposed dissertation project, we will focus our attention on a newly discovered candidate gene for vBMD: the ectonucleotide pyrophosphatase /phosphodiesterase 1 (ENPP1) gene (148).

### **2.6.3 Ectonucleotide pyrophosphatase / phosphodiesterase 1 (ENPP1)**

The transmembrane glycoprotein, ENPP1, also known as plasma-cell membrane glycoprotein 1 (PC-1), plays an important role in skeletal mineralization. The initial stages of mineralization begin in chondrocyte- and osteoblast-derived matrix vesicles (MVs) which contain calcium and inorganic phosphate ions (Pi). Crystals of

hydroxyapatite are formed within the MVs in growth plate cartilage and developing bone. Crystals of hydroxyapatite grow through the MV membrane to the extracellular environment. Exposure of the hydroxyapatite crystals to the extracellular milieu further enables growth and proliferation of the crystals. Inorganic pyrophosphate (PPi) inhibits the ability of Pi to crystallize with calcium to form hydroxyapatite and thereby suppresses hydroxyapatite deposition. Therefore, the balance between levels of Pi and PPi is crucial for normal mineralization to proceed (149).

The following figure showed the biological pathway of ENPP1 gene. Tissue-nonspecific alkaline phosphatase (TNAP), nucleotide pyrophosphatase phosphodiesterase 1 (NPP1) and multiple-pass transmembrane protein ANK are three important molecules identified as central regulators of extracellular PPi and Pi levels. TNAP, encoded by the *Akp2* gene, plays an important role in bone matrix mineralization by hydrolyzing PPi, whereas NPP1, encoded by ENPP1, regulates mineralization by generating PPi. The transmembrane protein, ANK, acts as a transport channel for PPi and therefore has a similar function to NPP1 in increasing extracellular PPi levels (149).



**Figure 2-1 Biological pathway of bone mineralization**

TNAP is an important promoter of mineralization because it catalyzes the hydrolysis of PPi thereby decreasing the concentration of this calcification inhibitor, while concomitantly increasing Pi levels. Mice with a deletion of the *Akp2* gene (*Akp<sup>-/-</sup>*) develop severe hypophosphatasia, a disease characterized by rickets, osteomalacia, spontaneous bone fracture and increased PPi levels. In contrast, NPP1 serves as a physiological inhibitor of calcification by generating PPi. In human infants, severe NPP1 deficiency was found to be associated with a syndrome of spontaneous infantile arterial and periarticular calcification (150,151). ENPP1 knockout mice show decreased levels of PPi and mineralization abnormalities that include osteoarthritis and ossification of the posterior longitudinal ligament of the spine. Studies by Suk et al found evidence for association between ENPP1 gene variation and osteoarthritis in Russian population (152).



Yerges et al. have recently identified that an intronic tagging SNP in ENPP1 is significantly associated with vBMD at the femoral shaft using QCT in older Caucasian men (148). We will test if tagging SNPs in ENPP1 are associated with DXA measures of aBMD, rates of decline in BMD, and pQCT measures of vBMD in men of African descent in the current project.

## 2.7 LIMITATIONS OF THE EXISTING LITERATURE

Osteoporosis studies have historically been conducted in Caucasian women due to the higher prevalence of this skeletal condition in this population group. However, in more recent years there has been an increased awareness of the importance of male osteoporosis. Longitudinal studies of bone loss with aging in men are still lacking, especially in regard to the risk factors for accelerated bone loss. Although bone density measured by DXA (areal BMD) has been widely used to diagnose osteoporosis, this measure is known to be confounded by bone size, where higher areal BMD may reflect bigger bone size, not necessarily higher bone density. Few population studies have used QCT or pQCT to characterize the correlates of volumetric BMD. In addition, there has been no study, to our knowledge, that thoroughly examines the potential anthropometric, medical, and lifestyle factors that may be associated with volumetric BMD. As with environmental factors, very few genetic association studies have assessed QCT or pQCT measures of bone related phenotypes.

Non-white populations have also been largely under-represented in epidemiological studies of osteoporosis, particularly in men. It is important to better understand and confirm the potential genetic and environmental factors that are associated with bone density and changes in bone density with aging using advanced techniques like pQCT in non-white populations.

## 2.8 SPECIFIC AIMS

The aim of *Research Article 1* was to better understand the age-related patterns and determinants of hip BMD changes (total hip and femoral neck) in men of African descent. We evaluated the magnitude of age-related changes in hip BMD across age and the effect of weight change on hip bone loss. We then assessed how changes in hip BMD correlate with demographic characteristics, anthropometric and body composition measures, lifestyle factors and medical history.

The aim of *Research Article 2* was to better understand the age-related patterns in trabecular and cortical vBMD at the radius and tibia among men of African heritage. We also sought to identify the correlates of vBMD, including demographic characteristics, anthropometric and body composition measures, lifestyle factors, dietary intake, medical history, and medication, related to trabecular and cortical vBMD at the radius and tibia, respectively.

The aim of *Research Article 3* was to examine single nucleotide polymorphism associations in the ENPP1 gene and BMD measurements, including rate of decline in aBMD and aBMD at the total hip and femoral neck, and trabecular and cortical vBMD at the radius and tibia, in men of African descent.

We used longitudinal and cross-sectional data from the Tobago Bone Health study to perform analyses for these three research articles. The Tobago Bone Health study is a large scale population study of Afro-Caribbean men aged 40 and older on the island of Tobago.

**3.0 DETERMINANTS OF BONE LOSS IN MEN OF AFRICAN ANCESTRY:  
THE TOBAGO BONE HEALTH STUDY**

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### 3.1 ABSTRACT

Very little is known about the magnitude, pattern and determinants of bone loss with advancing age among men, particularly among those of African descent. We examined the rate of decline in hip bone mineral density (BMD) and indentified potential factors associated with an accelerated loss of BMD among 1,691 men of African ancestry aged  $\geq 40$  years. BMD at the proximal femur was measured at study entry and after an average of 4.4 years by dual-energy X-ray absorptiometry. The overall rate of decline in femoral neck BMD was  $0.29 \pm 0.81\%/yr$  in the total sample ( $p < 0.0001$ ). However, a U-shaped relationship between advancing age and the rate of decline in BMD was observed. For example, the rate of decline at the femoral neck was  $-0.38 \pm 0.77\%/yr$  among men aged 40-44 years ( $P < 0.0001$ ), decelerated to  $-0.15 \pm 0.81\%/yr$  among men aged 50-54 yrs ( $P = 0.0026$ ) and then accelerated to  $-0.52 \pm 0.90\%/yr$  among those aged 75+ yrs ( $P < 0.0001$ ). Men who lost  $\geq 5\%$  of their initial body weight during follow-up had a significantly greater rate of decline in BMD than those who remained weight stable or gained weight ( $p < 0.0001$ ). The relationship between weight loss and bone loss was more pronounced among men who were older and leaner at study entry ( $P < 0.03$  for interactions). We also observed a strong impact of advanced prostate cancer and its treatment with androgen deprivation on the rate of decline in BMD. Men of African ancestry experience substantial bone loss with advancing age that appears to be comparable to the rate of loss among Caucasian men in other longitudinal studies.

Additional studies are needed to better define the natural history and factors underlying bone loss with aging in men of African ancestry.

### **3.2 INTRODUCTION**

Although osteoporosis is more prevalent among women than men, men also experience substantial bone loss and an increase in fracture incidence with advancing age. However, little information exists about the natural history, magnitude and correlates of bone loss with aging among men, especially among men of African ancestry. Osteoporosis is a global public health problem and as the population ages more men throughout the world will develop osteoporosis and its associated fractures, including men of African ancestry(1). The lower prevalence of osteoporosis in men of African ancestry compared with other ethnic and racial groups has led to the belief that osteoporosis represents less of a problem in this population. However, the African ancestry population is expected to comprise a growing proportion of the incidence and economic burden of osteoporosis-related fractures over the next 20-50 years in both the U.S. (2) and world-wide (1). These demographic trends underscore the importance of better understanding the natural history and determinants of bone loss and osteoporosis in men of all ages and racial/ethnic background.

Most longitudinal studies of bone mineral density (BMD) changes with aging in men have been conducted among Caucasian men in North America (3-8), Europe (9-11) and Australia (12). These studies have identified body weight and changes in body weight as the major correlates of bone loss with aging in men. Smoking has also been

reported as a potential risk factor for bone loss in some studies (3,4,7,13,14), whereas other lifestyle factors such as alcohol consumption, calcium intake and physical activity have been inconsistently related to bone loss (3,4,7,12). To our knowledge, only a single study has characterized the magnitude and correlates of bone loss in men of African descent (14). In this study, older age, lower initial body weight and smoking were correlated with a greater decline in BMD among 119 African American men aged  $\geq 65$  years. The aim of the present study was to examine further the magnitude, age-related patterns and correlates of the decline in hip BMD with aging in a large cohort of middle-aged and elderly men of African ancestry.

### **3.3 METHODS**

#### **3.3.1 Study population**

The population-based Tobago Bone Health Study was first conducted on the Caribbean island of Tobago in 2000 as described previously (15,16). In brief, recruitment was accomplished by word of mouth, hospital flyers and radio broadcasting. To be eligible, men had to be 40 years and older, ambulatory and not terminally ill. Questionnaires were administered to obtain information on demographic characteristics, occupation, medical history, and lifestyle related factors. A total of 2,652 men completed an initial dual-energy x-ray absorptiometry (DXA) scan for assessment of BMD and body composition at that visit. The self-reported ethnicity of the cohort is 97% African, 2% East Indian, <1% white, and <1% "other".

In late 2004, participants were re-contacted for a second DXA scan to assess the rate of change in BMD. A total of 1,748 men (70% of survivors) returned for the follow-up exam. We excluded 36 men who identified themselves with ethnicity other than Afro-Caribbean and 21 men who had incomplete data. We also subsequently excluded 233 men with prostate cancer and/or history of androgen deprivation therapy for prostate cancer from analysis because of their strong influence on BMD in this population. The final study sample for the present analysis was 1,458 men. The Institutional Review Boards of the University of Pittsburgh and the Tobago Ministry of Health and Social Services approved this study and all participants provided written informed consent before data collection.

### **3.3.2 Densitometry**

BMD was measured at the proximal femur and sub-regions at both the baseline and follow-up visits using a single Hologic QDR 4500W densitometer (Hologic, Inc., Bedford, MA). The left hip was scanned unless the participant had a fracture or a total hip replacement. Trained and certified technicians performed the DXA scans and followed a strict protocol for both visits. Longitudinal machine stability was assessed from plots of daily spine phantom scans, and reviewed monthly. A weekly print out of QC plots was generated to detect short-term inconsistencies and long-term drift. The scanner was stable throughout the course of the study.



### **3.3.3 Anthropometric and body composition assessments**

Body weight was measured in kilograms with participants wearing light clothing and without shoes, using a calibrated balance beam scale at both visits. Height was measured in centimeters without shoes, using a wall-mounted height board. Whole body fat and lean mass were also measured using DXA. Left and right grip strength was measured with a hand-grip dynamometer as a surrogate for upper body and overall strength (Preston Grip Dynamometer, JA Preston 136 Crop.). Average grip strength was based on two repeated measurements from left and right hands.

### **3.3.4 Other measurements**

Questionnaires were administered by trained interviewers and nurses to obtain information on demographic characteristics, lifestyle factors as well as medical history. In the current analysis, we used information from the baseline exam to assess potential factors related to the subsequent rate of decline in BMD. Mixed African ancestry was defined by self-report of one to three African-descent grandparents. Other factors that were assessed included history of cigarette smoking, alcohol consumption, time spent watching television, time spent walking, medical history of fracture, hypertension, coronary heart disease (CHD), stroke, diabetes, chronic bronchitis, and arthritis. We also asked participants to rate their overall health status compared to others of their age. Diagnosis of prostate cancer, advanced prostate cancer (prostate specific antigen >40 or Gleason score >7), and androgen deprivation therapy (ADT, use of Leuprorelin or orchiectomy) were also recorded at baseline.

### 3.3.5 Statistical Analysis

The annualized rate of change in BMD during follow-up was calculated as the percent BMD change from baseline to follow-up divided by duration in years between the two scans. Percent change in body weight or body composition was calculated as the difference between baseline and follow-up measures divided by baseline measures and multiplied by 100. We also categorized percent weight change into 3 groups: weight gain (>5% weight gain), weight stable (weight change between -5% and 5%) and weight loss (>5% weight loss).

We first compared the baseline characteristics between men who did and did not return for the follow-up visit, regardless of their race and ethnicity, using analysis of covariance (ANCOVA) for continuous variables with age adjustment and Chi-square tests for dichotomous variables. Analysis of variance (ANOVA) and ANCOVA were used to evaluate the annualized percentage rate of change in BMD across different age categories (e.g. 5-year age groups or greater than 55 years) and across weight change groups. We evaluated the age-adjusted and age- and weight-adjusted contribution of each individual variable to the annualized rate of change in BMD by using linear regression analysis. The strength of the association is expressed as an absolute difference in units of change chosen to approximate one standard deviation (SD) in the distribution for each continuous variable or null category for dichotomous variables. The formula used to calculate the absolute difference in rate of change in BMD per unit change (SD) of the independent variable was:  $(\beta) = ((\text{unstandardized } \beta \times \text{unit change in independent variable}))$ . The corresponding 95% confidence intervals were calculated using the following formula:  $((\beta \times \pm 1.96 \times \text{standard error}) \times \text{unit change})$ . We also evaluated the

interactions between weight change and age as well weight change and BMI on the rate of BMD decline using ANCOVA.

Multiple linear regression analysis was performed using a stepwise procedure to determine the potential independent correlates of the annualized rate of change in BMD. Variables from the age- and weight- adjusted univariate model with a P value <0.10 were further entered in the multiple linear regression model. Age was forced into all models. We assessed multi-collinearity by inspecting the variance inflation factor (VIF). Due to the high correlation of body weight with lean and fat mass, we developed two different multiple linear regression models: 1) models with body weight only; and 2) models substituting fat and lean mass for body weight. Statistical analyses were performed using the Statistical Analysis System (SAS, version 9.1; SAS Institute, Cary, NC).

### **3.4 RESULTS**

Table 1 shows the baseline characteristics of men who did and did not attend the follow-up exam. As expected, men who did not return were older, weighed less, and had lower baseline BMI than those who participated in the follow-up exam. Non-participants also were more likely to smoke, report poorer health status and have a greater prevalence of hypertension, heart disease, diabetes and arthritis. Non-participants also had significantly lower hip BMD than participants at the baseline exam.

### **3.4.1 Rate of change in BMD and androgen deprivation**

The average length of time between DXA scans was  $4.4 \pm 0.8$  years (range, 1.1 to 6.9 years). Men with advanced prostate cancer and men who had a history of androgen deprivation therapy for prostate cancer had a significantly greater rate of decline in BMD compared to their counterparts. For example, the average rate of decline in BMD was  $0.137 \pm 0.588$  %/yr at the total hip and  $0.333 \pm 0.841$  %/yr at the femoral neck (data not shown) for all men. The rate of decline in BMD was 0.78%/yr greater at the total hip and 0.58%/yr greater at the femoral neck for men with advanced prostate cancer (prevalence, 2%) compared to men without prostate cancer (both,  $p < 0.0001$ ). Men with non-advanced prostate cancer (prevalence, 11%) had a 0.08% greater ( $p = 0.08$ ) annualized rate of decline in BMD at the total hip and 0.15% greater ( $p = 0.02$ ) at the femoral neck, compared to men without prostate cancer. Approximately 6% of the study population reported that they had either taken Leuprorelin or underwent orchiectomy for prostate cancer. Men on either of these treatments experienced an approximately 0.4%/yr greater ( $p < 0.0001$ ) rate of decline in total hip and femoral neck BMD compared to men who had neither of these treatments. Because prostate cancer and its treatment by androgen deprivation had a strong impact on the rate of decline in BMD, we excluded the 233 men with prostate cancer or who had undergone androgen deprivation from subsequent analyses.

### **3.4.2 Rate of change in BMD and age group**

Total hip and femoral neck BMD declined by  $0.10 \pm 0.55\%/yr$  and  $0.29 \pm 0.81\%/yr$  among the men who did not have prostate cancer or who had undergone ADT ( $p < 0.0001$  for both). To examine the age-related patterns in the rate of decline in BMD among these men, we stratified the total sample by 5 year age groups using age at study entry (Figure 1). The rate of decline in BMD across age groups appeared to have a U-shape relationship. Men aged 40-44 years had a significantly greater rate of decline in BMD than those aged 45-49 years and 50-54 years at both the total hip and femoral neck. Thereafter, the rate of decline in BMD accelerated with advancing age. For example, the rate of decline in total hip BMD was  $-0.10 \pm 0.55\%/yr$  among men aged 55-59 years ( $P = 0.009$ ) and increased to  $-0.48 \pm 0.60\%/yr$  among men aged 70+ years ( $P < 0.0001$ ). Similar results were observed at the femoral neck.

### **3.4.3 Age-adjusted correlates of the rate of change in BMD**

Table 2 shows the age-adjusted association of participant characteristics with the rate of decline in BMD at the total hip and femoral neck. Age was inversely and significantly correlated with the decline in BMD. For example, the rate of decline in total hip BMD increased by  $0.084\%/yr$  every 10 years.

A 10 kg increase in body weight was associated with a slower rate of decline in total hip BMD. Body composition measures from DXA were significantly associated with the rate of decline in hip BMD. Whole body fat, lean mass and percent body fat

were all positively and significantly associated with the decline in hip BMD in age-adjusted analysis. Initial BMD was not related to the subsequent rate of decline in BMD.

We also evaluated the relationship between annualized percent rate of decline in BMD and change in body weight and body composition over the follow-up period. Men gained an average of  $0.2 \pm 6.2\%$  body weight during follow-up ( $P=0.20$ ). As expected, fat mass increased ( $P<.0001$ ) whereas lean mass decreased non-significantly ( $P=0.25$ ) during the follow-up period. In age-adjusted analysis, men who lost weight during follow-up had a significantly greater mean rate of decline in BMD. For example, every 6% decrease in body weight from baseline was associated with a 0.11%/yr decrease in total hip and a 0.14%/yr decrease in femoral neck BMD. Similar associations were observed for the change in fat mass and lean mass.

None of the lifestyle related characteristics examined were significantly associated with the annualized rate of decline in BMD including current and past smoking history, time spent watching television, and alcohol intake. Diabetes was present in about 11% of the cohort and was associated with a greater decline in total hip, but not femoral neck, BMD in age-adjusted analysis. Because there appeared to be a u-shaped relationship between age and the rate of decline in BMD, we also examined the correlates of bone loss in stratified analyses among men aged 40-54 and 55+ and found similar results as those in the total cohort (data not shown).

We further evaluated the association of age, BMI, weight change and the interactions of these variables with the rate of decline in BMD in stratified analyses (Tables 3 and 4). At both the total hip and femoral neck, men who lost 5% or more of their baseline body weight had a significantly greater decline in BMD compared to men

who had remained weight stable or who gained 5% or more body weight. There was also a significant loss of BMD among men who remained weight stable during follow-up. However, men who gained at least 5% body weight during follow-up did not experience a significant decline in hip BMD.

We also found a significant interaction effect of age at study entry and weight change on the rate of decline in hip BMD (Table 3). The effect of weight loss on the decline in BMD was significantly greater (total hip,  $p=0.006$ ; femoral neck,  $p=0.018$ ) among older (age  $\geq 55$  yrs) than younger (age  $<55$  yrs) men. Finally, we also observed a significant interaction effect of initial BMI and subsequent weight change on the rate of decline in total hip BMD (Table 4). Although weight gain was associated with an overall slower rate of decline in femoral neck BMD, this effect was not apparent among the leanest men. Men with a low initial BMI (BMI  $< 25.0$  kg/m<sup>2</sup>) experienced a more pronounced rate of decline in BMD even if they had gained weight during follow-up.

#### **3.4.4 Multiple Linear Regression**

The results from multiple linear regression analyses of the independent correlates of the rate of decline in decline are shown in Table 5. Age(-), BMI(+), weight change(+), and grip strength(+) were significant correlates of the rate of decline in BMD in multiple regression analysis. Diabetes was no longer a statistically significant correlate of the rate of decline in total hip BMD in the multivariable model. Multivariate models only explained 5-6% of the variance in the rate of decline in BMD.

### 3.5 DISCUSSION

Compared with women, much less is known about the magnitude and correlates of bone loss with aging in men, especially among non-white men. To our knowledge, only a single longitudinal study to date has evaluated age-related bone loss in men of African ancestry and that study only included 119 men aged 65 and older (14). Thus, one aim of the current study was to examine the pattern of BMD loss at the proximal femur over 4 years in a large population sample of middle-aged and elderly men of African ancestry. A primary finding of our study is that BMD loss at the proximal femur is substantial and may begin early in life among men of African ancestry. This early decline in BMD may reflect, in part, an early loss of trabecular bone mass and a later loss of cortical bone mass from the proximal femur. Indeed, others have recently observed a loss of trabecular BMD well before middle age among men (17,18). However, the pattern of BMD loss with advancing age in our study appeared to be non-linear with a deceleration in the rate of loss between ages 40-54 and an acceleration in the rate of loss thereafter that continued unabated into the 7<sup>th</sup> decade of life. A progressive acceleration of BMD loss with advancing age has also been observed among Caucasian men(3,13). Past epidemiologic studies of the decline in BMD have largely focused on Caucasian men aged  $\geq 65$  years and may have thus missed this early decline in hip BMD.

In our study of Afro-Caribbean men aged 40-92 years, the overall unadjusted rate of decline in BMD at the femoral neck was  $-0.29 \pm 0.81$  %/yr and was  $-0.47 \pm 0.89$  %/yr among men aged 65 years and older. These rates of decline in BMD were surprisingly very similar to the rates of BMD decline reported in other longitudinal studies of Caucasian men(3-5,7,9). For example, in the Framingham Osteoporosis Study, the rate of



decline in femoral neck BMD was  $-0.38\%/yr$  among 278 Caucasian American men aged 67-90(4). In the Rotterdam Study, the rate of decline in femoral neck BMD was  $-0.4\%/yr$  in 1856 Caucasian European men aged  $>55$  yrs(3). In the Rancho Bernardo study, the rate of bone loss at the femoral neck was  $-0.34\%/yr$  in 500 Caucasian American men aged 45-92 yrs(7). In the Network in Europe for Male Osteoporosis, the rate of decline in BMD at the femoral neck was  $-0.48\%/yr$  among  $\sim 1300$  Caucasian European men aged 50-80 yrs(11). Finally, in the Baltimore Men's Osteoporosis Study, the rate of decline in femoral neck BMD was  $-2.1\%/yr$  in 349 Caucasian American and  $-1.1\%/yr$  in 119 African American men aged 60-74 yrs(14). The high rate of BMD loss in this study may reflect the use of different densitometers at the initial and follow-up exams. Comparisons across these studies are difficult to make and should be interpreted cautiously due to the differences in follow-up time, densitometers used, sample sizes, geographic areas, and age distributions of the populations studied. Nonetheless, the rate of decline in femoral neck BMD with aging in our cohort of Afro-Caribbean men is very consistent with the majority of these other studies of Caucasian men.

To better understand the factors that might influence the rate of BMD loss with age in men of African ancestry, we characterized a number of anthropometric, lifestyle and medical variables and examined the relation of these variables to the rate of BMD loss at the proximal femur. In addition to advanced age, leanness at study entry and weight loss during follow-up were particularly important independent correlates of an increased rate of BMD loss with age. A positive association between body weight or BMI and BMD among middle-aged and elderly men has been well-documented in Caucasians and African Americans(19-24). Weight loss with aging is also a consistent predictor of

the rate of loss in BMD (4,6-8,25). In the present study, men who lost 5% or more of their baseline body weight had an accelerated rate of decline in BMD compared to men who remained weight stable or gained weight. The effect of weight loss on the age-related decline in BMD was more profound among men aged 55 and older in our study. In the Framingham, Rancho Bernardo, Osteoporotic Fractures in Men (MrOS) and EPIC studies, Caucasian men who lost 5% or more of their baseline weight also had a significantly greater rate of BMD loss at the proximal femur than those who gained 5% or more of their baseline weight(4,6-8). The importance of weight loss as a risk factor for accelerated loss of BMD among middle-aged and elderly men may be explained by underlying illness that results in poor health and physical inactivity(8,25), to declines in muscle mass and strength, to decreased mechanical loading on weight-bearing skeletal sites (26,27), to a decrease in adipose tissue mass which is an important source of estrogens in men(28), or to a combination of these factors.

Other frequently examined predictors of the age-related loss of BMD such as physical activity, smoking, calcium intake and medical conditions have yielded inconsistent results across studies(3,4,7,12). We also examined several of these variables that might affect the rate of decline in BMD with aging. Greater grip strength was associated with a slower loss of BMD, even after adjusting for age, body weight and weight change. The association with grip strength may be explained, in part, by increased physical activity and lean mass.

We were unable to document an association between smoking and the rate of BMD loss in our study. Some(22,29-31), but not all(19,32,33), studies suggest that smokers have lower BMD than non-smokers in cross-sectional analyses. In Caucasian

men, smokers had a greater decline in hip BMD with age compared to non-smokers(3,4,7,25). We were also unable to document an association between alcohol intake and the rate of decline in BMD. Moderate alcohol consumption has been associated with greater BMD in several(22,23,34), but not all(13,19), cross-sectional studies of middle-aged and elderly Caucasian men. Alcohol consumption was associated with a slower rate of age-related decline in BMD in some(7,35), but not all(4,25) longitudinal studies. The absence of a significant relationship with smoking and alcohol drinking in the current study may be due to a low prevalence of these behaviors.

Two of the strongest contributors to the rate of decline in BMD in our study were advanced prostate cancer and ADT for prostate cancer. The association was independent of other covariates including body weight and weight change during follow-up. Androgens increase bone formation and decrease bone resorption and ADT may disrupt this balance resulting in bone loss(36). ADT has been associated with a higher prevalence of osteoporosis among prostate cancer patients(37). Most of the longitudinal population-based studies in men did not examine the relationship between prostate cancer and changes in BMD with aging. Prostate cancer and its treatment might be particularly important risk factors for bone loss in men of African descent given its higher prevalence in men of African compared with Caucasian descent.

Our study has several limitations. The small proportion of older-aged men may have limited our ability to estimate the rate of bone loss in these men. Although walking is a common form of physical activity in this population, our questionnaire estimates of walking hours might not have been an accurate reflection of total physical activity levels. In addition, our DXA measures of areal BMD cannot provide insight on age-related loss

of trabecular and cortical bone mass or bone geometry. Three dimensional measures of trabecular and cortical volumetric BMD and bone structure would provide important insight on the aging skeleton in this population.

In conclusion, although the prevalence of osteoporosis is higher among Caucasian than African ancestry men, men of African ancestry appear to experience a substantial loss of BMD with aging that may be comparable to the rate of loss in Caucasian men. Advancing age, lower body weight, increased weight loss, advanced prostate cancer and its treatment by androgen deprivation were identified as potential risk factors for accelerated loss of BMD in our study. However, these factors only explained 6% to 7% of the variation in the rate of decline in BMD with aging. These findings suggest that many other undetermined variables, including inherited factors, may contribute to age-related loss of BMD in men of African ancestry. A more complete understanding of BMD changes with aging in men of African ancestry will require a broader examination of the potential determinants.

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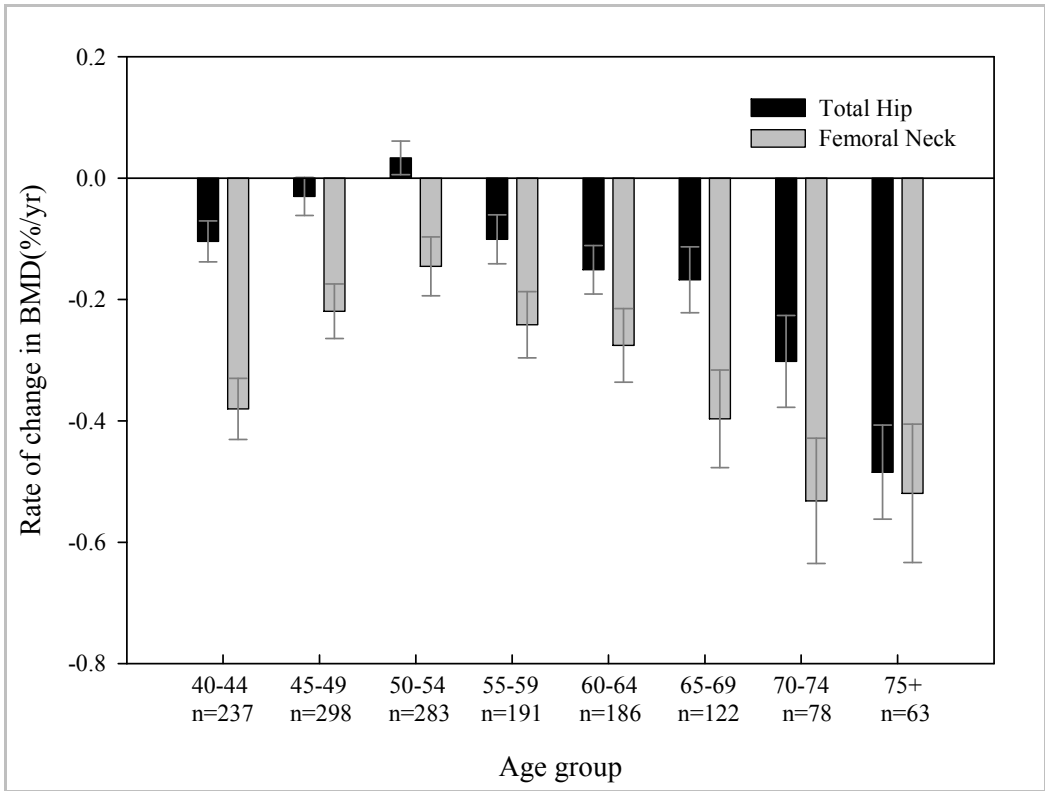


**Table 3-1 Comparison of selected baseline characteristics among men who participated and did not participate in the follow-up exam**

	Participants (n=1748)	Non-participants (n=904)	P-value
Age (yrs)	56.4±10.5	59.1±12.0	<.0001
Height (cm)	174.8±6.6	174.6±6.7	.3810
Weight (kg)	83.6±14.2	81.2±14.6	<.0001
BMI (kg/m <sup>2</sup> )	27.3±4.1	26.5±4.2	<.0001
Grip strength (kg)	43.0±9.8	40.6±11.1	<.0001
Whole body fat (%)	20.1±5.8	19.9±6.2	.5877
Total hip BMD (g/cm <sup>2</sup> )*	1.150±.147	1.123±.155	<.0001
Femoral neck BMD (g/cm <sup>2</sup> )*	0.998±.147	0.961±.150	<.0001
Worse health compared to 12 months ago (%)	12.1	18.0	<.0001
Worse health compared to men with the same age (%)	2.5	5.5	.0001
Ever Smoker (%)	38.2	48.8	<.0001
Current smoker (%)	11.6	18.8	<.0001
Drank alcohol > 4 times a week	16.2	18.8	.0975
Fractured bone (%)	19.8	20.8	.5385
Hypertension (%)	28.9	34.2	.0052
Heart Disease (%)	3.4	5.9	.0026
Diabetes (%)	11.2	14.8	.0084
Arthritis (%)	11.7	15.5	.0064
Prostate cancer (%)	13.8	14.2	.7633
ADT treatment (%)	5.7	1.7	<.0001

\*Age-adjusted

ADT, androgen deprivation treatment



**Figure 3-1 Annualized rate of change in hip BMD by age group**

\* Group-specific rate of change in BMD was not significantly different from zero. Rates of change in BMD were significantly different from zero for all other groups. P-values for overall association with age was <0.0001 for total hip and 0.0002 for femoral neck. Men with prostate cancer or who had undergone androgen deprivation therapy were excluded from the analysis.

**Table 3-2 Correlates of the rate of change in hip BMD\***

Variables (baseline)	Mean ± SD or prevalence (%)	Unit	Age-adjusted rate of change in BMD (95%CI)	
			Total Hip	Femoral Neck
<i>Demographic characteristics</i>				
Age (years)	54.8±10.0	10	<b>-0.084 (-0.112, -0.055)</b>	<b>-0.053 (-0.095, -0.011)</b>
Mixed African ancestry	11.2%	Yes	0.037 (-0.052, 0.126)	0.105 (-0.028, 0.238)
<i>Occupational history</i>				
Ever work on farm	51.1%	Yes	-0.009 (-0.065, 0.048)	-0.023 (-0.107, 0.061)
Ever work on fishing boat	17.0%	Yes	-0.012 (-0.087, 0.062)	-0.061 (-0.172, 0.051)
<i>Anthropometric &amp; DXA measures</i>				
Body weight (kg)	84.0±14.2	10	<b>0.040 (0.020, 0.059)</b>	<b>0.065 (0.035, 0.094)</b>
BMI	27.3±4.1	4.1	<b>0.057 (0.029, 0.085)</b>	<b>0.094 (0.052, 0.137)</b>
Height	175.1±6.5	6.5	<b>0.029 (0.000, 0.058)</b>	0.032 (-0.011, 0.075)
Grip strength	44.0±9.6	9.6	0.030 (-0.003, 0.063)	<b>0.074 (0.025, 0.124)</b>
Total hip BMD (g/cm <sup>2</sup> )	1.16±0.14	0.14	0.004 (-0.024, 0.032)	-
Femoral neck BMD (g/cm <sup>2</sup> )	1.00±0.15	0.15	-	0.008 (-0.037, 0.052)
Fat mass (kg)	16.3±6.5	6.5	<b>0.069 (0.041, 0.097)</b>	<b>0.101 (0.059, 0.143)</b>
Lean mass (kg)	64.9±8.5	8.5	<b>0.034 (0.005, 0.064)</b>	<b>0.064 (0.021, 0.108)</b>
Body fat (%)	19.7±5.7	5.7	<b>0.079 (0.051, 0.108)</b>	<b>0.104 (0.061, 0.146)</b>
Weight change (%)	0.2±6.2	6.2	<b>0.108 (0.078, 0.137)</b>	<b>0.158 (0.114, 0.201)</b>
Fat mass change (%)	6.4±18.1	18.1	<b>0.062 (0.033, 0.091)</b>	<b>0.101 (0.059, 0.143)</b>
Lean mass change (%)	-0.1±3.6	3.6	<b>0.132 (0.103, 0.161)</b>	<b>0.136 (0.092, 0.180)</b>
<i>Lifestyle characteristics</i>				
Ever smoker	38.5%	Yes	0.009 (-0.049, 0.066)	0.029 (-0.057, 0.115)
Current smoker	12.0%	Yes	-0.064 (-0.150, 0.023)	-0.091 (-0.220, 0.038)
TV watching ≥21 hrs per week	31.1%	Yes	0.036 (-0.025, 0.097)	0.027 (-0.063, 0.118)
Drink alcohol ≥4 times per week	15.9%	Yes	0.001 (-0.078, 0.080)	0.026 (-0.093, 0.144)
<i>Medical History</i>				
Ever fractured bone	20.2%	Yes	-0.015 (-0.085, 0.055)	-0.007 (-0.111, 0.098)
Hypertension	27.6%	Yes	0.037 (-0.027, 0.102)	0.065 (-0.031, 0.161)
Diabetes	10.7%	Yes	<b>-0.102 (-0.195, -0.008)</b>	-0.028 (-0.167, 0.111)
Arthritis	10.6%	Yes	0.015 (-0.080, 0.111)	0.053 (-0.089, 0.195)

\*Expressed as absolute difference in annualized rate of change in BMD change per unit of the predictor variable. Men with prostate cancer or who had undergone androgen deprivation therapy were excluded from the analysis

Bold: p-value<0.05

**Table 3-3 Mean annualized rate of change in hip BMD by category of percent weight change and Age**

Category of weight change	Mean annualized % change in hip BMD(95%CI)*		
	Overall cohort	Age	
		Age 40-54	Age 55+
Total hip		n=768	n=590
Weight loss(n=216)	-0.340(-0.413, -0.267)	-0.204(-0.325, -0.085)	-0.418(-0.509, -0.326)
Stable weight(n=878)	-0.062(-0.097, -0.026)	-0.030(-0.077, 0.016)	-0.106(-0.162, -0.051)
Weight gain(n=274)	0.003(-0.031, 0.067)	0.070(-0.009, 0.149)	-0.122(-0.230, -0.015)
Femoral neck**		n=765	n=596
Weight loss(n=220)	-0.609(-0.716, -0.502)	-0.437(-0.615, -0.259)	-0.705(-0.839, -0.572)
Stable weight(n=874)	-0.252(-0.305, -0.199)	-0.273(-0.342, -0.204)	-0.222(-0.304, -0.141)
Weight gain(n=273)	-0.088(-0.182, 0.007)	-0.048(-0.165, 0.069)	-0.161(-0.321, -0.002)

Weight loss was defined as a loss of 5% or more of body weight from the baseline exam.

Weight gain was defined as a gain of 5% or more of body weight from the baseline exam.

\* Adjusted for BMI

\*\*The interaction term between weight change and age categories was significant for femoral neck only (p=0.03). Men with prostate cancer or who had undergone androgen deprivation therapy were excluded from the analysis.

**Table 3-4 Mean annualized rate of change in hip BMD by category of percent weight change and BMI**

Category of weight change	Mean annualized % change in hip BMD(95%CI)*			
	Overall cohort	BMI		
		BMI<25kg/m <sup>2</sup>	BMI 25.0-29.9 kg/m <sup>2</sup>	BMI≥30kg/m <sup>2</sup>
Total hip**		n=385	n=655	n=318
Weight loss(n=210)	-0.308(-0.381, -0.235)	-0.279(-0.447, -0.112)	-0.289(-0.393, -0.186)	-0.327(-0.458, -0.196)
Stable weight(n=874)	-0.066(-0.102, -0.030)	-0.186(-0.253, -0.118)	-0.043(-0.093, 0.007)	0.037(-0.039, 0.113)
Weight gain(n=274)	-0.014(-0.078, 0.051)	-0.027(-0.132, 0.078)	-0.091(-0.190, 0.008)	0.140(0.004, 0.276)
Femoral neck		n=390	n=657	n=314
Weight loss(n=214)	-0.585(-0.692, -0.478)	-0.598(-0.841, -0.355)	-0.663(-0.816, -0.510)	-0.436(-0.630, -0.242)
Stable weight(n=874)	-0.252(-0.305, -0.199)	-0.381(-0.481, -0.282)	-0.228(-0.303, -0.154)	-0.146(-0.260, -0.032)
Weight gain(n=273)	-0.106(-0.201, -0.011)	-0.271(-0.427, -0.113)	-0.049(-0.196, 0.099)	0.079(-0.125, 0.283)

Weight loss was defined as a loss of 5% or more of body weight from the baseline exam.

Weight gain was defined as a gain of 5% or more of body weight from the baseline exam.

\* Adjusted for age

\*\*The interaction term between weight change and BMI categories was significant for total hip only (p=0.02). Men with prostate cancer or who had undergone androgen deprivation therapy were excluded from the analysis.

**Table 3-5 Multivariable correlates of the annualized rate of change in BMD in older Afro-Caribbean men**

Variable	Unit	Rate of change in BMD (95%CI) per unit	
		Total Hip <sup>1</sup>	Femoral Neck <sup>2</sup>
		(n=1358)	(n=1370)
Age (years) <sup>3</sup>	10	<b>-0.056 (-0.0856, -0.027)</b>	0.026 (-0.025, 0.078)
BMI (kg/cm <sup>2</sup> )	4.1	<b>0.073 (0.043, 0.102)</b>	<b>0.101 (0.057, 0.146)</b>
Weight change (%)	6.2	<b>0.112 (0.082, 0.142)</b>	<b>0.167 (0.123, 0.211)</b>
Grip strength (kg)	9.6	-	<b>0.055 (0.004, 0.106)</b>
Model R <sup>2</sup>		0.07	0.06

<sup>1</sup>Age, BMI, weight change, grip strength, and diabetes were entered into the model

<sup>2</sup>Age, BMI, weight change and grip strength were entered into the model

<sup>3</sup>Age was forced into the model

Bold: p-value less than 0.05

Men with prostate cancer or who had undergone androgen deprivation therapy were excluded from the analysis.

- : variable was entered into the model but not a significant in the final model

#### **4.0 DETERMINANTS OF TRABECULAR AND CORTICAL VOLUMETRIC BONE MINERAL DENSITY IN MEN OF AFRICAN HERITAGE**

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#### 4.1 ABSTRACT

Quantitative computed tomography (QCT) is a 3-dimensional imaging technique that is able to distinguish trabecular and cortical bone and provide a measure of volumetric bone mineral density (vBMD). Very few studies have examined the factors related to vBMD in men, especially men of African heritage. The present study evaluated comprehensively the relationship of vBMD with demographic, anthropometric, medical and behavioral factors in a large cohort of men of African heritage (n=1,901) aged 40 years and older. Trabecular and cortical vBMD were measured by peripheral QCT (pQCT) at the radius and tibia. Trabecular vBMD decreased dramatically before age of 50 and then remained similar or decreased slightly thereafter. In contrast, cortical vBMD decreased steadily with advancing age. Step-wise multiple linear regression analysis identified, age, body weight, cigarette smoking, and a history of type II diabetes and prostate cancer as the major correlates of vBMD. However, different relationships between cortical and trabecular vBMD and skeletal site were observed for several variables. Our findings suggest that there are different age patterns and correlates for trabecular and cortical vBMD in men of African ancestry. A better understanding of the mechanisms underlying these differential associations may reveal new insight into the etiology of age-related bone loss and osteoporosis.

## 4.2 INTRODUCTION

Bone mineral density (BMD) measured by dual-energy x-ray absorptiometry (DXA) has been widely used to diagnose osteoporosis and to identify subjects at risk for fracture and factors for osteoporosis. However, DXA is a two-dimensional imaging technique that measures areal BMD (aBMD), cannot distinguish between trabecular and cortical bone, and is confounded by differences in bone size. Unlike DXA, quantitative computed tomography (QCT) measures volumetric BMD (vBMD), provides a separate measure of trabecular and cortical BMD, and is not confounded by bone size differences between individuals.

Factors related to DXA-measured aBMD have been well-established. In contrast, few studies have comprehensively characterized the anthropometric, medical and behavioral factors related to trabecular and cortical vBMD. Moreover, there is limited information available on the correlates of vBMD in populations of African descent (1-3). In the present study, we examined the factors related to trabecular and cortical vBMD in a large population-based study of men of African ancestry. The aim of our study was to identify the correlates of vBMD and to compare the factors associated with cortical and trabecular vBMD.



## 4.3 METHODS

### 4.3.1 Study subjects

Between 1997 and 2003, 3,170 men were recruited for a population-based prostate cancer screening study on the Island of Tobago, Trinidad & Tobago (4). Briefly, the Tobago Prostate Cancer Survey is an observational cohort study of prostate cancer prevalence and incidence in otherwise healthy men aged 40 years and older. To be eligible, men had to be ambulatory, non-institutionalized and not terminally ill. Recruitment for the survey was accomplished by flyers, public service announcements, posters, informing health care workers at local hospital and health centers, and word of mouth. Approximately 60% of all age-eligible men on the island participated and participation was representative of the island Parishes. The cohort is 97% African, 2% East Indian, <1% white, and <1% "other" as defined by paternal and maternal grandparent's ethnicity.

Between 2004 and 2007, men were invited back for a follow-up examination and to complete a peripheral QCT (pQCT) scan. A total of 2031 men in the prostate cohort (70% of survivors) returned for the visit. At the follow-up visit, we also recruited 451 new participants. Of them, 2153 underwent pQCT scan at the radius and tibia. We also excluded men with incomplete data or of non Afro-Caribbean origin. The current analysis is limited to the 1,901 men of African descent with complete pQCT scans and available information from questionnaire interview by the time the analysis was complete.

### **4.3.2 Anthropometric and body composition measurements**

Body weight (in kilograms) was measured with light clothing and without shoes using a calibrated balance beam scale. Height (in centimeters) was measured without shoes using a wall-mounted height board. Two height measurements were made and the average used in analysis. Waist circumference was measured at the umbilicus with an inelastic tape measure. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Handgrip strength was measured in kilograms for both the left and right hands as a surrogate for upper body and overall strength using a dynamometer (Preston Grip Dynamometer, JA Preston 136 Crop.). Body composition (bone mineral-free lean mass and fat mass) was measured using a Hologic QDR-4500W DXA scanner (Hologic Inc., Bedford, MA). For all participants, the same scanner was used and DXA scans were completed using the array beam mode. Standardized positioning and utilization of QDR software was based on the manufacturer's recommended protocol. Scans were analyzed with QDR software version 8.26a. To ensure consistency, the DXA technician scanned a spine phantom daily and completed a weekly quality control whole body air scan, prior to completing any participant scans.

### **4.3.3 Other measurements**

Trained interviewers and nurses administered questionnaires to participants. Questionnaires gathered information pertaining to demographic characteristics, medical history, fracture history, physical activity, and lifestyle variables. We focused on potential correlates of vBMD based on the body of literature for men and women.

Ethnicity was self-reported and participants provided detailed information on the ethnic origin of their parents and grandparents. Respondents were assigned to an ethnic group if they reported that all four grandparents as belonging to that group. Afro-Caribbean men who reported having less than 4 African grandparents were categorized as mixed African ethnicity. Occupational history was measured as a dichotomous variable and included several common occupations.

Participants were asked whether they had been diagnosed by a health care provider with selected conditions including cardiovascular disease, diabetes, hypertension and prostate cancer. We also obtained information on personal and parental fracture history. Disability was assessed with questions about the degree of difficulty (no difficulty; some difficulty; much difficulty; or unable to perform activity) in six activities of daily living that involved the back (bending down to pick up light-weight objects, lifting a 10-pound object from the floor, reaching for objects just above the head, putting on socks or stockings, getting in and out of an automobile, and standing for 2 hours). Subjects answered any difficulty or unable to perform any of the six activities due to back pain were considered with “difficulty performing daily activity due to back pain”.

Smoking status was categorized as never, past and current by asking men whether they had smoked at least 100 cigarettes in their lifetime. Men who smoked fewer than 100 cigarettes were considered to have never smoked. Weekly alcohol drinking in the past 12 months was categorized as none, occasional drinking, and 1-3, 4-7, 8-14, 15-21, 22-27 and more than 28 drinks per week. Physical activity was assessed by the frequency and duration of walking in the past 7 days for exercise, to work, the store or church. We also used hours of television watching per week as a surrogate of physical inactivity.

Men also reported their daily consumption of coffee, tea and soda (not decaffeinated). We assumed that one cup of coffee, tea and soda contained 95, 55 and 45mg of caffeine, respectively. Dietary calcium intake was assessed 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 cuisine. Supplemental intake of calcium and vitamin D was also assessed.

#### **4.3.4 Peripheral QCT**

Peripheral QCT was performed at the non-dominant forearm and left tibia (4% and 33% of the total length of forearm and tibia), two skeletal sites that are subjected to different weight bearing, using the Stratec XCT-2000 scanner (Stratec Medizintechnik, Pforzheim, Germany). Technicians followed a stringent protocol for patient positioning and scanning. A scout view was obtained prior to the pQCT scan to define an anatomic reference line for the relative location of the subsequent scans at the radius and tibia. Tibia length was measured from the medial malleolus to the medial condyle of the tibia, and forearm length was measured from the olecranon to the ulna styloid process. The scans at the 4% radius and tibia sites represent predominantly trabecular bone, whereas the scans at the 33% sites represent predominantly cortical bone. A single axial slice of 2.5mm thickness with a voxel size of 0.5mm and a speed of 20 mm/s is taken at all locations. Image processing was performed using the Stratec software package (Version 5.5E). To determine the total and trabecular vBMD ( $\text{mg}/\text{cm}^3$ ) at the 4% site of the radius and tibia, identical parameters for contour finding and separation of trabecular and cortical bone are: contour mode 2, Threshold= $169 \text{ mg}/\text{cm}^3$ ; peel mode 1, area=45%. To

determine the total and cortical vBMD ( $\text{mg}/\text{cm}^3$ ) at the 33% site of the radius and tibia, identical parameters are: mode 2, Threshold=169  $\text{mg}/\text{cm}^3$ ; cortmode 1, Threshold=710  $\text{mg}/\text{cm}^3$ . pQCT scans were stable throughout the study. The short-term in vivo precision of pQCT measurements was evaluated in 30 subjects. All CVs for measures of pQCT BMD were 2.1%.

#### 4.3.5 Statistical Analysis

Analysis of variance (ANOVA) was used to compare the unadjusted skeletal site-specific vBMD across 5-year age groups. We also evaluated the age-adjusted, and age and weight-adjusted (data not shown) association of each predictor with cortical and trabecular vBMD at the radius and tibia using linear regression analysis. The relationships between potential correlates and vBMD were expressed as a one unit increase for categorical variables or approximately a one standard deviation (SD) increase for continuous variables, along with 95% confidence intervals. The formula used to calculate the percent difference in vBMD per unit change of independent variable was:  $((\beta \text{ coefficient} * \text{unit}) / \text{mean vBMD}) * 100$ . The corresponding confidence intervals were calculated as:  $((\beta \text{ coefficient} \pm 1.96 * \text{standard error}) * \text{unit}) / \text{mean vBMD} * 100$ . In order to identify the independent correlates, multiple linear regression analysis was performed separately using a stepwise procedure for trabecular and cortical vBMD at the radius and tibia. Age was forced into each multiple linear regression model. Variables with a p-value less than 0.10 from the age-adjusted univariate linear regression model were entered into the multiple variable model. We also assessed the multi-collinearity of predictor variables using the variance inflation factor (VIF). If there was evidence of collinearity, the model

was re-evaluated. If variables were from the same domain (e.g., “ever smoked” and “currently smoke”), we selected the variable with a stronger association with vBMD. In addition, multiple linear regression analyses of vBMD at each skeletal site were performed for three separate models that included anthropometric or body composition variables: 1) using BMI; 2) using body weight and height modeled separately; and 3) using total body fat and lean mass. Results were similar and thus only the results for BMI are shown. All statistical analyses were performed using the Statistical Analysis System (SAS, version 9.1; SAS Institute, Cary, NC).

#### 4.4 RESULTS

The mean age of the population was  $59.1 \pm 10.4$  years old (range 40-92 years old). Approximately 9% of the men reported having at least one non-African grandparent. The mean values and standard deviation (SD) of trabecular and cortical vBMD were  $206 \pm 49$  and  $1213 \pm 23$   $\text{mg}/\text{cm}^3$  at the radius, and  $228 \pm 41$  and  $1177 \pm 24$   $\text{mg}/\text{cm}^3$  at the tibia. Figure 4-1 and 4-2 shows the unadjusted age-related patterns for trabecular and cortical vBMD at each skeletal site. The pair-wise t-test showed that the oldest age group (75+) had a 17% and 5% lower trabecular vBMD than the youngest age group (40-44) at the radius ( $p < 0.0001$ ) and tibia ( $p = 0.10$ ), respectively. Men aged 45-49 had a 9% ( $p = 0.01$ ) and 4% lower ( $p = 0.01$ ) trabecular vBMD than those aged 40-44 at the radius and tibia, respectively, but trabecular vBMD did not differ substantially between the older age groups. Cortical vBMD was 2% ( $p < 0.0001$ ) and 1% ( $p = 0.0003$ ) lower for the oldest age group at the radius and tibia, respectively, compared to the youngest age group.

#### 4.4.1 Age-adjusted regression results

Each SD (4.3 kg/m<sup>2</sup>) increase in BMI was associated with a 3.0% and 4.6% higher trabecular vBMD and 0.37% and 0.36% lower cortical vBMD at the radius and tibia, respectively (Table 4-1). In general, the body weight and body composition related variables were positively correlated with trabecular vBMD, whereas these variables were negatively associated with cortical vBMD. In addition, every 6.7cm increase in height was associated with a 4% lower trabecular vBMD at both the tibia and radius, but a 0.1% higher cortical vBMD at the radius.

The prevalence of self-reported diabetes was 16.4% and was positively associated with vBMD at all 4 skeletal sites with an approximately 4% and 5% higher trabecular vBMD but only 0.4% higher cortical vBMD than men without diabetes. Mixed African ancestry, prostate cancer history and androgen deprivation therapy (orchiectomy or currently taking Leuprorelin) was associated with a 0.3% to 0.9% lower cortical vBMD but these variables were not associated with trabecular vBMD. Men who had experienced a previous fracture had lower trabecular and cortical vBMD, but the association was only significant for trabecular vBMD at the radius and cortical vBMD at the tibia. Use of thiazide or non-thiazide diuretics was not associated with trabecular or cortical vBMD. Glucocorticoids use was low in this population and was not significantly associated with vBMD at either skeletal site.

Men who smoked more than 100 cigarettes in their lifetime had a 4-5% lower trabecular vBMD and approximately 0.2% lower cortical vBMD than nonsmokers. Moreover, the effect of smoking on vBMD was greater for current smokers than past smokers, compared with nonsmokers. Alcohol drinking and physical activity were not

correlated with vBMD. Fifteen percent of the men chewed animal or fish bones at least 5 days a week. Bone chewing was associated with a 2-3% higher trabecular vBMD at both skeletal sites but was not associated with cortical vBMD.

#### **4.4.2 Results from the multiple linear regression model**

Age(-), body weight(+), height(-), diabetes(+), personal fracture history(-), cigarette smoking (-), and bone chewing(+) explained 6% of the variation in trabecular vBMD at the radius (Table 4-2). These variables, except fracture history, explained 10% of the variation in trabecular vBMD at the tibia. Multiple linear regression models explained 16 and 13% of the variation in cortical vBMD at the radius and tibia, respectively. At the radius, age(-), body weight(-), height(+), grip strength(-), mixed African ethnicity(-), diabetes(+), prostate cancer(-), androgen deprivation therapy(-), and smoking(-) were significant correlates of cortical vBMD. However, for cortical vBMD at the tibia, only age(-), weight(-), diabetes(+) and androgen deprivation therapy(-) remained in the final model.

Multiple linear regression models substituting BMI or body fat and lean mass for body weight yield similar results (data not shown). BMI was positively associated with trabecular vBMD, but negatively associated with cortical vBMD. In the models that included body fat and lean mass, lean mass was a significant positive correlate of trabecular vBMD but negative correlate of cortical vBMD. Fat mass and vBMD were only significantly correlated at the tibia. Fat mass was positively associated with trabecular vBMD but was negatively associated with cortical vBMD at the tibia.



## 4.5 DISCUSSION

To better understand the factors that influence bone mass in men of African heritage, we characterized a number of anthropometric, lifestyle and medical variables and examined the relation of these variables to BMD at the radius and tibia in a large population sample of middle-aged and elderly men. Several features of our study were unique, including the large sample size of Afro-Caribbean men, careful measurement of variables, focus on both cortical and trabecular volumetric BMD, and the wealth of information available about the study cohort. The results validate associations described previously among Caucasian men, such as the major importance of body weight, but also illuminate previously unrecognized relationships.

Bone mineral density decreases with advancing age in men of all race and ethnic background, and this trend most likely contributes to the increase in fracture rates with aging. In the current study, we observed different age-related patterns for cortical and trabecular vBMD. Similar results have been observed in other recent studies, but these reports have included only Caucasian men. We found that cortical vBMD appeared to decrease more slowly with advancing age than trabecular vBMD and the age pattern appeared to be more linear for cortical vBMD. Cross-sectionally, the largest decline in trabecular vBMD occurred among men aged 40-44 and 45-49 years and then trabecular vBMD appeared to decline more slowly at the radius or remained stable at the tibia. Although our study did not include men aged 40 and below, our results among men of African ancestry appear to be consistent with the recent observations of an early reduction of trabecular vBMD with aging in men (5,6).

DXA measures of areal BMD are one of the most important predictors of osteoporotic fracture risk. Fewer studies have examined pQCT or QCT measures of vBMD and fracture in men, particularly among men of African ancestry. Among men and women, those with vertebral fracture have lower cortical and trabecular vBMD compared to controls (7). Each SD decrease in cortical, but not trabecular, vBMD at the radius was associated with a 16-fold risk of fracture among 52 hemodialysis patients (8). In our study, men who had experienced a previous fracture had significantly lower trabecular vBMD at the radius independent of other variables.

Previous studies using ancestry informative molecular markers in this Afro-Caribbean population have indicated that the ancestral proportions are 94.0% African, 4% European and 1% Native American. Despite the low level of admixture in this population, we found that mixed African ancestry was associated with lower cortical vBMD at the radius. These results are consistent with previous studies which have observed lower aBMD among African Americans who have greater European ancestry (9). The high proportion of African ancestry and negative impact of admixture on vBMD implies that the Tobago population may benefit from a greater prevalence of protective alleles of African origin.

Previous studies have found that greater body weight is associated with higher areal BMD in Caucasians and African Americans (10-12). In the present study, greater body weight, fat mass and lean mass were all associated with higher trabecular vBMD. In contrast, these variables were associated with lower cortical vBMD. Our results are consistent with the recent findings of Lorentzon et al. who showed that greater fat mass is associated with lower cortical vBMD at the tibia (13). Increased adipocytes in the bone

marrow cavity among heavier subjects may suppress osteoblastogenesis and contribute to decreased cortical vBMD by producing adipokines in the local bone micro-environment (14).

In our study, diabetes was associated with greater cortical and trabecular vBMD and remained a significant correlate after adjusting for age, body weight and other covariates. A higher trabecular vBMD was observed in diabetic than non-diabetic black women and white men in the Health, Aging, and Body Composition Study (15). A similar association was not observed among white women or black men in the same study. In the Diabetes Heart Study, trabecular vBMD was not associated with diabetes independently from BMI (16).

Androgens are a strong positive regulator of bone mass in men. Several studies have revealed that androgen deprivation for treatment of prostate cancer is associated with a marked loss of bone mass (17-19). However, studies evaluating the effect of androgen deprivation on bone mass in men of African descent are limited. Agarwal et al. reported a significant loss of trabecular vBMD at the lumbar spine after orchiectomy in Indian patients (20). We found significantly lower cortical, but not trabecular, vBMD among men who had a history of androgen deprivation therapy in the current study.

Cigarette smoking was associated with lower trabecular but not cortical vBMD in our study. The GOOD study also did not observe a difference in cortical vBMD between Caucasian European men who did and did not smoke (21). The effect of smoking on trabecular vBMD in the current study was greater for current smokers, but was also evident among past smokers. This suggests that smoking may have a long-lasting effect on vBMD. The association with vBMD was also observed in the multivariable model,

indicating that the association was independent of other intervening variables such as body weight, alcohol intake and physical activity. Nicotine may directly affect bone metabolism and bone mass by inhibiting the proliferation of osteoprogenitor cells in a concentration-dependent manner (22,23).

Our multivariate models explained up to 16% of the variance in cortical vBMD. Only 10% of the variance in trabecular vBMD could be explained by the variables studied. These estimates compare well with other reports of DXA measures of areal BMD in men and women (24-28) and trabecular vBMD in men (3). However, these findings raise the possibility that many other undetermined variables, including inherited factors, contribute to cortical and trabecular vBMD in men of African heritage.

In multivariate analyses, we found no association of vBMD with several lifestyle and behavioral variables including physical activity and intake of alcohol, caffeine and dietary calcium. Use of diuretics was also not associated with vBMD in the current study. Although dietary calcium intake was not associated with vBMD, we did observe an association between chewing bones and increased trabecular vBMD in multi-variate models. The absence of an association with some variables could reflect low statistical power to detect an association or the inherent difficulty in quantifying some variables with questionnaires.

This study has several potential limitations. First, the cross-sectional design limits our ability to establish temporal relationships with trabecular and cortical vBMD. Confirmation of our results with longitudinal evaluations would be useful, particularly for establishing the tempo and natural history of trabecular and cortical bone loss in this population. We also examined Afro-Caribbean men who were volunteers and their

characteristics may differ from those of other groups. Information collected by questionnaires depended on participants' recall and this may have limited our ability to detect relationships. On the other hand, our study has several unique features including its large size, focus on individuals of African heritage, careful delineation of trabecular and cortical BMD with QCT, and the wealth of information available on the study cohort.

In summary, our study of a large group of older men of African ancestry supports the importance of several factors associated with volumetric BMD. From a clinical perspective, these findings reinforce the need to avoid excessive thinness and cigarette smoking, a negative impact of androgen deprivation for prostate cancer, and the importance of calcium for preserving vBMD. Our study also revealed a potentially negative impact of increased body weight and fat mass on cortical vBMD.

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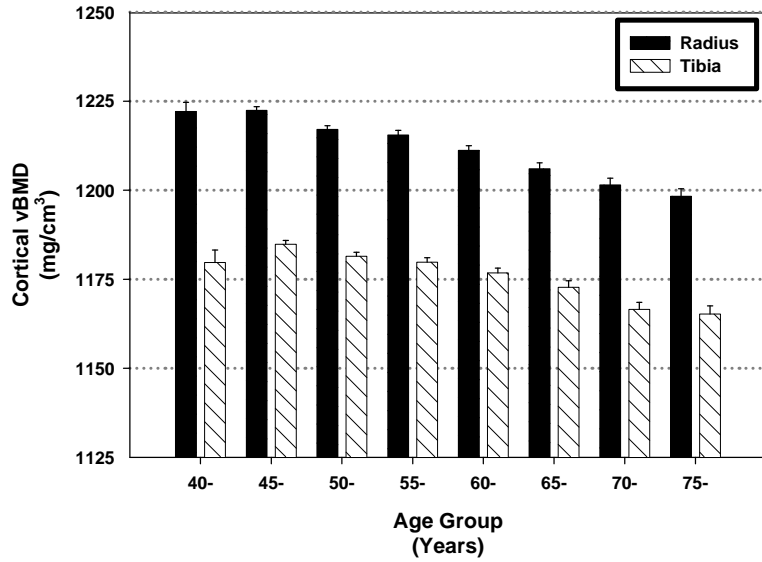


Figure 4-1 Trabecular vBMD by age group (unadjusted)

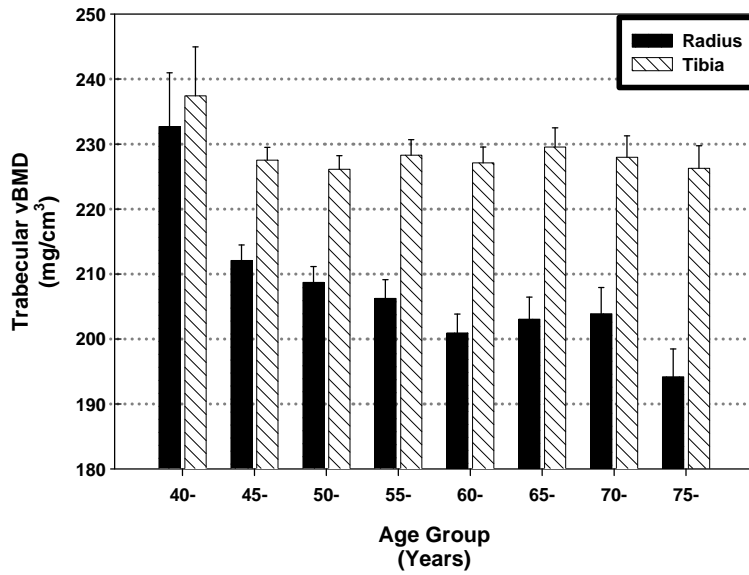


Figure 4-2 Cortical vBMD by age group (unadjusted)

**Table 4-1 Correlates of trabecular and cortical vBMD**

Variable	Mean ( $\pm$ SD) or frequency (prevalence)	Unit	Age- and weight- adjusted percent difference change in vBMD per unit change (95%CI)			
			Radius		Tibia	
			Trabecular vBMD	Cortical vBMD	Trabecular vBMD	Cortical vBMD
Age(yrs) <sup>§</sup>	59.1 $\pm$ 10.4	10	<b>-2.62(-3.65, -1.58)</b>	<b>-0.62(-0.70, -0.54)</b>	-0.09(-0.88, 0.69)	<b>-0.52(-0.61, -0.43)</b>
Body weight (kg)*	83.9 $\pm$ 14.5	10	<b>1.11(0.35, 1.87)</b>	<b>-0.26(-0.31, -0.20)</b>	<b>2.36(1.79, 2.94)</b>	<b>-0.32(-0.37, -0.25)</b>
Standing height (cm)	175.0 $\pm$ 6.7	6.7	<b>-4.07(-5.25, -2.89)</b>	<b>0.11(0.02, 0.20)</b>	<b>-4.18(-5.05, -3.30)</b>	-0.08(-0.17, 0.02)
BMI(kg/m <sup>2</sup> )*	27.4 $\pm$ 4.3	4.3	<b>3.00(1.93, 4.07)</b>	<b>-0.37(-0.45, -0.28)</b>	<b>4.63(3.83, 5.42)</b>	<b>-0.36(-0.45, -0.27)</b>
Waist circumference (cm)	93.1 $\pm$ 10.7	10.7	0.76(-1.46, 2.97)	0.03(-0.13, 0.20)	<b>2.70(1.06, 4.35)</b>	0.15(-0.03, 0.33) <sup>¶</sup>
Grip strength (kg)	42.7 $\pm$ 10.1	10.1	0.21(-1.19, 1.60)	-0.09(-0.19, 0.01) <sup>¶</sup>	<b>-1.24(-2.29, -0.20)</b>	<b>-0.12(-0.23, -0.00)</b>
Daily dietary calcium intake (kg)	465.4 $\pm$ 219.3		0.60(-0.48, 1.68)	0.02(-0.07, 0.10)	0.67(-0.13, 1.48) <sup>¶</sup>	-0.00(-0.09, 0.08)
Daily caffeine intake in top quartile	364(20.0)		0.47(-2.29, 3.23)	-0.20(-0.41, 0.00) <sup>¶</sup>	0.99(-1.07, 3.05)	-0.10(-0.32, 0.12)
Total body fat mass (kg)*	17.6 $\pm$ 6.9	6.9	<b>1.53(0.45, 2.62)</b>	<b>-0.30(-0.38, -0.22)</b>	<b>3.29(2.48, 4.11)</b>	<b>-0.33(-0.42, -0.24)</b>
Total body lean mass (kg)*	64.2 $\pm$ 8.7	8.7	<b>1.50(0.33, 2.67)</b>	<b>-0.88(-1.09, -0.67)</b>	<b>6.88(4.75, 9.01)</b>	<b>-1.16(-1.39, -0.93)</b>
Percent fat mass (%)*	21.0 $\pm$ 5.9	5.9	<b>5.15(1.24, 9.06)</b>	<b>-0.36(-0.49, -0.24)</b>	<b>4.69(2.48, 5.90)</b>	<b>-0.36(-0.50, -0.23)</b>
Mixed African ancestry	170(8.9)		-1.20(-4.95, 2.55)	<b>-0.37(-0.65, -0.08)</b>	2.09(-0.70, 4.89)	<b>-0.31(-0.61, -0.01)</b>
Diabetes	311(16.4)		<b>3.45(0.48, 6.42)</b>	<b>0.40(0.18, 0.62)</b>	<b>5.22(3.01, 7.43)</b>	<b>0.37(0.13, 0.62)</b>
Hypertension	620(32.8)		0.31(-2.09, 2.71)	0.04(-0.14, 0.22)	1.17(-0.63, 2.96)	0.10(-0.09, 0.29)
CVD	67(3.5)		2.08(-3.74, 7.9)	0.20(-0.24, 0.64)	3.17(-1.30, 7.63)	0.07(-0.42, 0.55)
Prostate cancer	291(15.3)		-0.25(-3.43, 2.92)	<b>-0.39(-0.63, -0.15)</b>	0.03(-2.36, 2.43)	<b>-0.33(-0.59, -0.07)</b>
Androgen deprivation therapy	61(3.3)		2.18(-4.05, 8.41)	<b>-0.84(-1.33, -0.35)</b>	0.64(-4.04, 5.32)	<b>-0.88(-1.42, -0.35)</b>
Good overall health status	1718(91.3)		1.74(-2.14, 5.62)	-0.04(-0.33, 0.25)	0.27(-2.65, 3.20)	-0.29(-0.61, 0.02) <sup>¶</sup>
Ever fracture	367(19.3)		<b>-3.24(-5.97, -0.51)</b>	-0.18(-0.39, 0.02) <sup>¶</sup>	-1.52(-3.55, 0.51)	<b>-0.26(-0.48, -0.04)</b>
Mother had fracture	80(4.9)		-4.64(-9.98, 0.69) <sup>¶</sup>	0.03(-0.37, 0.43)	-1.50(-5.45, 2.46)	-0.04(-0.47, 0.38)
Father had fracture	68(4.3)		-0.80(-6.62, 5.02)	-0.34(-0.78, 0.10)	0.50(-3.77, 4.78)	-0.38(-0.84, 0.09)
Paternal or maternal fracture history	141(9.3)		-2.84(-6.98, 1.30)	-0.16(-0.47, 0.15)	-0.54(-3.59, 2.51)	-0.26(-0.59, 0.08)
Difficulty perform daily activity due to back pain	176(9.8)		-2.89(-6.77, 0.98)	-0.20(-0.49, 0.10)	-1.20(-4.17, 1.76)	-0.11(-0.43, 0.22)
Ever smoked	619(32.7)		<b>-5.16(-7.44, -2.88)</b>	<b>-0.19(-0.36, -0.02)</b>	<b>-3.89(-5.59, -2.19)</b>	-0.18(-0.36, 0.01) <sup>¶</sup>
Smoking status						
Past smoker vs. non smoker	420(22.2)		<b>-3.74(-6.36, -1.12)</b>	-0.16(-0.35, 0.04)	<b>-3.06(-5.02, -1.09)</b>	-0.12(-0.36, 0.06)
Current smoker vs. non smoker	199 (10.5)		<b>-8.17(-11.77, -4.58)</b>	<b>-0.27(-0.54, -0.00)</b>	<b>-5.61(-8.26, -2.96)</b>	-0.23(-0.52, 0.06)
Alcohol intake $\geq$ 1 drink/wk	358(18.9)		-1.96(-4.71, 0.79)	0.13(-0.08, 0.33)	-1.47(-3.51, 0.57)	0.12(-0.10, 0.34)
TV watching $\geq$ 14 hours/wk	722(38.2)		0.01(-2.21, 2.22)	0.12(-0.04, 0.29)	-0.58(-2.24, 1.07)	<b>0.19(0.01, 0.37)</b>
Walk $\geq$ 3.5 hours/wk	545(29.1)		-0.72(-3.11, 1.68)	0.06(-0.12, 0.24)	0.29(-1.49, 2.08)	-0.00(-0.20, 0.19)
Walk $\geq$ 5-7days/wk	1192(63.1)		0.18(-2.06, 2.42)	-0.11(-0.28, 0.06)	1.13(-0.55, 2.79)	-0.03(-0.21, 0.15)
Bone chewing $>$ 5 days/wk	278(14.8)		<b>3.32(0.27, 6.37)</b>	0.01(-0.22, 0.23)	<b>2.38(0.10, 4.65)</b>	0.01(-0.24, 0.25)

**Table 4-1 (continued) Correlates of trabecular and cortical vBMD**

≥1 glass/d milk during teens	1588(84.2)	0.57(-2.38, 3.52)	-0.02(-0.24, 0.20)	0.73(-1.48, 2.94)	0.07(-0.17, 0.31)
≥1 glass/d milk at age 18-50 y	1260(67.0)	1.28(-1.05, 3.61)	0.02(-0.15, 0.19)	1.24(-0.50, 2.97)	0.03(-0.16, 0.21)
Calcium supplement ≥3 times/wk	323(17.5)	-1.45(-4.31, 1.41)	0.04(-0.17, 0.26)	-1.27(-3.40, 0.86)	-0.05(-0.28, 0.18)
Vitamin D supplement ≥3 times/wk	221(12.1)	0.66(-2.68, 3.99)	-0.03(-0.28, 0.22)	1.31(-1.19, 3.80)	-0.24(-0.51, 0.03)
Use of nonthiazide diuretics	26 (1.4)	-1.65(-11.11, 7.80)	-0.03(-0.78, 0.72)	0.06(-6.96, 7.08)	-0.45(-1.25, 0.35)
Use of thiazide diuretics	195 (10.5)	1.20(-2.40, 4.80)	0.01(-0.26, 0.28)	0.61(-2.11, 3.32)	0.07(-0.22, 0.36)
Use of glucocorticoid	12 (0.64)	-2.60(-16.14, 10.9)	-0.26(-1.32, 0.79)	-3.00(-13.99, 7.99)	-0.12(-1.29, 1.06)

§ Unadjusted

\*Age adjusted only

¶ Variables with p-value between 0.05 and 0.10

**Table 4-2 Significant correlates of vBMD in stepwise multiple linear regression models.**

Variables	Unit	Percent change in vBMD per unit change (95%CI)			
		Radius		Tibia	
		Trabecular vBMD <sup>1</sup>	Cortical vBMD <sup>2</sup>	Trabecular vBMD <sup>3</sup>	Cortical vBMD <sup>4</sup>
N		1868	1805	1828	1801
Age (yrs)	10	-3.30 (-4.42, -2.17)	-0.70 (-0.81, -0.60)	-0.44 (-1.27, 0.39)*	-0.63 (-0.72, -0.54)
Weight (kg)	10	1.83 (1.00, 2.65)	-0.28 (-0.34, -0.22)	4.52 (3.63, 5.40)	-0.31 (-0.38, -0.25)
Height (cm)	6.7	-3.87 (-5.06, -2.68)	0.15 (0.05, 0.24)	-3.96 (-4.83, -3.07)	-
Grip strength (kg)	10.1	-	-0.13 (-0.24, -0.02)	-	-
Mixed African ancestry		-	-0.37 (-0.66, -0.09)	-	-
Diabetes		3.40(0.46, 6.35)	0.39(0.16, 0.61)	5.18 (3.02, 7.35)	0.41 (0.17, 0.65)
Prostate cancer		-	-0.26(-0.52, -0.01)	-	-
Prostate cancer treatment		-	-0.68(-1.19, -0.18)	-	-0.86 (-1.40, -0.33)
Any fracture		-3.01 (-5.71, -0.30)	-	-	-
Smoking					
Past smoker vs. nonsmoker		-2.95 (-5.57, -0.33)	-0.20 (-0.40, -0.002)	-2.48 (-4.40, -0.55)	-
Current smoker vs. nonsmoker		-7.30 (-10.89, -3.72)	-0.28 (-0.55, -0.004)	-4.84 (-7.43, -2.24)	-
Chew bones >5 days/wk		3.25 (0.25, 6.25)	-	2.32 (0.11, 4.53)	-
R <sup>2</sup>		0.06	0.16	0.10	0.13

<sup>1</sup> Age, weight, height, diabetes, prostate cancer treatment, any fracture, smoking status, and bone chewing were enter into the model

<sup>2</sup> Age, weight height, grip strength, caffeine intake (dichotomous), mixed African ancestry, reported/diagnosed prostate cancer, diabetes, prostate cancer treatment and smoking (past and current smoker vs. non smoker) were entered into the model

<sup>3</sup> Age, weigh, height, grip strength, diabetes, systolic blood pressure, daily calcium intake (continuous), smoking (past and current smoker vs. non smoker), and bone chewing were entered into the model

<sup>4</sup> Age, weight, grip strength, diabetes, mixed African ancestry, reported/diagnosed prostate cancer, prostate cancer treatment, any fracture, ever smoked (dichotomous), and good overall health were entered into the model.

\* P-value> 0.5 (Age was forced into the multiple linear regression model)

**5.0 ASSOCIATION OF COMMON ECTONUCLEOTIDE  
PYROPHOSPHATASE/PHOSPHODIESTERASE 1 (ENPP1) GENE VARIANTS WITH  
BONE MINERAL DENSITY**

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## 5.1 ABSTRACT

Bone mineralization is a tightly controlled process that determines the quality of bone. The ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) gene product plays an important role in this process. However, very little is known about the potential effect of common variation in this gene on bone mineral density (BMD) in humans. The present study aimed to examine the associations of 34 single nucleotide polymorphism (SNP) in the *ENPP1* gene region with several BMD measures in 1,139 Afro-Caribbean men aged  $\geq 40$ . Phenotypes included areal BMD (aBMD) at the proximal femur and the rate of proximal femur bone loss determined over an average of 4.4 years by dual-energy X-ray absorptiometry and trabecular and cortical volumetric BMD (vBMD) at the radius and tibia, measured by quantitative computed tomography. The 34 SNPs captured 77% of the common genetic variation in the *ENPP1* gene region. We found that SNPs rs6936129 and rs9398995 were associated with accelerated areal bone loss at the femoral neck and areal BMD at the total hip ( $p < 0.01$ ). Significant associations ( $p < 0.05$ ) were also observed between several SNPs (rs13211931, rs6939185, rs703184, rs7775386, rs9493110, rs7769993 and rs9373000, rs1830971, rs858339 and rs7749493) and cortical vBMD. Our findings suggest a strong association between common variation in *ENPP1* gene and the rate of bone loss and BMD in men of African heritage. More studies are needed to confirm and explore these relationships in greater detail.

## 5.2 INTRODUCTION

Osteoblasts mineralize bone matrix by promoting hydroxyapatite crystal formation and growth, which provides collagen with structural rigidity and load-bearing strength. Bone mineralization is a tightly regulated process that occurs initially in matrix vesicles (MVs) that contain calcium and inorganic phosphate ions (Pi)(1,2). Pi crystallizes with calcium to form hydroxyapatite within the MVs (3,4). Inorganic pyrophosphate (PPi) determines the rate of hydroxyapatite crystal formation in bone by inhibiting the ability of Pi to crystallize. Thus, the balance between levels of Pi and PPi is important for normal bone mineralization to proceed(5). Nucleotide pyrophosphatase /phosphodiesterase 1, encoded by the ectonucleotide pyrophosphatase /phosphodiesterase 1 (ENPP1) gene, is a central regulator of the extracellular PPi pool and Pi level. ENPP1 regulates bone mineralization by generating PPi from nucleotide triphosphates, which serves as a source of Pi. The PPi serves further to antagonize the ability of Pi to crystallize with calcium to form hydroxyapatite and thereby reduces hydroxyapatite deposition in bone matrix (5).

*ENPP1* has been intensively studied as a candidate gene for diabetes, obesity and insulin resistance(6-13) due to its ability to inhibit insulin signaling(7). Two meta-analyses have reported that individuals who were homozygous for the minor allele of the K121Q polymorphism (rs1044498) were more likely to be diabetic(11,14). Recently, studies have examined the effect of ENPP1 on bone mineralization in animal models (5,15-20). These studies observed hypermineralization and low PPi levels in *ENPP1* knockout mice(5,15,19,20). Another study found that *ENPP1* deficiency effects on mineral deposition might be skeletal site-specific

and observed a lower BMD level in the long bones of *ENPP1* knockout mice compared to wild-type mice(20). In the present study, we examined the association between common single nucleotide polymorphisms (SNPs) in the human *ENPP1* gene and measures of bone mineral density and bone loss in a large population of men of African heritage.

## 5.3 METHODS

### 5.3.1 Study population

All men were participants in the Tobago Bone Health Study, a study of 2652 community-dwelling men aged 40 years and older who resided on the island of Tobago. Men who were ambulatory, not terminally ill and who had not undergone a bilateral hip replacement were recruited between 2000 and 2004 and completed an areal BMD (aBMD) assessment using dual-energy X-ray absorptiometry (DXA). Details of this study have been described elsewhere(21). In 2004, participants were asked to return for a follow-up exam and to have a second measure of aBMD by DXA. Approximately 70% of survivors returned for the follow-up exam and 434 new participants were also recruited. At this exam, men also completed a peripheral quantitative computed tomography (pQCT) scan of the tibia and radius. Blood samples and anthropometric measures were also collected and questionnaires about lifestyle and medical factors were completed. Samples were batch shipped from Tobago to Pittsburgh for long-term storage by overnight shipment with packed dry ice. Genomic DNA was extracted from 5ml frozen blood clots (baseline sample) or whole blood (follow-up) using the Qiagen DNA Blood Kit (Qiagen, Inc., Valencia, CA). Written informed consent was obtained from each participant using forms



and procedures approved by the University of Pittsburgh Institutional Review Board, the U.S. Surgeon General's Human Use Review Board, and the Tobago Ministry of Health.

### **5.3.2 Bone Measurements**

Areal BMD (aBMD) at the proximal femur and its subregions was measured by DXA at both the baseline and follow-up visits using the same Hologic QDR-4500W densitometer (Hologic Inc., Bedford, MA). Technicians followed the same DXA scanning protocol at both visits. The left hip was scanned unless fractured or a hip replacement had occurred. Quality control assessments were performed weekly to ensure that the scanner was stable throughout the study.

Volumetric BMD (vBMD) was measured using pQCT (XCT-2000, Stratec Medizintechnik, Pforzheim, Germany). pQCT scans were performed at 2 skeletal sites (4% and 33%) at the non-dominant forearm and left tibia. The scans at the ultra-distal region of radius and tibia sites represent predominantly trabecular bone, whereas the scans at the shaft regions represent predominantly cortical bone.(22). The radius and tibia were scanned because these two skeletal sites are subjected to different weight bearing.

Technicians followed stringent protocols for patient positioning and scanning. A scout view was obtained prior to the pQCT scan to define an anatomic reference line for the relative location of the subsequent scans (4% and 33% of the total length) at the radius and tibia. Tibia length was measured from the medial malleolus to the medial condyle of the tibia, and forearm length was measured from the olecranon to the ulna styloid process. A single axial slice of 2.5mm thickness with a voxel size of 0.5mm and a speed of 20 mm/s is taken at all locations. Image processing was performed using the Stratec software package (Version 5.5E). To determine the trabecular vBMD ( $\text{mg}/\text{cm}^3$ ) at the 4% site of the radius and tibia, identical

parameters for contour finding and separation of tabecular and cortical bone were: contour mode 2, Threshold=169 mg/cm<sup>3</sup>; peel mode 1, area=45%. To determine the cortical vBMD (mg/cm<sup>3</sup>) at the 33% site of the radius and tibia, identical parameters were: mode 2, Threshold=169 mg/cm<sup>3</sup>; cortmode 1, Threshold=710 mg/cm<sup>3</sup>

### 5.3.3 SNP selection

The ENPP1 gene is located on Chromosome 6 (position 132,170,849-132,257,988) with a size of 87,140 bps. We used a two-stage strategy to prioritize SNPs for genotyping. First, we employed a tagging SNP approach to capture common genetic variation across the ENPP1 gene region. We initially identified 149 SNPs across the ENPP1 gene region (including 10kb downstream and 10kb upstream of the transcript) using publicly available SNP data from Phase II of the International HapMap project (<http://www.hapmap.org>) that were obtained using samples from the African Yoruban (YRI) population in Ibadan, Nigeria. A subset of informative SNPs was then selected from this larger reference SNP panel using a pair-wise correlation method with  $r^2 \geq 0.80$  and minor allele frequency (MAF)  $\geq 0.05$  using the program HClust (23,24). In brief, HClust identifies clusters of correlated SNPs. For each cluster, the SNP that is most correlated with all other SNPs in the same cluster is then identified as a tag SNP for that particular cluster. Using this approach, we identified 52 SNPs (36 singletons and 16 tag SNPs) from the 91 SNPs in the reference SNP panel that had a MAF  $\geq 0.05$ .

We then used the Function Analysis and Selection Tool for Single Nucleotide Polymorphisms (FASTSNP)(<http://fastsnp.ibms.sinica.edu.tw>)(25) and the EIDorado tool (version 4.5) from the Genomatix software package (Genomatix Suite release 3.4; <http://www.genomatix.de>) to prioritize the 36 singleton SNPs for genotyping based on their

predicted functional effects. FASTSNP identifies SNPs that may alter: 1) the amino acid in the encoded protein to one with different structural characteristics or create a premature termination of an amino-acid sequence; 2) an exonic splicing enhancer/silencer binding site in a coding sequence that may affect splicing regulation; 3) a consensus splicing site sequence; 4) a putative binding site for a transcription factor in the promoter or an intronic region; or 5) a 3' untranslated region motif likely to be involved in post-transcriptional regulation. EIDorado identifies SNPs that may create or abolish a putative transcription factor binding site in the promoter/regulatory region of genes by using a large library of weight matrices(26) and was used to analyze 7 SNPs that could not be analyzed by FASTSNP. Potentially non-functional singleton SNPs were not selected for genotyping leaving 31 SNPs in the panel of tag SNPs. In addition to these 31 SNPs, we also included SNPs that were previously identified to be significantly associated (nominal  $p < 0.05$ ) with vBMD in Caucasian men aged  $\geq 65$  years in the Osteoporotic Fractures in Men Study (MrOS) (27,28). The 6 SNPs that were significantly associated with vBMD in MrOS and that had a MAF  $> 0.05$  and that were not monomorphic in YRI samples were selected. The final SNP panel included a total of 37 SNPs of interest.

#### **5.3.4 Genotyping**

Genotyping was performed using pre-designed TaqMan SNP genotyping assays (Applied Biosystems). Genotyping was completed according to the manufacture's protocol on a 7900HT Fast Real-Time PCR system (Foster City, CA). The reactions were cycled with standard TaqMan conditions (50°C for 2 min hold, 95°C for 10 min hold, 95°C for 15 sec and 60°C for 1 min for 40 cycles and then cool down to 4°C). The genotypes were called with the Applied Biosystems SDS 2.2.2 software package. SNP rs9493119 failed to be manufactured and was replaced by

rs9493118 from the same tag SNP cluster. Each SNP was tested for Hardy-Weinberg equilibrium (HWE) using a chi-square goodness-of-fit test. Three SNPs (rs9493105, rs7773477 and rs9493116) were not in Hardy-Weinberg Equilibrium ( $p < 0.001$ ) and were dropped from further analysis. Two of these SNPs (rs9493105 and rs7773477) had no replacement and one SNP (rs9493116) was replaced by rs7769712 from the same cluster. SNP rs9402345 had a MAF  $< 0.05$  in our population and was removed from the analysis. Our final working genotype set consisted of 34 SNPs. The average genotyping completeness rate was 95.9%. The average genotyping consensus rate among the 5% blind replicate samples was 99.4% with individual consensus rates for all SNPs  $\geq 97.2\%$ . We estimated using the program Tagger (<http://www.broad.mit.edu/mpg/haploview> (29)) that the 34 genotyped SNPs captured 77% of the common variation in the ENPP1 gene region .

### **5.3.5 Statistical analysis**

All single SNP associations were tested assuming additive, dominant and recessive inheritance models using linear regression or analysis of covariance (ANCOVA). For the additive model, a linear regression model was constructed to test whether carrying 0, 1, or 2 minor alleles had a linear effect on the bone related trait. The continuous phenotypes were regressed on the allele count (0, 1, 2). For dominant (AA vs. AB+BB) and recessive (AA+AB vs. BB) inheritance models, ANCOVA was used to test the association between genotype and phenotype. All models were adjusted for age, weight and height. We also performed multiple-testing correction of p-values with permutation using R project (<http://www.r-project.org>). Study-wise p-values were obtained empirically for each phenotype with the consideration of the best inheritance models associated with each SNP. This p-value represented the overall impact of the 34 genotyped SNPs

on the selected phenotype, rather than any particular SNP. Statistical analysis was performed using the Statistical Analysis System (SAS, version 9.1; SAS Institute, Cary, NC).

## 5.4 RESULTS

The population sample consisted of 1,139 Afro-Caribbean men aged 40 and older who self-reported non-mixed African ancestry. Their mean ( $\pm$  SD) age was  $58.7 \pm 10.1$  years (Table 5-1). Information on the 34 genotyped ENPP1 SNPs, including NCBI dbSNP reference number, chromosome position, major and minor alleles, minor allele frequencies in Tobago and Yoruban (YRI) population from Ibadan, Nigeria from Phase II of the International HapMap project ([www.hapmap.org](http://www.hapmap.org)) and number of subjects with each genotype in current study, and tests of HWE are shown in Table 5-2. Minor allele frequencies were very comparable to those in the YRI sample from the HapMap project and all SNPs were in HWE in the current study ( $P > 0.01$ ). The results of single SNP association analyses and rate of change in aBMD during the 4.4 years of follow-up are shown in Figure 5-1 and Table 5-3. We plotted the transformed ( $-\log_{10}$ ) p-values from the best fitting model for each SNP. We further summarized the results for SNPs that were significantly associated with the rate of change in aBMD (nominal  $p < 0.05$ ). Of the 34 SNPs tested, only one SNPs(rs9398995) was associated with bone loss at the total hip (nominal  $p < 0.05$ ). For this SNP, men who were homozygous for the minor allele experienced twice the rate of decline in aBMD than those who were homozygous for the major allele. At the femoral neck, there were five additional SNPs that showed a significant association with the rate of decline in aBMD with a nominal  $p < 0.05$ . Except for rs2021966, the minor alleles for all the other 4 SNPs were associated with a greater decline in aBMD. The strongest SNP association

was for rs6936129, where the best fitting model was additive ( $p=0.0035$ ). Men who were homozygous for the minor allele of this SNP experienced a 2.5-fold greater rate of decline in aBMD compared to those who were homozygous for the major allele, whereas men who were heterozygous experienced a more intermediate rate of bone loss.

The SNP association results with cross-sectional areal and volumetric BMD measures are presented in the same format in Figure 5-2 and Table 5-4. For the aBMD measures, nominally significant associations were only observed at the total hip, but not femoral neck. Three SNPs achieved nominal significance for aBMD at the total hip with rs9398995 showing to strongest evidence of association in the recessive inheritance model ( $p=0.0063$ ). Men who were homozygous for the minor allele had approximately  $0.05 \text{ g/cm}^2$  lower hip aBMD than those who were homozygous for the major allele. Similar to the results for the decline in aBMD, SNPs associated with total hip BMD in cross-sectional analyses were largely concentrated in the 5' flanking region and intron 1 of ENPP1.

Significant associations between vBMD were more likely to be observed in cortical than trabecular vBMD (Figure 5-2 and Table 5-4). For cortical vBMD, 7 SNPs (rs13211931, rs6939185, rs703184, rs7775386, rs9493110, rs7769993 and rs9373000) at the radius and 4 SNPs (rs13211931, rs1830971, rs858339 and rs7749493) at the tibia were significant at  $p<0.05$ . SNP rs13211931 showed the strongest association with cortical vBMD at the tibia and was the only SNP with a significant association with cortical vBMD at both the radius and tibia. For example, men who had two copies of the minor allele had  $12 \text{ mg/cm}^3$  or approximately  $\frac{1}{2}$  standard deviation lower cortical vBMD at the radius compared to men who were homozygous for the major allele.

The associations between ENPP1 SNPs and trabecular vBMD were not as strong as they were for cortical vBMD. Similar to rs13211931, rs6916495 was associated with trabecular vBMD at both the radius and tibia. The effect of this SNP on trabecular vBMD appeared to be best modeled as a recessive mode of inheritance. For instance, men who were homozygous for the minor allele had an approximately 12-13% and 8-9% or approximately ½ standard deviation lower trabecular vBMD at the radius and tibia, respectively, than those with the other genotypes.

Nine of the SNPs that showed significant results among Caucasians in the MrOS study (nominal  $p < 0.05$ ) did not seem to have very strong associations with any of the BMD measures in the Afro-Caribbean men. Four of the 9 SNPs (rs6939185, rs858339, rs6916495 and rs7768480) had a nominal  $p < 0.05$ .

The study-wise empirical p-value generated from the permutation test for each phenotype was between 0.0014 and 0.0017. Thus, none of our single SNP associations with bone loss or BMD remained significant after the correction for multiple testing.

## 5.5 DISCUSSION

Osteoporosis is a common disorder characterized by a loss of bone mineral and structure leading to compromised bone strength. Despite the lower prevalence of osteoporosis in men than women, the number of older men with low BMD is still substantial(30). Evidence from twin and family studies indicates that the heritability for aBMD ranges from 50% to 85% (31-33). Although most of the heritability studies for aBMD were in Caucasians, men and women of African heritage also have a high heritability of aBMD (34). In contrast to the heritability of cross-sectional measures of aBMD, the heritability of bone loss is less well defined. Nonetheless,

heritability estimates for bone loss with age range from 15% to 45% (35-38). Although previous studies have demonstrated an important role of ENPP1 in bone mineralization in animal models, the effect of ENPP1 gene variation on aBMD and bone loss in humans is unknown. Our results suggest a novel association between common ENPP1 allelic variation and aBMD and the rate of bone loss in men of African heritage.

Most association and linkage studies of BMD in humans have relied solely on DXA measures of areal BMD as the phenotype. DXA provides a two-dimensional measure of BMD and is known to be confounded by bone size. Furthermore, DXA yields a measure of integral BMD – trabecular and cortical BMD combined. On the other hand, 3-dimensional QCT enables a separate measure of volumetric BMD in the trabecular and cortical bone compartments. Although less well studied than DXA measures of aBMD, a high heritability has been demonstrated for trabecular vBMD (~70%) (34,39) and there is evidence for both shared and unique loci for cortical and trabecular vBMD (34). However, the genetic variants contributing to vBMD variation remain poorly defined. Results from the current study suggest a role of genetic variation in ENPP1 in determining vBMD with more consistent effects on cortical than trabecular vBMD.

Although our study is the first, to our knowledge, to investigate ENPP1 and measures of bone health, other studies have investigated this gene in relation to other disease related endpoints. In studies of humans, SNP rs1044498 which creates a missense substitution was found to be related to arterial calcification (40-42), osteoarthritis(18) and ossification of posterior longitudinal ligament of the spine(43). This SNP has also been associated with diabetes (6,10-14). In the current study, we have found no significant association between this particular amino acid substitution and measures of aBMD, vBMD or the rate of hip bone loss.



SNP rs6936129 in the promoter region was highly and significantly associated with femoral neck bone loss with evidence of an additive mode of inheritance. Although there were only 13 men homozygous for the minor allele of rs6936129, the 180 men who were heterozygous for this variant also experienced 55% greater bone loss than those who were homozygous for the major allele. This SNP is predicted *in silico* by FASTSNP to create a putative binding site for the pro-inflammatory transcription factor, STAT, which may regulate expression of ENPP1 in response to cytokines.

Only one SNP, rs9398995, showed a strong association with aBMD at the total hip. This SNP is located in intron 1 and is predicted to generate a putative binding site for the homeobox transcription factor, CDXA, which is not known to regulate ENPP1 gene expression. There were five SNPs that were associated with cortical vBMD at  $p < 0.01$  (rs13211931, rs7749493, rs703184, rs9493110 and rs9373000). None of these five SNPs were in the same linkage disequilibrium (LD) blocks defined by using a solid spline approach ( $D' > 0.80$ ) to analyze Phase II HapMap SNPs in the Yoruban population from Nigeria, Africa. SNPs rs13211931 and rs7749493 were associated with cortical vBMD at the tibia, whereas rs703184, rs9493110 and rs9373000 had strong associations with cortical vBMD at the radius. SNP rs7749493 lies in intron 5 of ENPP1 and is predicted to create a putative binding site for the transcription factor, RUNX1/CBFA2/AML1, which is involved in skeletal development. SNP rs703184 lies in intron 1 of ENPP1 and is predicted to create a putative binding site for the SRY-related high-mobility-group box (SOX) family of transcription factors. The SOX family controls cell fate and differentiation and plays a role in skeletogenesis (44).

Although a high heritability of trabecular vBMD was observed in previous studies (34,39), no SNP in ENPP1 appeared to be associated with trabecular vBMD at a  $p < 0.01$ . Among

the 9 SNPs that showed a statistically significant association with vBMD ( $p < 0.05$ ) at the femoral shaft among Caucasian men in the MrOS study, only 4 were associated with aBMD or vBMD in our study of Afro-Caribbean men. However, none of these SNPs were associated at  $p < 0.01$ . The low level of concordance between SNPs and BMD between two studies may be explained by the different linkage disequilibrium patterns Caucasian and African individuals, different skeletal sites studied or differences in the characteristics of the cohorts. Nonetheless, our results suggest a “gene level” replication of associations between common genetic in ENPP1 and BMD in men of diverse ethnicity/race.

Our study has several notable strengths including its large cohort of middle- and older-aged men who were recruited from a relatively homogeneous population. In addition, we identified and genotyped 34 SNPs across the gene region which provide good coverage of the common genetic variation in the ENPP1 gene. Our study also had limitations. We evaluated a large number of SNPs, several skeletal phenotypes and tested for 3 models of inheritance for each SNP and phenotype. None of our SNPs achieved statistical significance after controlling for multiple comparisons and our results will require replication and further evaluation in a larger sample size and in additional populations.

In conclusion, our study suggests a possible novel role of common genetic variation in ENPP1 and several measures of bone health including areal BMD, volumetric BMD and the rate of bone loss in a large male cohort of African ancestry. This is the first study to examine the association between ENPP1 gene variation and BMD in humans. Additional studies are needed to explore the association between ENPP1 gene variants, bone mineralization and BMD.

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**Table 5-1 Selected characteristics of Afro-Caribbean men (n=1139)**

<b>Characteristics</b>	<b>Mean ± SD</b>
Age (yrs)	58.7 ± 10.1
Height (cm)	175.2 ± 6.8
Weight (kg)	84.0 ± 14.4
BMI (kg/m <sup>2</sup> )	27.3 ± 4.2
Annualized rate of change in BMD (%/yr)	
Total hip BMD	-0.118 ± 0.562
Femoral neck BMD	-0.296 ± 0.823
Proximal femur area BMD (g/cm <sup>2</sup> )	
Total hip BMD (g/cm <sup>2</sup> )	1.157 ± 0.145
Femoral neck BMD (g/cm <sup>2</sup> )	1.000 ± 0.149
Trabecular volumetric BMD (mg/cm <sup>3</sup> )	
Radius	207.2 ± 49.2
Tibia	229.4 ± 40.4
Cortical volumetric BMD (mg/cm <sup>3</sup> )	
Radius	1214.8 ± 22.6
Tibia	1179.0 ± 23.1



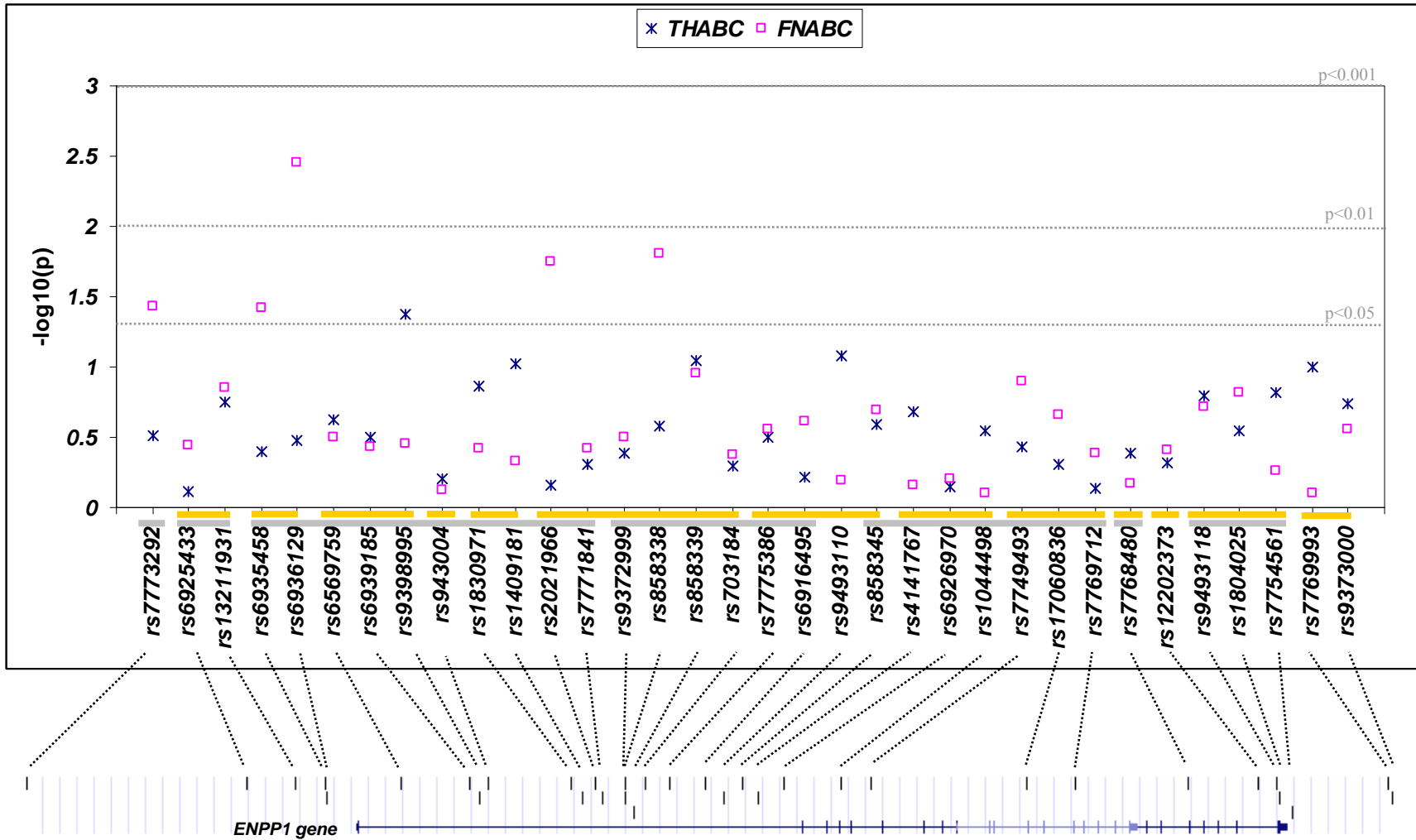
**Table 5-2 Information on the genotyped SNPs in the ENPP1 gene**

dbSNP rs number	Major/minor allele	Chrom. Position (bp)	MAF		N in each genotype*			HWE p-value
			Afro-Caribbean	YRI	NN	Nn	Nn	
rs7773292	C/T	132141454	0.22	0.19	681	349	60	0.069
rs6925433	A/G	132161059	0.19	0.21	713	309	46	0.071
rs13211931	G/T	132165417	0.09	0.09	897	177	12	0.328
rs6935458	A/G	132168013	0.43	0.43	373	501	223	0.021
rs6936129	G/C	132168133	0.09	0.07	875	168	13	0.130
rs6569759	G/A	132174809	0.23	0.28	658	378	71	0.095
rs6939185	G/A	132180880	0.09	0.11	917	180	14	0.131
rs9398995	T/C	132181896	0.21	0.18	702	337	55	0.065
rs943004	G/A	132182569	0.23	0.13	656	405	49	0.174
rs1830971	T/C	132190046	0.25	0.23	615	412	72	0.714
rs1409181	C/G	132190993	0.29	0.30	560	457	97	0.729
rs2021966	T/C	132192132	0.29	0.28	556	452	100	0.546
rs7771841	G/A	132192798	0.21	0.20	678	366	51	0.773
rs9372999	C/A	132194845	0.27	0.29	575	411	79	0.631
rs858338	G/T	132194900	0.13	0.12	843	240	20	0.542
rs858339	T/A	132195590	0.32	0.24	521	473	124	0.261
rs703184	G/C	132196688	0.10	0.09	898	187	12	0.540
rs7775386	C/T	132198842	0.49	0.44	296	528	270	0.246
rs6916495	C/T	132201958	0.11	0.09	867	210	16	0.421
rs9493110	C/T	132203671	0.24	0.32	620	423	58	0.197
rs858345	G/A	132205310	0.35	0.34	458	475	141	0.301
rs4141767	A/G	132206700	0.41	0.41	389	521	183	0.687
rs6926970	A/C	132208983	0.19	0.15	729	321	55	0.013
rs1044498	C/A	132214061	0.13	0.08	856	205	29	0.483
rs7749493	G/T	132216752	0.23	0.19	665	362	69	0.032
rs17060836	T/C	132230641	0.23	0.25	630	395	53	0.379
rs7769712	A/C	132235088	0.32	0.32	478	463	99	0.397
rs7768480	G/A	132245125	0.46	0.42	339	522	251	0.062
rs12202373	T/C	132251394	0.08	0.10	916	151	12	0.045
rs9493118	C/T	132253058	0.32	0.31	518	455	124	0.112
rs1804025	A/G	132253227	0.23	0.20	669	366	73	0.019
rs7754561	G/A	132254387	0.11	0.07	851	207	20	0.078
rs7769993	A/G <sup>§</sup>	132262945	0.43	0.47	371	520	210	0.240
rs9373000	A/G	132263399	0.38	0.44	433	508	163	0.471

<sup>§</sup> The major allele in YRI population was G allele

\*NN/Nn/nn: N=major allele; n=minor allele

Chrom indicates position on chromosome 6 from NCBI Genome Build 36; MAF, minor allele frequency; YRI, Yoruban population from Ibadan, Nigeria; HWE; Hardy-Weinberg Equilibrium.



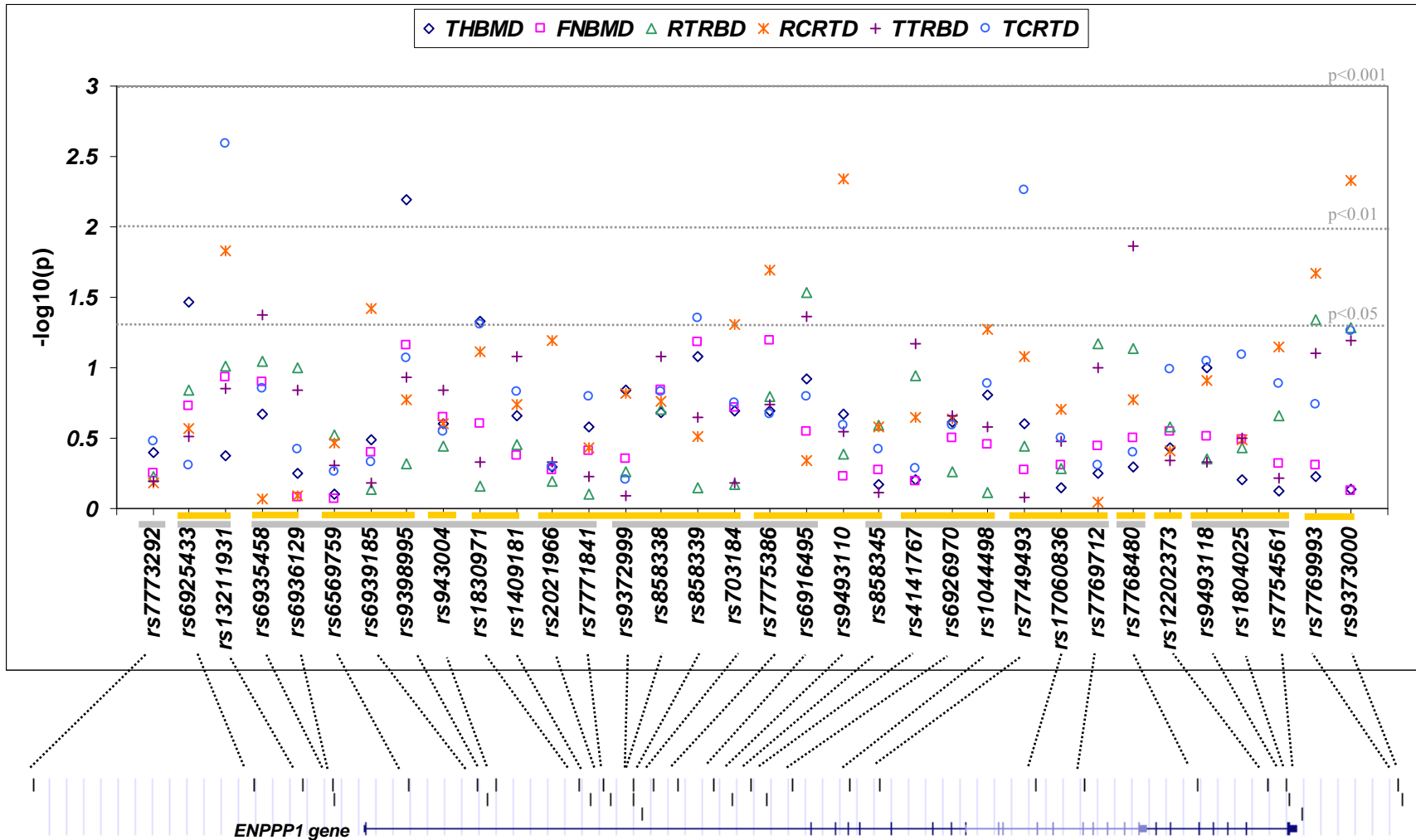
**Figure 5-1 Schematic of negative log 10 of p-value for 34 single SNP association tests with longitudinal aBMD changes at the total hip and femoral neck.** ENPP1 SNPs are represented on the X axis according to their positions in the gene from 5' to 3' direction. Dotted lines represent p-value thresholds of 0.05, 0.01 and 0.001. The smallest p-value for each SNP is plotted. Models were adjusted for age, weight and height. THABC: Total hip annualized aBMD change rate; and FNABC: Femoral neck annualized aBMD change rate.

**Table 5-3 Association of ENPP1 SNPs and rate of decline in aBMD per year (%/yr)**

SNP	Genotype mean(standard error)			p-value	Best fitting model
	1/1	1/2	2/2		
<b>Total hip</b>					
rs9398995	-0.100(0.021)	-0.125(0.030)	-0.262(0.074)	0.0423	Recessive
<b>Femoral neck</b>					
rs7773292	-0.291(0.031)	-0.288(0.043)	-0.514(0.104)	0.0366	Recessive
rs6935458	-0.253(0.042)	-0.290(0.036)	-0.379(0.054)	0.0383	Additive
rs6936129	-0.269(0.027)	-0.417(0.062)	-0.679(0.223)	0.0035	Additive
rs2021966	-0.308(0.034)	-0.326(0.038)	-0.115(0.081)	0.0177	Recessive
rs858338	-0.279(0.028)	-0.312(0.052)	-0.727(0.180)	0.0156	Recessive

Genotype-specific means were adjusted for age, weight and height.

1 indicates major allele; 2 minor allele.



**Figure 5-2 Schematic of negative log 10 of p-value for 34 single SNP association tests with cross-sectional aBMD at the total hip and femoral neck, and trabecular and cortical vBMD at the radius and tibia.**

ENPPP1 SNPs are represented on the X axis according to their positions in the gene from 5' to 3'. Dotted lines represent p-value thresholds of 0.05, 0.01 and 0.001. The smallest p-value for each SNP is plotted. Models were adjusted for age, weight and height. THBMD: Total hip aBMD; FNBMD: femoral neck aBMD; RTRBD: trabecular vBMD at the radius; RCRTD: cortical vBMD at the radius; TTRBD: trabecular vBMD at the tibia; and TCRTD: cortical vBMD at the tibia.

**Table 5-4 Association of ENPP1 SNPs and bone mineral density**

SNP	Genotype Mean(standard error)			p-value	Best fitting model
	1/1	1/2	2/2		
<b>Total hip aBMD (g/cm<sup>2</sup>)</b>					
rs6925433	1.156(0.005)	1.156(0.007)	1.198(0.019)	0.0338	Recessive
rs9398995	1.159(0.005)	1.160(0.007)	1.110(0.018)	0.0063	Recessive
rs1830971	1.159(0.005)	1.159(0.006)	1.128(0.015)	0.0470	Recessive
<b>Radius trabecular vBMD (mg/cm<sup>3</sup>)</b>					
rs6916495	208.1(1.635)	207.4(3.324)	181.6(12.040)	0.0294	Recessive
rs7769993	207.4(2.489)	205.9(2.102)	213.9(3.309)	0.0460	Recessive
<b>Radius cortical vBMD (mg/cm<sup>3</sup>)</b>					
rs13211931	1214.0(0.704)	1217.4(1.586)	1225.4(6.084)	0.0147	Additive
rs6939185	1215.3(0.694)	1213.5(1.57)	1203.8(5.619)	0.0380	Additive
rs703184	1215.5(0.703)	1213.8(1.544)	1203.3(6.078)	0.0049	Additive
rs7775386	1216.3(1.224)	1215.5(0.916)	1212.4(1.282)	0.0201	Recessive
rs9493110	1213.2(0.847)	1216.9(1.025)	1216.5(2.782)	0.0046	Dominant
rs7769993	1213.0(1.095)	1216.5(0.925)	1215.1(1.456)	0.0215	Dominant
rs9373000	1212.6(1.009)	1216.4(0.931)	1215.9(1.643)	0.0047	Dominant
<b>Tibia trabecular vBMD (mg/cm<sup>3</sup>)</b>					
rs6935458	232.7(2.011)	228.0(1.736)	226.8(2.608)	0.0416	Dominant
rs6916495	230.2(1.323)	227.2(2.689)	209.8(9.741)	0.0429	Recessive
rs7768480	228.3(2.104)	227.6(1.695)	234.8(2.445)	0.0137	Recessive
<b>Tibia cortical vBMD (mg/cm<sup>3</sup>)</b>					
rs13211931	1178.1(0.730)	1183.2(1.646)	1185.6(6.314)	0.0026	Additive
rs1830971	1180.2(0.876)	1177.7(1.071)	1176.7(2.558)	0.0492	Dominant
rs858339	1177.9(0.950)	1180.8(0.997)	1179.6(1.941)	0.0450	Dominant
rs7749493	1179.1(0.845)	1177.8(1.144)	1186.2(2.627)	0.0055	Recessive

Genotype-specific means were adjusted for age, weight and height.

1 indicates major allele; 2, minor allele; aBMD, areal BMD; vBMD, volumetric BMD

## 6.0 GENERAL DISCUSSION

### 6.1 SUMMARY

Osteoporosis is a growing public health problem in men of all races and ethnicities. Much less is known about age-related bone loss and osteoporosis in men relative to women, especially in men of African heritage. This dissertation investigated the determinants of bone mineral density in a large cohort of Afro-Caribbean men aged 40 and older from the Tobago Bone Health Study. Three broad aims of this dissertation project were to: 1) examine the patterns and determinants of longitudinal age-related hip bone loss measured by DXA; 2) examine the age-related pattern and correlates of volumetric BMD and to compare and contrast findings for trabecular and cortical vBMD measured by pQCT; and 3) evaluate the relationship of polymorphisms in the *ENPPI* gene with bone loss and BMD.

In the bone loss analyses, we observed that men of African descent had a comparable rate of decline in hip BMD to published data in Caucasian men (2-4,6,8) and the rate of bone loss across 5-year age groups appeared to have a U-shape relationship. Men aged 40-44 had a significantly greater rate of decline in BMD than those aged 45-49 and 50-54 at both the total hip and femoral neck. Thereafter, the rate of decline in BMD accelerated with advancing age through the 7<sup>th</sup> decade. We also identified low body weight, weight loss, prostate cancer and androgen deprivation treatment for prostate cancer as potential determinants of accelerated bone loss in

this population. However, the variation in rate of bone loss explained by these factors was only 6% to 7%. Our study also confirmed the modulating effects of weight loss and older age on bone loss that has been observed in previous studies of Caucasian men (3,5-7,73).

In the analysis of vBMD, we observed a non-linear reduction of trabecular vBMD with aging where a larger decline was found among men aged 40-44 and 45-49 years, compared with those aged 50 and older. This finding is consistent with the recent observation of an early reduction of trabecular vBMD with aging in Caucasian men (131,134). In contrast, cortical vBMD appeared to decline more slowly and steadily with aging than trabecular vBMD, which was also observed in another study (131). Body weight appeared to be a protective factor for trabecular vBMD but a risk factor for cortical vBMD. The reverse relationship between body weight and cortical vBMD is consistent with another recent study (153). Diabetes and bone chewing were also identified as beneficial correlates of vBMD, whereas cigarette smoking, prostate cancer and treatment for prostate cancer with androgen deprivation were identified as potential risk factors for lower vBMD. Moreover, these determinants did not have the same effects on vBMD across different skeletal sites and only explained 6% to 16% variation in vBMD.

In the genetic analysis, several polymorphisms in the *ENPP1* gene were found to be associated with bone loss and areal and volumetric BMD independent of age, weight and height. For femoral neck bone loss, total hip BMD and trabecular vBMD, only one SNP was associated with each of these phenotypes with a nominal p-value<0.01. However, there were 4 SNPs that appeared to have strong association with cortical vBMD at P<0.01. The commonly tested missense SNP in *ENPP1* (rs1044498) that has been associated with insulin resistance and

abnormal calcification (150,151,154-158) was not associated with any BMD measures in our study.

## 6.2 CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Our study sought to determine potential demographic, anthropometric, medical, behavioral and genetic factors associated with bone loss and BMD in a large cohort of men with a high African ancestral proportion. Our study also used a unique imaging scan data obtained by pQCT that provided a true measure of volumetric BMD for cortical and trabecular bone. To our knowledge, this is first study to examine the age-related patterns and determinants of trabecular and cortical vBMD, and the second and largest study to examine age-related bone loss and its determinants in a group of middle- and older age men of African descent. This is also the first study, to our knowledge, to examine the relationship between *ENPP1* gene variants and vBMD in men of African descent.

Indeed, we were able to demonstrate a significant rate of decline in BMD among this population despite their high BMD and low osteoporosis risk. We also identified several anthropometric, behavioral and medical factors that may be associated with accelerated bone loss and lower vBMD. However, these factors only account for a small proportion of the variation in age-related bone loss and vBMD. This suggests that many other undetermined variables, including inherited factors, may contribute to age-related bone loss and vBMD. We were also able to establish an association of SNPs in the *ENPP1* gene with bone density, especially cortical vBMD, and the rate of hip bone loss in this cohort.



Our study had several potential limitations including: 1) a small proportion of older-aged men that may have limited our ability to estimate age-related patterns in vBMD and the rate of bone loss among these older men; 2) DXA measures of bone loss that are unable to provide insight on age-related loss of trabecular and cortical bone; 3) questionnaire assessments of medical and behavioral history that may be subject to recall bias and misclassification; 4) cross-sectional measures of vBMD that limits our ability to establish temporal relationships with trabecular and cortical vBMD; and 5) the use of multiple SNPs and inheritance models to test the association between *ENPP1* gene variants and bone measures generates issues of multiple comparisons and possible false positive findings; However, our study also had notable strengths including: 1) its large sample size and focus on men of African descent with low Caucasian admixture; 2) careful measurement of variables; 3) focus on both cortical and trabecular volumetric BMD; and 4) the wealth of information available about the study cohort. Our results validated associations described previously among Caucasian men, such as the major importance of body weight in determining BMD and bone loss, but also illuminate several previously unrecognized relationships.

Additional longitudinal studies are needed to confirm our findings of a non-linear relationship of age and bone loss in men of African heritage. Specially, the impact of aging on trabecular and cortical volumetric BMD in this population deserves further investigation. Although osteoporosis largely occurs later in life, there is a need to recruit younger adults for longitudinal studies of bone loss. With the increasing body of evidence showing that development of osteoporosis and osteoporotic fractures is due to factors other than or in addition to low bone density, it will be important to characterize other bone health parameters, such as

cortical and trabecular thickness and bone structural geometry and to identify the behavioral and genetic determinants of these parameters. In addition, more studies are also needed to understand the differences in trabecular and cortical bone loss with aging and the correlates of this loss including behavioral/lifestyle and medical characteristics, and hormonal and genetic factors.

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

### **6.3 PUBLIC HEALTH SIGNIFICANCE**

Osteoporosis, an important risk factor of osteoporotic fractures, is a public health problem that is not only prevalent in women but also in men. In the US alone, 1-2 million Caucasian men are affected with osteoporosis, and another 8-13 million are affected with osteopenia (20). As lifespan increases worldwide in the next several decades, the prevalence of osteoporosis and its associated fractures is expected to increase significantly not only among Caucasians, but also among people of African descent (21-23). In addition, despite the lower prevalence of osteoporosis and fractures in people of African ancestry, a higher mortality after a fracture has been observed among African Americans and men, compared to Caucasians and women (1,26,27). Moreover, the direct medical costs for osteoporotic fractures is greater than the projected annual cost of stroke, breast cancer, diabetes, or chronic lung disease (30) and the total cost due to osteoporotic fractures is predicted to increase more in African Americans than Caucasians (28).

Identifying factors associated with the rate of bone loss and low BMD may facilitate the development of strategies to better prevent osteoporosis. Our study revealed that men of African descent may have a comparable rate of hip bone loss with Caucasian men, and also confirmed an important role of low body weight and its weight loss in accelerated bone loss with age which are both potentially modifiable risk factors.

We reported in our study that behavioral factors only account for a small proportion of the variation in vBMD and rate of decline in aBMD. With the high heritability observed in BMD (11-13) , it is likely that genetic factors contribute greatly to the remaining unexplained variation. The present study also demonstrated a relationship of allelic variants in *ENPP1*, a gene involved in bone mineralization, with BMD and bone loss. Further research on the genetic susceptibility to low bone density and accelerated bone loss may help to identify new therapeutic targets to treat or prevent osteoporosis.

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