ADIPOSITY, RACE AND BONE STRENGTH

by

NORMAN KENNETH POLLOCK

(Under the Direction of Richard D. Lewis)

ABSTRACT

To date, studies have not investigated the relationships between adiposity, race and bone strength indices measured by three-dimensional bone imaging techniques. The purpose of this research is to determine the associations between measures of adiposity and bone strength, using pQCT, and whether these relationships vary by race. The first study (Chapter 3) addresses the relationships of percent body fat and bone strength parameters, assessed by pQCT, in predominately white late adolescent females (*N*=115; aged 18-19 years), taking into consideration surrogates of muscle force [i.e., MCSA and bone length]. Bone measurements in normal- and high-fat groups were also compared. Results showed that excess weight in the form of fat mass does not provide additional benefits, and may potentially be negative, for bone in late adolescent females. The second study presented in Chapter 4 was conducted in 18-19 year old white (*n*=25) and black (*n*=25) females individually matched on age, height, FFST mass, and weight to determine whether there are racial differences in bone strength parameters, assessed by pQCT. Results suggested that at the tibia, differences in bone strength are evident between black and white females; however, at the radius, these differences are less clear. In Chapter 5, relations between total fat mass and pQCT-assessed trabecular and cortical bone measurements within the tibia and radius were investigated in black females (*N*=48; aged 18-22 years). Since height, limb lengths

and surrogates of muscle loads may confound total fat mass and bone outcome variables, these fat and bone relationships were observed independent of the following variables: height, limb lengths for each respective bone site, FFST mass, and MCSA for each respective bone site. The second objective was to compare tibial and radial bone measurements between two adiposity groups defined as having normal and high percentages of body fat, before and after controlling for differences in the same confounding variables. Consistent with the adiposity and bone strength analyses in a predominately white sample of late adolescent females, these findings in black females entering adulthood also suggest that excess adiposity levels may adversely influence the overall strength of cortical bone at appendicular skeletal sites.

INDEX WORDS: pQCT, LATE ADOLESCENT, BONE STRENGTH, BODY COMPOSITION, OBESITY, RACE, ETHNICITY, AFRICAN AMERICAN, ADIPOSITY, OBESITY, BONE GEOMETRY, QUANTITATIVE COMPUTED TOMOGRAPHY

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by

NORMAN KENNETH POLLOCK

B.S.Ed., The University of Georgia, 1998

B.S.F.C.S., The University of Georgia, 2002

M.S., The University of Georgia, 2004

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NORMAN KENNETH POLLOCK

Major Professor:	Richard D. Lewis
Committee:	Clifton A. Baile Mark W. Hamrick Daniel B. Hall Ruth B. Harris

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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DEDICATION

This thesis is dedicated to my dear wife, Traci, for all her support and love. Thank you for coming into my life because none of this would mean anything without you.

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CHAPTER 1

INTRODUCTION

The incidence of obesity, defined as an excess storage of fat tissue, has progressively escalated in the United States (US). It is estimated that since the 1970's, obesity rates have doubled in adults aged 20 years or older and has tripled in children and adolescents aged 6 to 19 years.[1, 2] The direct costs associated with obesity in the US is approximately $80 billion per year, having represented 9% of the national health expenditures in 1998.[3] Furthermore, obesity is associated with many other debilitating diseases, such as type 2 diabetes mellitus, hypertension, coronary heart disease, and some cancers.[4] Thus, obesity has become a serious national health concern.

Osteoporosis, another major health problem, is characterized by low bone mass and microarchitectural deterioration leading to a reduction in bone strength with a resulting increase in the susceptibility to fracture.[5] Approximately 200 million people worldwide are affected by osteoporosis, and the prevalence is continuing to increase, primarily due to the aging of the population.[6, 7] The prevalence of osteoporosis in the US is expected to increase from 10 to 12 million among individuals over the age of 50 years by 2010, and to nearly 14 million by 2020.[8] An important consequence of osteoporotic fractures is the enormous economic burden. The estimated US healthcare costs amounted to $18 billion in 2002, and is projected over the next decade to approach $45 billion per year.[9]

Obesity and osteoporosis are two complex diseases with multifactorial etiologies and only recently, has the possibility been raised that the two diseases could be linked.

Similarities between obesity and osteoporosis include the following:

- Both diseases are affected by genetic and environmental factors, or the interaction between them, and there is some overlap between the genetic and environmental factors influencing both diseases.[10]
- Normal aging processes are generally associated with an increase in percentage body fat mass and a gradual loss in bone strength.[11]
- Bone remodeling and adiposity are both regulated through the hypothalamus and sympathetic nervous system.[12]
- Adipocytes (the cells for storing fat) and osteoblasts (the cells for forming bone) both derive from mesenchymal stem cells.[13]

Elucidation of the parallel mechanisms between fat and bone is vital not only for understanding basic biology, but also for the improvement of public health.

It is often implied that a high areal bone mineral density (aBMD) is associated with a high body weight and increased bone strength. Overweight children have been reported to have higher lumbar spine or total bone mineral content (BMC) relative to height, maturation and/or fat-free soft-tissue mass.[14-16] Leonard et al.[15] observed that overweight, based on BMI-for-age percentiles, in boys and girls aged four to 20 years, was a predictive factor for higher lumbar spine aBMD corrected for height. Ellis et al.[14] grouped children by percent body fat (<25%, 25-30% and >30% body fat) and found that the >30% body fat group had significantly higher total body BMC relative to height.

This paradigm that a higher body weight is associated with improved bone strength has recently been questioned.[10] Goulding and colleagues[17, 18] observed that overweight children had lower lumbar spine and total body BMC and bone area relative to weight compared to children

with normal BMI-for-age percentiles. Moreover, Goulding et al.[19] found that a high body weight, independent of total body lean mass, contributed to fracture risk in children and adolescents who had fractured their forearms repeatedly. In another fracture study of females, four to 15 years of age, it was found that those who sustained a fracture were more overweight and had a smaller forearm cross-sectional area compared to girls who did not experience a fracture.[20]

In both research and clinical settings, the current methodology used to assess bone strength and to predict fracture risk is based on aBMD assessed by dual energy X-ray absorptiometry (DXA).[21, 22] The methodology used to estimate bone strength in these aforementioned childhood investigations was DXA, and the limitations associated with its 2-dimensional imaging may have contributed to these contrasted findings.[14-20, 23-25] The use of DXA for assessment of bone strength is limited by its 2-dimensional capabilities for determining the material properties and therefore its inability to assess the geometric properties of bone. More accurate predictions of bone strength require 3-dimensional bone assessments of both the material (e.g., mineral density) and geometric (e.g., size and shape) properties of bone.[21, 26-28] Quantitative computed tomography (QCT) and peripheral QCT (pQCT) have the capability to determine, in three dimensions, the volumetric BMD and geometry of the bone as well as a strength-strain index that can be calculated from the geometric and material measurements. More importantly, QCT and pQCT can differentiate trabecular and cortical bone, which is their main advantage over DXA.[29, 30] Separating the two types of bone is extremely advantageous in bone and adiposity research, since each may respond differently to stimuli such as hormonal changes and weight-bearing forces.

Recently, we reported that excess weight in the form of fat mass does not provide additional benefits to material and geometric measurements of bone strength, relative to site-

specific muscle size and bone length, in predominately white late adolescent females, aged 18 to 19 years (Chapter 3).[31] Using pQCT, we found in high-fat (>32 percentage body fat) females that polar strength strain index, an estimate of torsional bone strenth,[32] was lower that normal-fat (<32 percentage body fat) females at cortical bone sites of the tibia (8.7%) and radius (8.0%). In another study, consisting of 13 to 21 year old males and females, Janicka et al.[30] found that total fat mass was negatively associated with bone structure, assessed by 3-dimensional imaging, at axial and appendicular sites, after accounting for total body lean mass and height. Why bone strength and structure are not adapting appropriately to the excess weight of fat mass relative to the surrogates of loading forces is unknown.

Although osteoporotic fractures occur at twice the rate in white compared to black females, recent reports have suggested that approximately 1.5 million black women have poor bone health and are at-risk for skeletal fractures.[33-37] The higher osteoporotic fracture rates in white compared to black females may be related to a racial dimorphism in skeletal strength that is thought to emerge during growth. However, the actual existence of racial differences in bone strength has been a controversial issue, primarily due to the methodology employed, the skeletal sites measured and the statistical adjustments of data.

Whether racial/ethnic differences in bone strength exist is a controversial issue, primarily due to how and what skeletal site is measured and its interpretation. Blacks have been shown to have higher aBMD than whites, which tracks from prepuberty[38, 39] to late puberty.[40-42] Bell and colleagues[40] found that black compared to white children, between the ages of 7 and 12 years, have higher spine aBMD. By contrast, Wang et al.[43] and Hui et al.[44] reported no racial differences in aBMD or bone mineral content (BMC) at the lumbar spine, assessed by DXA, in 4 to 25 year olds. The discrepancies among the aforementioned DXA studies[38-44] may be explained

by the authors various combinations of control variables used in the statistical analyses in an attempt to minimize the influence of confounders such as sex, height, weight, age, body composition, and sexual maturation.

To date, only two 3-dimensional imaging studies have investigated the effects of race on bone strength parameters in children, while taking into consideration bone size and body size differences.[45, 46] Gilsanz and colleagues[45] used QCT to assess lumbar spine volumetric BMD (vBMD) in 75 pairs of black and white females between the ages of 2 and 20 years, matched for age and sexual maturation. The lumbar spine vBMD did not differ between prepubertal black and white females. However, in the late puberty analyses, the black females were found to have 23% greater lumbar spine vBMD than white females. In the second QCT investigation[24] the same group of investigators matched 80 pairs of black and white males and females, aged 8 to 18 years, for age, sex, weight, height, and sexual maturation to assess racial differences in vBMD and cross-sectional area at the lumbar spine and femoral midshaft. At the lumbar spine, blacks were found to have higher vBMD than whites, but similar cross-sectional area. Conversely, at the femur, no differences were found in vBMD; however, the black versus white females had greater cross-sectional area, which the authors attributed to their longer femoral bone length. [24] The axial skeleton advantages described in black children are likely important determinants of greater resistance to skeletal fractures later life.[45, 46] These meticulously designed studies, using 3-dimensional imaging, provide significant clues to racial differences in bone strength at weight-bearing skeletal regions. Similar investigations are warranted at non-weight bearing skeletal sites, particularly since an increase in distal forearm fractures in children and adolescents have been observed over the past few decades.[47]

Currently, only one study has investigated bone and fat relations in black children.[48] Afghani and Goran[48] found an inverse correlation between subcutaneous abdominal adipose tissue, using QCT, and BMC, measured by DXA, in white children, but not in black children. They also found an inverse association between intra-abdominal adipose tissue and BMC in black, but not in white children. Taylor et al.[49] found higher fracture rates in overweight versus non-overweight children and almost 47% of the fracture cases in the overweight group were among the black children (personal communication with author). Moreover, another study in postmenopausal adults indicated that for every unit increase in BMI, the odds of low lumbar spine aBMD decrease for whites but increase for blacks.[50] In light of these studies, reduced bone strength and the risk for osteoporotic fractures cannot be discounted in blacks, particularly young overweight black females. The National Health and Nutrition Examination Survey, 2003-2006,[51] estimated that the overweight prevalence among black females, aged 12 to 19 years, was almost double the rate of whites (28% versus 15%). Recently, we found in predominantly white females that excess weight in the form of fat mass does not provide additional benefits, and may potentially be negative, for adolescent bone (Chapter 3). Given that young black females are becoming exceedingly heavier, it is important to assess whether excess adiposity levels associated with being overweight may have negative consequences on bone health in blacks.

To date, studies have not investigated the relationships between race, adiposity and bone strength indices measured by 3-dimensional bone imaging techniques. The purpose of this research is to determine the associations between measures of adiposity and bone strength, using pQCT, and whether these relationships vary by race. The first study (Chapter 3) addresses the relationships of percent body fat and bone strength parameters, assessed by pQCT, in predominately white late adolescent females, taking into consideration surrogates of muscle

force [i.e., muscle cross-sectional area (MCSA) and bone length]. Bone measurements in normal- and high-fat groups were also compared. It was found that excess weight in the form of fat mass does not provide additional benefits, and may potentially be negative, for bone in late adolescent females.

In order to determine whether relationships between adiposity and bone strength vary by race, the study in Chapter 4 investigated racial differences in bone strength, assessed by pQCT, in black and white females. Because of the importance of body size on bone strength development, 25 whites and 25 blacks were individually matched on age, height, fat-free soft-tissue mass, and weight. Our data suggest that at a weight-bearing site (tibia), differences in bone strength are evident between black and white females; however, at a non-weight bearing site (radius), these differences are less clear.

In Chapter 3 we determined relationships between adiposity and bone strength measurements, using pQCT, in 115 late adolescent females, however, only 2 of the participants were black. As a result, we sought to investigate in a larger sample of young African American females the relations between total fat mass and pQCT-assessed trabecular and cortical bone measurements within the tibia and radius (Chapter 5). Since height, limb lengths and surrogates of muscle loads [e.g., total body fat-free soft tissue (FFST) mass and/or MCSA] may confound total fat mass and bone outcome variables, we elected to observe these fat and bone relationships independent of the following variables: height, limb lengths for each respective bone site, FFST mass, and MCSA for each respective bone site. The second objective was to compare tibial and radial bone measurements between two adiposity groups defined as having normal and high percentages of body fat, before and after controlling for any differences in the same confounding variables (i.e., height, limb lengths for each respective bone site, FFST mass, and MCSA for

each respective bone site). Consistent with our adiposity and bone strength analyses in a predominately white sample of late adolescent females, our findings in black females entering adulthood also suggest that excess adiposity levels may adversely influence the overall strength of cortical bone at appendicular skeletal sites.

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CHAPTER 2

REVIEW OF THE LITERATURE

In the past decade there has been interest in understanding the impact of excess adiposity on bone health not only in whites but also in other race/ethnicities. In this review, the following topics will be discussed that provide the basis for better understanding the interrelations between bone, fat and race: bone biology, noninvasive assessment of bone strength, muscle-bone unit, adiposity and bone, and the relations between race/ethnicity and the skeleton.

Bone biology

Bone is a dynamic tissue that continually adapts to functional needs to produce a structure that is strong enough to prevent fractures in most activities. The skeleton serves the function of movement, acts as a protector of vital organs, provides an environment for storage of calcium and phosphorus, and serves as a site for blood cell formation.[1] Ninety-eight percent of bone is an organic matrix made up of type I collagen and noncollagenous proteins, while the remaining 2% is composed of inorganic material, consisting of enmeshed hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] containing primarily calcium and phosphorus.[2] The architecture of the skeleton adapts to provide adequate strength and mobility so that bones do not break when subjected to substantial impact, even the loads placed on bone during high-impact activity.

Types of bone

The osseous compartments of long bones generally fall into two categories, cortical (compact) bone and trabecular (cancellous) bone (Figure 2.1). Cortical bone is the dense tissue forming the outer shell of bone and ranges in porosity from 5 to 10%.[3] The skeleton is

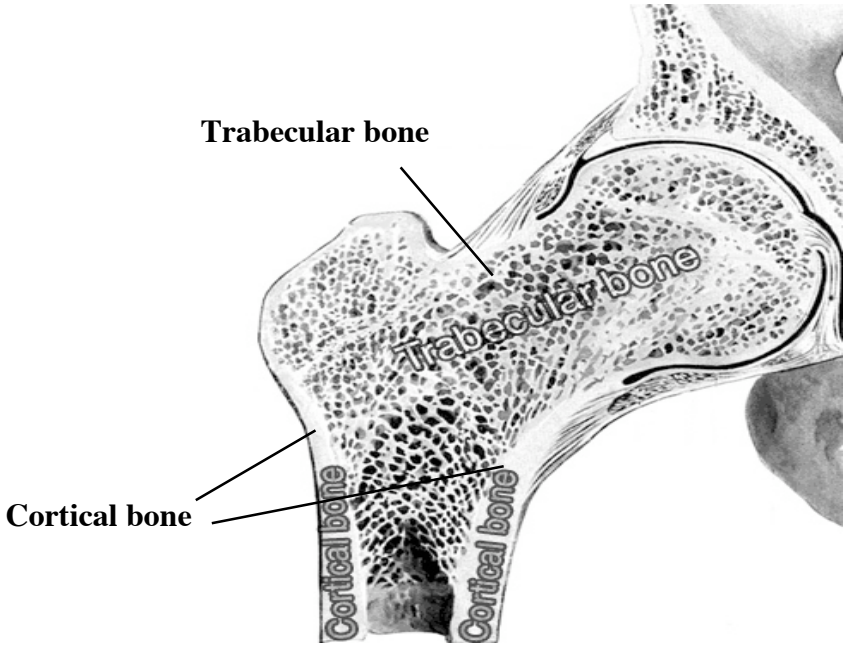

Figure 2.1 **Trabecular and cortical bone at the femoral neck**. Adapted from Gregory et al., 2004. [4]

approximately 75 to 80% cortical bone by mass with the remaining 20-25% being trabecular bone.[1] Trabecular bone forms a matrix of thin plates, approximately 0.10 mm wide and 1 mm long,[5] which are distributed throughout a space encompassed by cortical bone. Trabecular bone is found in the vertebral bodies, the ends of long bones and in some flat bones and has a porosity of 75 to 95%,[1] giving it a sponge-like appearance. The greater porosity of trabecular bone gives it greater surface area to volume ratio compared to cortical bone; consequently, trabecular bone is thought to experience higher metabolic activity and turnover.[3, 6] Additionally, the small diameters of individual trabeculae make them susceptible to erosion in the presence of resorptive trends in bone remodeling, and once they are dissociated, it is thought that they cannot be reformed.[7]

Modeling and remodeling of bone

To maintain its functions, bone tissue is constantly turned over by processes referred to as modeling and remodeling.[2] Modeling refers to alterations in the shape of bone, whereas remodeling refers to turnover of bone that does not alter its shape.[2] However, these two processes occur often simultaneously and the distinctions between them may not be as distinct as once thought.[8]

Bone formation begins in utero and continues throughout adolescence until skeletal maturity.[2] Formation of bone within cartilage enables longitudinal skeletal growth, and enlarged width is a result of modeling within the organic matrix membrane and deposition of new bone on the existing surface.[1, 2] Following skeletal maturity, remodeling continues throughout life in order to maintain an adequate structure within a safety margin of normal mechanical demands. This is balanced by the cost of excessive bone mass on mobility.[9] Remodeling also provides a mechanism to repair the damage created in bone by repetitive cycles of mechanical loading, and it enables the alteration of the essential minerals by increasing or decreasing their concentration in serum.[10]

Bone undergoes both formation and resorption throughout the lifecycle. Three types of bone cells, osteoblasts, osteocytes, and osteoclasts, are primarily involved with either formation or resorption. To form bone, mesenchymal stem cells produce osteoprogenitor blood cells that likely differentiate into single nucleated, bone-forming osteoblasts.[11] Later, these cells mature into osteocytes and lose some of their cell organelles once incorporated into the bone matrix within the lacunae.[11] Once established within the cell matrix, bone formation by these cells ceases and the tissue becomes highly mineralized. The osteocytes facilitate communication between adjacent cells within the mineralized matrix via gap junctions.[11]

Bone resorption occurs primarily as a function of the multi-nucleated, large cell osteoclast that may originate from circulating mononuclear progenitor cells.[12] The characteristic feature of the osteoclast is the ruffled border surrounded by a ring of contractile protein. This border serves to attach the osteoclast to the bone surface and create what is known as the extracellular bone-resorbing compartment.[13] Lysosomal enzymes are actively synthesized in the osteoclast and then secreted, via the ruffled border, into the extracellular bone-resorbing compartment where a high concentration of enzymes develops to resorb bone.[3] The acidic environment digests the noncollagenenous link between hydroxyapatite crystals and collagen, allowing calcium to be released from the skeleton.[3] Together, the osteoblast, osteocyte, and osteoclast comprise the small basic multicellular units (BMU) where the process of remodeling occurs within the cortical and trabecular bone.[14] The fact that osteoclastic bone resorption and osteoblastic bone formation follow each other is fundamental to the concept of the BMU, which describes a packet of bone being resorbed or rebuilt.[15] Figure 2.2 depicts the coupling of osteoclast and osteoblast function. Bone resorption initiates bone formation, which, under balanced conditions, restores lost bone.[3] These active bone cells, which are known to act in response to various environmental signals including chemical, mechanical, electrical, and magnetic stimuli, are essential for the modeling and remodeling processes within bone.[2, 16, 17] The balance between modeling and remodeling differs between the growing and adult skeleton. In the former, modeling is the dominant mode, whereas in the latter, remodeling is dominant.[18]

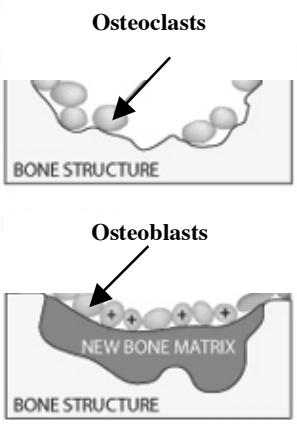

Figure 2.2. **Osteoblast and osteoclasts**. Adapted from Marks et al., 2002[4]

Modeling, seen in early childhood up to early adulthood, is the process in which bones become larger, heavier, and denser; hence, osteoblastic activity exceeds osteoclastic activity. This uncoupled process with osteoblasts and osteoclasts improves bone strength not only by adding mass, but also by expanding the periosteal (outer) and endosteal (inner) diameters of bone (Figure 2.3).[19]

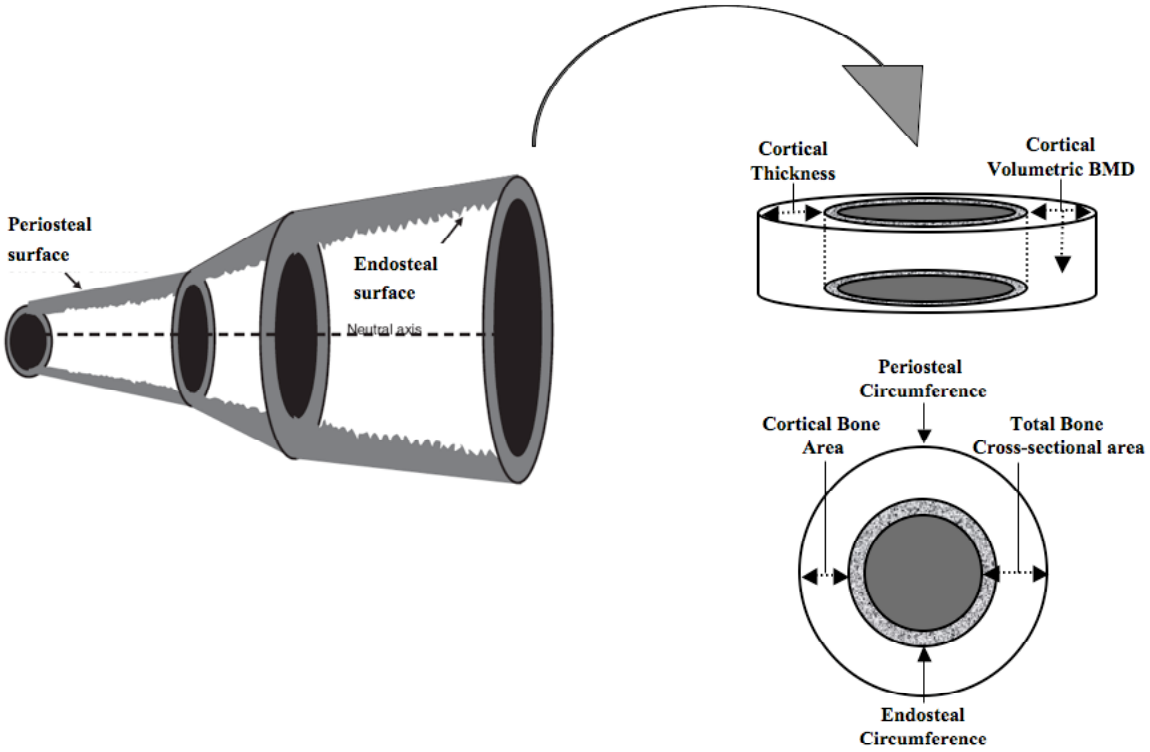

Figure 2.3. **A theoretical model of long bone development during the lifecycle, along with bone geometry parameters currently assessed by 3-dimensional imaging**. Adapted from Seeman et al, 2008[44] and Pollock et al., 2007[20]

Bone remodeling begins to take over in adulthood, where bone mass undergoes constant and equal removal of old bone and renewal with newly formed bone.[7] An equilibrium exists between bone resorption and formation until the fourth or fifth decade of life, when bone resorption begins to supercede the continually declining bone formation process.[18] In the situations of an aging skeleton, the balance between the amount of bone resorbed and formed is

shifted in favor of resorption, thereby resulting in a net loss of bone.[21, 22] Bone remodeling supports response and adaptation to mechanical stresses and metabolic demands of the body, as well as repairing skeletal damage, preserving bone strength, and maintaining mineral homeostasis throughout adulthood.[16, 21, 23] This process is regulated by complex interactions between genetic, hormonal, and environmental factors working to preserve the mechanical structure of the skeleton.[16, 22]

Form follows formation

Bone has been defined as an organ of optimal structural design to serve its functions.[24] A whole bone's mechanical integrity depends mostly on the size, cross-sectional geometry, mass distribution, and internal architecture, whereas material properties vary less, and thus seem less important.[25] Although both cortical and trabecular bone have the same material properties, the difference in mineral distribution and microachitecture within and between the texture of these two compartments, explains the differences in the mechanical properties of specific bone and parts of bones.[9] Generally, biomechanical properties can be material and structural.[2, 26]

Inorganic matrix, such as mineral mass, mainly determines bone's rigidity as a material whereas the organic component of the tissue is responsible for the elasticity of the material, allowing the transient deformation of the bone under the applied loads.[27] After the external load is removed, bone will return to its original shape unless the applied loads exceed the yield point.[25] Without sufficient elastic properties, bone would be brittle and unable to withstand any substantial forces.

Mechanical properties of whole bone depend on the orientation of the applied forces, and bone optimally withstands loads that are applied in the direction of customary loading.[28] The form of long bones, as thick-walled tubes and a dense cortical bone diaphysis, provide adequate

stiffness against torsion and bending with the minimal mass required.[29] The longitudinal orientation of the osteons explain, in part, why diaphyseal cortical bone is strong in both tension and compression when it is loaded parallel rather than perpendicular to its long axis.[30] In the metaphysis and epiphyses, the wide bone ends filled with trabecular bone broading the bone end to form an articular surface.[30] They also help cope with axial compression and spread out the applied load across the synovial cartilage.[28] Similarly, in short and flat bones, the thin cortices are supported by trabecular bone inside. This structural form resists compression and impacts better than would cortical bone alone by allowing deformation to occur.[27] The arrangement of trabecular bone in positions of maximum stresses is such that the greatest strength is secured with minimum material.[25]

Although applied force can be directed to bone from any angle producing any set of complex stress patterns, all stresses can be resolved into three types: tension, compression, and shear.[31, 32] The three stress types can result in a variety of complex loading configurations and may lead to different fracture patterns.[3, 33] Tensile force can produce a failure in bone when a tendon or ligament inserted into bone undergoes loading and detaches itself from the bone by pulling a piece of bone off with it.[9] These types of fractures occur particularly during childhood, because before the growth plates are closed, the structure of bone is relatively frail.[34, 35] The common vertebral fracture sustained in osteoporotic patients is an example of the failure of bone as result of compressive loading configuration.[36] Bending results in a combination of tensile and compressive stresses, and torsion produces shear stresses along the entire length of bone and can result in a spiral fracture.[2]

Achieving and maintaining mechanically appropriate bone mass and structure by loading is better understood at the tissue level than at the cellular level.[37] The skeleton's ability to adapt

to the functional demands was recognized over a century ago, and has been since referred as Wolff's law.[38] The basic premise of this law is that an individual's level of activity transforms the mass and morphology of the skeleton such that it is sufficient to withstand functional loads but not so much as to make transportation a metabolic liability.[39, 40] At the tissue level, this can be obtained by adjusting characteristics of bone strength (mass, size, shape, cortical thickness, and geometry) in a direction that tends to keep the internal strains within an acceptable biological level.[41] Modeling improves geometric properties by adding material where customary deformation is greatest.[19]Adding new bone on the periosteal surface and resorbing bone on the endocortical site increases section modulus and hereby diminishes the relative deformation of the predisposed load.[19] Endocortical strains have been estimated to less than those at the periosteum, and are thus explained to be insufficient to initiate a bone formation response.[41] However, even a lower threshold at the endocortical site may be adequate to maintain bone mass and geometry, and possibly important to prevent cortical thinning during aging.[18] At the distal part of the lower extremities, where locomotion and body weight induced strains are higher, the remodeling threshold could also be higher compared with proximal parts of these bones.[18]

A number of specific components have been proposed as the dominant stimulus for transformation of mechanical stimuli into biochemical signals for bone formation. Besides the different characteristics of strain (magnitude, cycles, rate, and distribution), these include prostaglandin release, shear-induced fluid flow, electric potentials, piezoelectric currents, microdamage, and hormonally mediated mechanisms.[26, 27, 30, 42] Evidence from experimental studies suggest that cyclic loading can move strain-mediated fluid-flow through the canalicular channels, and that shear stresses generated on bone cells seem to be proportional to the rate of loading.[26, 42] Deformation and fatigue damage experienced by the whole bone due to mechanical

loading may also form an amplified strain. [26] The sensor for these signals is likely the lining cell-osteocyte complex.[26]

Bones must be stiff so that they do not bend when loaded; however, bones must also be flexible so they can absorb the energy imposed by loading.[43, 44] The foremost purpose of bone modeling and remodeling is to adapt both the material (e.g., mineral density and mass) and geometric (e.g., size and shape) properties of bone to prevailing loads, ultimately, to resist skeletal fracturing.[45] The contributions made by differences in material and geometric properties of bone to disparities among the population in skeletal fracture remain poorly defined.[44] The challenge, however, is to measure these specific determinants of bone strength.

Noninvasive measurement of bone strength

The innovation of new research tools for the evaluation and characterization of bone strength has greatly broadened our understanding of skeletal fractures. Bone fragility is influenced by bone size, shape, structure, microarchitecture, and quantity and quality of the tissue.[27, 28, 32, 36] In both research and clinical settings, the current method of choice to assess bone strength and fracture risk is based on noninvasive assessment of bone mineral by dual energy X-ray absorptiometry (DXA).[28, 46] Though bone mineral measurements are useful, the regional averaging obscures structural differences or alterations that may be critical determinants of bone strength.[47] Consequently, measurements of bone mineral should be complemented by methods that provide mechanically more relevant information on bone structure. However, despite the evidence of the importance of structural architecture in the assessment of bone integrity, it is not yet fully understood whether or which structural measures will prove to be indices of fracture likelihood. As a result, the need for more harmless, accurate, reproducible and inexpensive bone

measurement techniques that can measure both the material and structural properties of different sites of the skeleton is evident.[44]

Dual energy X-ray absorptiometry

DXA was introduced commercially in the late 1980's and it determines bone mineral content (BMC; grams) and bone area (cm^2) in order to calculate areal bone mineral density (aBMD; g/cm^2), its most relevant index of bone strength in adults. DXA has many benefits, which include: precise measurements, short scan times, low radiation dose and aBMD predicts the fracture risk at the population level. [48] Consequently, it is suitable for most clinical purposes.

The normalization of BMC by the "projected" bone area partially reduces the effect of body size.[49] While minimizing the differences between large-boned and small-boned individuals, aBMD facilitates the assessment of what is "normal" in the process of screening, which actually has been a principal target market for the technology.[49] Although aBMD values are good predictors of fracture risk in older adults at the population level, they cannot specifically identify individuals who will eventually get a fracture.[50]

To improve the predictive value of DXA data, analytic strategies have been proposed to assess bone's volumetric density and structure. For example, bone mineral apparent density (BMAD), assuming a constant cylindrical shape of bone, can be calculated as BMC per estimated total bone volume.[51, 52] This adjustment for estimates of bone size was proposed in order to alleviate the effects of variation in regional bone size and shape during the skeletal growth.[51] BMAD, however, has not achieved accepted use in the bone densitometry field. One reason for this can be its inability to improve the predictive value of aBMD for future hip fractures.[53] On the other hand, the Hip Structure Analysis (HSA) software has been shown to predict breaking strength of the femoral neck and hip fracture better than aBMD alone.[47, 54] HSA

might prove to be a reasonable enhancement of DXA densitometry in clinical practice; and recently, this methodology has been incorporated into Hologic's (Hologic, Inc., Bedford, MA) DXA bone densitometers.[55]

The planar nature of DXA, however, makes the assessment of the geometry and true composition of bone inaccurate; and therefore, the evaluation of bone fragility of an individual is likely to be an approximation at best. In addition, DXA measurements are subjected to considerable patient-specific inaccuracies, and can thus seriously mislead the diagnostic or prognostic interpretations of individual bone fragility.[56] Likewise, the relevance of the 2-dimensional nature of DXA and its aBMD value for estimating bone strength at the individual level has been challenged, and the need for additional sophisticated noninvasive methods to characterize bone strength more accurately has been recommended.[44, 57-59]

Peripheral quantitative computed tomography

Compared to DXA, the advantage of quantitative computed tomography (QCT) is its ability to determine, in three dimensions, the volumetric BMD (vBMD) of both trabecular and cortical bone compartments at any skeletal site.[49] QCT also provides assessment of bone size and geometry, without the influence from body size or skeletal size, which is a main advantage when used to assess and compare bone development during childhood.[60] However, the primary disadvantage of QCT is its high radiation doses, making it unsuitable for use in children.[61] Moreover, QCT scanners are expensive, large, non-portable machines that require costly maintenance.[61] These disadvantages, nevertheless, have partially been overcome by the recent development of peripheral QCT (pQCT), which are smaller, more mobile, lower in radiation and less expensive that the QCT.[61]

Peripheral QCT is a type of computed tomography that, like the QCT, provides a 3-dimensional assessment of bone size and geometry of the appendicular skeleton. In addition, pQCT can give analysis of cortical and trabecular vBMD and derivation of specific geometric parameters of cortical bone from cross-sectional images. Although the pQCT method is not routinely used currently in the U.S. for clinical purposes, its popularity has grown in Europe among pediatricians and pediatric bone researchers in the U.S. The first commercial pQCT scanners were produced in Germany and became available by the early 1990s.[62] An advantage of the new technology was its ability to separate trabecular bone tissue, generally found at the end of the long bones, from cortical bone tissue found in the shaft.[62] By the mid to late 1990s, the technology was being assessed for accuracy and precision[63] and being used to investigate geometric and biomechanical properties of bone,[64] establish reference data,[65]correlate muscle and bone strength[66] and obtain measurements in children.[67]

pQCT outcome measurements

Peripheral QCT images are normally acquired at the radius and/or tibia. The duration of time for scanning study participants varies depending on scan speed, voxel size and number of image slices chosen by the operator. Before scanning the subject, a scout view is obtained to locate the endplate of the bone and a reference line is selected. Images of the bone are then taken at the pre-measured distances from the reference line, generally a distance equal to a percentage of the bone length from the distal end (Figure 2.4). Typically, measurements are taken at two or more sites along the radius or tibia, where one site is composed predominately of trabecular bone (4% or 8% site) and the other mostly of cortical bone (14%, 20% or 33% site).

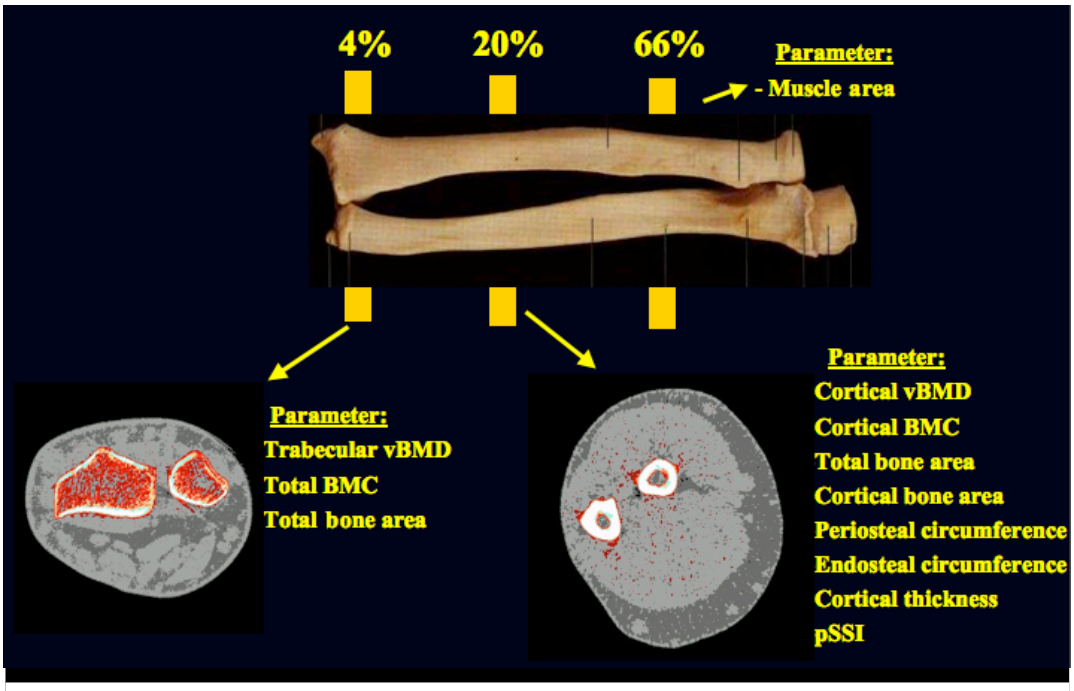

Figure 2.4 **Peripheral QCT slices at the 4%, 20% and 66% from the distal end of the radius can be obtained to calculate bone and muscle parameters.**

A greater number of bone outcomes and sites (along the radius and tibia) can be assessed by pQCT in comparison to DXA; however, there is no established consensus as to which are the most valuable to assess bone strength or predict fracture risk.

Measurements assessed by pQCT fall into broad categories of bone density, bone geometry, and bone strength. Table 2.1 defines the most commonly used pQCT outcome measures that can be assessed at the tibia and radius. The bone measurement of choice depends on whether it is a metaphyseal or diaphyseal site. For example, trabecular bone can only be measured at the metaphysis, since there is no trabecular bone at diaphyseal sites. Historically, the radius was measured by pQCT measurements because of its accessibility and vulnerability to fractures.[60, 67] However, the bones of children are smaller and thinner than those of adults, and are more subject to partial volume effects.[60, 68] Partial volume effect is related to the resolution of the

image and the size of the object being measured.[68] The pQCT bone image is processed from the attenuation of the X-ray beam for each voxel in the scan field. Voxels that are close to the edge of the bone are more likely to be comprised of both bone and soft tissue and will have a lower attenuation value than the voxels that are within the bone envelope and attenuated by bone only.[60] Smaller bones will have a higher proportion of voxels that are close to the bone edge and may thereby appear to have a lower density.[60] The tibia is larger than the radius, so some investigators have chosen the tibia for their measurement site to reduce partial volume effects.[60] The tibia is also a weight-bearing limb and is less susceptible to movement artifacts. Accordingly, there are several reasons why the tibia may be chosen for measurement.[60]

The use of pQCT in bone research has increased in the past decade. Investigations in healthy children have been done to test the effects of activity, bone loading, diet, pubertal stage and hormonal status on bone.[21, 69-73] Peripheral QCT measures of healthy children, although predominately white, have been used to establish reference ranges for bone growth patterns that can be used as a comparison when studying the bone status of children in disease states.[65, 68, 74] However, current published data on healthy children are not sufficient to serve as reference data for the clinical use of pQCT for fracture prediction or diagnosis of inadequate bone development. Until reference data are established, published reports can be used only for comparative purposes, provided the scan acquisition and analysis techniques are comparable.[60] Since cortical and trabecular bone respond differently to disease-related processes and medications, it is important to obtain information from both the diaphysis and the metaphysis for clinical assessment.[60] Further research is needed to determine the optimum pQCT scanning procedures and bone outcome measures for fracture risk prediction

Table 2.1. **Peripheral QCT bone measurements and their meanings**

VARIABLE NAME	DEFINITION (UNITS)
Bone mineral content (BMC) parameters	
Total BMC	**Total bone mineral content (mg)**
Trabecular BMC	**Trabecular bone mineral content (mg) at metaphyseal site only**
Cortical BMC	**Cortical bone mineral content (mg)**
Bone mineral density (vBMD) parameters	
Total vBMD	**Total volumetric bone mineral density (mg/cm^3)**
Trabecular vBMD	**Trabecular volumetric bone mineral density (mg/cm^3) at metaphyseal site only**
Cortical vBMD	**Cortical volumetric bone mineral density (mg/cm^3)**
Structural and geometrical parameters	
Total area	**Total bone cross-sectional area (mm^2)**
Trabecular area	**Trabecular bone cross-sectional area (mm^2) within the total bone cross-sectional area**
Cortical area	**Cortical bone cross-sectional area (mm^2) within the total bone cross-sectional area**
Cortical thickness	**Cortical shell thickness (mm)**
Periosteal circumference	**Outer diameter of bone (mm)**
Endosteal circumference	**Inner diameter of bone**
Cross-sectional moment of inertia	**Cross-sectional moment of inertia (mm^4): $p/4(R^4_o - R^4_i)$; where R_o = the outer radius and R_i = the inner diameter, indicative of bone bending strength**

VARIABLE NAME, cont.	DEFINITION (UNITS), cont.
Polar moment of inertia	**Polar moment of inertia (mm^4): $p/2(R^4_o - R^4_i)$; where R_o = the outer radius and R_i = the inner diameter, indicative of strength in torsion**
Section modulus	**Section modulus (mm^3): Polar moment of inertia divided by the maximum distance to the centroid. This measure is indicative of shearing strength**
Polar strength-strain index	**Polar strength-strain index (mm^3): Also known as *polar stress-strain index* and *polar strain-strength index*. This measures takes into account the material properties by multiplying the section modulus by the quotient of Cortical vBMD and maximum normal cortical density, which under normal physiologic conditions is estimated to be 1200 mg/cm^3**
Bone strength index	**Total area x Total $vBMD^2$**

Muscle-bone unit

During growth, two processes continually pose a challenge to bone stability: 1) the increase in bone length (total height and/or limb length) and 2) the increase in muscle force.[28] Longitudinal growth increases lever arms and bending moments and therefore leads to greater bone deformation.[28] Muscle force can also increase bone deformation during muscle contraction.[28] Body weight alone puts a relatively small load on the skeleton, but the effect of weight at many skeletal locations is amplified by muscle action.[45, 75] Thus, increases in bone length, muscle force and body weight with developmental growth create the need for adaptational changes in bone mass and architecture in order to ensure stability. This concept is the essence of Harold Frost's mechanostat model of bone physiology, which has practical consequences for clinical assessment of bone development in children.[41, 76-79]

Since so many morphological features (i.e., total body height, various limb lengths, body composition, etc.) interact to create a specific loading environment for the skeleton, analysis of

bone measurements should require an integrated approach.[45, 80] It has been suggested that bone measurements should be assessed relative to indices of muscle strength and lever arm length.[41] Incorporating surrogates for muscle strength (e.g., fat-free soft-tissue by DXA or muscle cross-sectional area by pQCT), bone strength (e.g., section modulus by DXA or polar strength strain index by pQCT) and lever arm length (e.g., height or limb length) into regression models or as a ratio, may provide a more accurate estimate of the bone strength relative to a mechanical load for an individual child.[41,45, 80] In this way, when a bone measurement is adjusted for a site-specific muscle size and/or strength as well as a site-specific bone length, we can more clearly determine whether there is a non-mechanically induced defect (e.g., decreased modeling due to disease or medication) in the way bone develops.

It is difficult, however, to test whether the osteogenic stimuli created by increased forces acting on bones, due to larger and stronger muscles, lead to a proportional increment in bone strength because a number of independent factors are known to influence both muscle and bone development. This includes common genes regulating both muscle and bone size, and external or intrinsic stimuli such as nutritional or hormonal factors.[81, 82] Although there are cross-sectional studies showing that muscle and bone are highly correlated and that young athletes have both greater muscle and bone mass than controls, this does little to prove causation.[70, 83] Similarly, there have been few longitudinal trials that have specifically shown that an increase in muscle size and strength translates to a proportional increment in bone strength.[84-86]

Unilateral sports, such tennis and squash, provide a unique model to examine the effects of loading on muscle and bone because any differences between the playing and non-playing arm are independent of the effects of genes, nutrition and hormones. Daly et al.[87] found that in pre, peri- and post-pubertal female tennis players, muscle and bone cross-sectional areas, measured

by QCT, were significantly greater in the playing versus non-playing arms. However, the side-to-side differences in muscle cross-sectional area only accounted for 16% of the variance of the differences in bone cross-sectional area. This suggests that muscle size alone was not a good indicator of the muscle strains on bones that stimulated an adaptive skeletal response. It is likely that the greater bone size and strength in the playing arm was associated with increased forces at the tennis racket-hand interface associated with the high-speed acceleration and deceleration with the racket-ball impact. Consistent with these findings, Rittweger and colleagues,[88] having used pQCT to measure the muscle cross-sectional area and cortical bone area at the tibia, observed that female volleyball players had significantly higher cortical bone area than controls, despite no differences in muscle cross-sectional area. This may indicate that the sport-specific training that some athletes receive may lead to training adaptations and/or improvements in the force production capacity of muscle that influences muscle strength independent of muscle size.[80]

It is known that certain types and amounts of loading strain on the bone applied through weight-bearing activities are necessary to create sufficient muscular forces in order to stimulate an adaptive skeletal response.[89] For example, in young swimmers and cyclists, the mechanical loading from muscle pull at bone site appears to be ineffective at enhancing bone mineral accrual, and astronauts typically experience a reduction in bone mineral, despite physical training.[90-92] Clearly, the large forces applied to the lower limbs during the landing phase of volleyball are much greater compared to the low-gravity of swimming or a revolution in cycling.

Despite the strong biomechanical link between muscle and bone, there remain many unanswered questions regarding the influence of loading on the muscle-bone relationship, particularly during growth. For instance, it is uncertain whether skeletal adaptations to increased loading during growth relate directly to the magnitude of the load from muscle pull or some

other aspects of muscle contraction (i.e., rate of force development).[93, 94] The results of animal studies indicate that the rate of loading may be more important than the magnitude in stimulating an osteogenic response, but in these experiments the bone is typically loaded directly rather than through the action of muscle pulling on bone at the site of attachment. [10, 37, 95] These results have not been clearly verified in humans because it is difficult to isolate strain magnitude from strain rate because large strain rates are usually combined with high magnitude loads. However, it has been consistently reported that athletes (sprinting, triple jump, gymnastics, volleyball) that experience strains, which are high in magnitude and rate, have very high aBMD.[96-100] Conversely, endurance athletes (i.e., middle distance runners) who typically experience strains, which are low in magnitude and rate, are often reported to have low aBMD.[101, 102] The lower aBMD, however, reported in endurance athletes may also be due in part to low body weight and menstrual disturbances.[103, 104]

Future studies examining the influence of growth and/or loading on the muscle-bone relationship need to consider specific muscle properties that contribute to the force and power producing capacity of muscles. Consequently, interpreting bone strength with respect to the loading strains incurred during growth is critical for an accuracy diagnosis and interpretation of the growing bone.

Adiposity and bone

Since obesity and osteoporosis have increased dramatically over the past few decades, researchers have been exploring and discovering noteworthy and complex relationships between the two disorders once thought to be mutually exclusive. Adipose tissue was once considered just a passive reservoir for energy storage, however it is now known to play a role in energy metabolism, neuroendocrine function and immune status. Likewise, analyses from cellular and

molecular studies also suggest that adipose tissue plays a significant role in bone metabolism.[105, 106] Mechanisms involving bone and fat are intricate by nature, since both adipocytes and osteoblasts originate from mesenchymal stem cells in bone marrow (Figure 2.5). The fate of their differentiation is determined by common factors, such as PPAR-γ, Wnt, TGF-β, leptin and estrogen.[106] Adipocytes can secrete biologically active molecules, such as leptin, estrogen, adiponectin, resistin and interleukin-6 and may be involved in bone metabolism (Figure 2.5).[107]

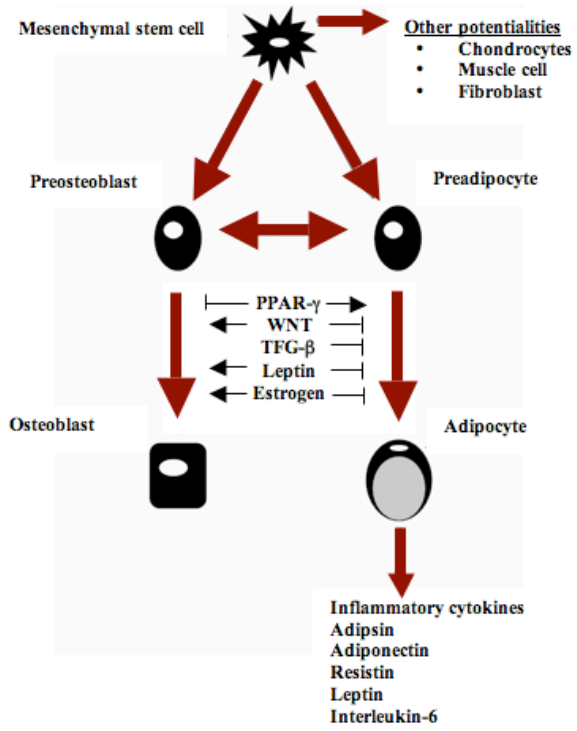

Figure 2.5 **The differentiation of adipocytes and osteoblasts from mesenchymal stem cells.** Adapted from Rosen and Bouxsein, 2006[106]

Factors regulating lipid metabolism may also have a significant effect on bone formation. Extra weight in the form of fat mass has been shown to stimulate bone growth through increased production of the hormones insulin, estrogen and leptin, all of which have demonstrated increases in markers of bone formation when administered in vivo.[108-112] Alternatively, excess adipose tissue has also been shown to hinder bone growth, in vitro, by enhancing the role of oxidized lipids in accelerating atherogenesis, thus activating calcifying vascular cells and inhibiting osteoblastic differentiation.[113] Moreover, bone marrow adipogenesis increases with conditions that induce bone loss, such as estrogen depletion,[114] disuse and

hindlimb unloading.[115, 116] Table 2.2 provides the hypothesized relationships regarding this complex interaction between adiposity and bone.

Table 2.2. **Possible links between adiposity and bone**

Positive effects on bone	Negative effects on bone
Increased loading on the skeleton[117]	Inflammatory cytokines impair bone formation[118]
Increased protection against falls[119]	Increased fracture risk[120]
Leptin directly stimulates bone formation[109]	Fatty acids increase bone resorption[121]
Increased aromatase activity increases estradiol, which leads to: • Decrease bone resorption[122] • Increase bone formation[111]	Leptin inhibits bone formation[110]
Increased insulin stimulates bone formation[123]	Increased insulin inhibits bone formation[124]
	PPAR-γ activation, which inhibits bone formation[125]

Fat and the bone relations in adults

In adults, it is thought that adiposity, via increased loading, seems to be a protective factor against osteoporotic fractures. For instance, in a meta-analysis of 60,000 men and women from 12 population-based cohorts, De Laet and colleagues[126] observed that a high body mass index (BMI) reduced the risk for osteoporotic fractures, although it was more evident in hip versus other types of fractures. This relationship seems plausible since extra weight from fat mass can potentially increase the load that the skeleton, particularly bones at the hip, must endure.

Currently, the accepted measurable bone determinant for fracture risk in adults is aBMD.[34, 127, 128] Studies have shown that high body weight or body mass index (BMI) is correlated with high aBMD, and that losing body weight leads to bone loss.[129-133] These fat and bone relations in adults have been found in both males and females, across a range of ages, and at various skeletal sites.[107, 129, 130] Evidence also supports the view that fat mass, a component of total body weight, has a similar beneficial effect on increasing aBMD, thereby potentially reducing the risk of osteoporosis-related fractures.[128] In otherwise healthy pre- and

postmenopausal women, total body fat was a positive predictor of aBMD throughout the skeleton.[129, 134, 135] Longitudinally, Wu et al.[136] showed that baseline fat mass and 10 year gains in fat mass were positive predictors of aBMD in postmenopausal women.

Other adult investigations, however, suggest that excess fat mass may not be beneficial to the skeleton. In a sample of 6,500 Chinese and Caucasian populations, fat mass and percent body fat were inversely related with aBMD and BMC after correcting for total body weight.[137] Before correcting for total body weight, positive associations were seen between fat mass and the bone variables, which were consistent with the aforementioned studies.[107, 129, 130] The authors[137] suggested that previous studies concluding positive relationship between obesity and bone may have been confounded by the loading effects of excess body weight. Similarly, Hsu et al.[138] observed in a cohort of 14,000 Chinese men and women, fat mass and percent body fat were negatively related to aBMD and BMC independent of body weight, physical activity and age. Further analysis in the study indicated that the risks of osteoporosis and nonspine fractures were significantly higher for subjects with a higher percentage body fat, independent of body weight.[138] In another study, Blum and colleagues[139] sought to investigate the associations of percent body fat and of serum leptin concentration with aBMD in 153 premenopausal Caucasian women. Percent body fat and leptin were each positively associated with aBMD at all the lumbar spine and hip. However, for a given body weight, aBMD was found to be inversely associated with percent body fat and leptin.

These conflicting results in adults suggest a complex relation between fat and bone and may be partially attributed to inter-study differences related to gender, hormonal status, sample size, race, activity levels, statistical analysis method or bone imaging technique.[140, 141] For example, Hsu et al.[138] also concluded that the lowest quartile of percentage fat mass had a higher

risk of osteoporosis than the highest quartile, in both Chinese men and women. However, a smaller study with 68 healthy white premenopausal women and 51 white men showed that aBMD was positively related to fat mass in premenopausal women, but not in men.[117] The same research group reported in another study[129] fat mass had a greater positive effect on bone in post- versus premenoupausal women. The study from Pluijm et al.[142] confirmed the beneficial effect of fat mass on aBMD in white women, but not in white men (n = 264 women and n = 258 men).[142] Castro et al.[143] reported that increased body weight is associated with high aBMD in white women, but with significantly lower aBMD in black women. These differential findings might imply that results from one ethnic group may not be transferable to another ethnic group, or that studies with a large sample size generally have sufficient power to detect associations that may be undetectable in a smaller sample. The qualitatively different relationship between fat and DXA-derived bone outcome is dependent upon whether the bone value is unadjusted or adjusted for variables such as weight status or activity levels[137-139, 144] indicates that selection of covariates may also contribute to the diverse findings. Finally, the use of only DXA instruments in fat and bone investigations may have also contributed to the mixed findings. Bone fragility is influenced not only by the amount of bone mineral but also by bone size, shape and structure.[27, 28, 32, 36] Bone may adapt in geometry in ways that may not be apparent in DXA bone outcomes. Future studies investigating the relationship between fat and bone in adults should consider the use of 3-dimensional imaging techniques to inquire about other components of bone strength.

Fat and bone relations during childhood

Between 12 and 18 years of age, the foundation of skeletal strength is being established by means of increased bone mineral accumulation and dynamic changes in bone size and shape.[145] Any disorder or condition that alters bone formation or enhances bone resorption during

this maturational period will lead to suboptimal skeletal development and presumably a greater risk of osteoporotic fracture later in life.[146, 147] Given that the prevalence of child and adolescent overweight has increased almost three-fold since the early 1970's,[148, 149] researchers have recently explored for potential links between childhood skeletal fractures and adiposity levels. For instance, Taylor and colleagues[120] reported that overweight children (BMI ≥95% for age) had significantly more documented skeletal fractures than non-overweight children. Goulding et al.[150] found that a high body weight, independent of total body lean mass, contributed to fracture risk in children and adolescents who had fractured their forearms repeatedly. Furthermore, in a fracture study of females four to 15 years of age, those who sustained a fracture were more overweight and had a smaller cross-sectional area at the non-fractured forearm compared to the non-fracture group.[151]

Studies that have investigated skeletal strength using DXA in overweight youth have shown mixed results. Overweight children have been reported to either have higher lumbar spine or total BMC relative to height, maturation, and/or fat-free soft-tissue mass[152-154] or lower BMC when corrected for their body weight.[155, 156] Leonard et al.[153] observed that overweight, based on BMI-for-age percentiles, in boys and girls aged four to 20 years, was a predictive factor for higher lumbar spine aBMD corrected for height. Ellis et al.[152] grouped children by percent body fat (<25%, 25-30% and >30% body fat) and found that the >30% body fat group had significantly higher total body BMC relative to height. In contrast, Goulding and colleagues[155, 157] observed that overweight children have lower lumbar spine and total body BMC and bone area relative to weight compared to children with normal BMI-for-age percentiles. Lastly, Afghani and Goran[158] reported an inverse correlation between subcutaneous abdominal adipose tissue and

BMC in white children, but not in black children. They also found an inverse association between intra-abdominal adipose tissue and BMC in blacks, but not in white children.

The discrepancies in the above studies are likely related to various statistical analysis method, childhood obesity classification and race. Whether correcting for differences in body weight or height is the most appropriate statistical approach is unclear, however, it is likely more important to consider site-specific muscle force and limb length on bone variable outcomes. Since the rate of bone formation during growth is highly influenced by the mechanical loading forces generated by muscle,[159] it has been proposed to not only consider the absolute bone measurements but also these measures relative to site-specific surrogates of muscle force and limb length.[80, 140, 160] Further insight would also be gained if body fatness were investigated in addition to BMI-for-age percentiles, since the health complications associated with overweight are related to the excess deposition of fat rather than absolute body weight.[161-163] Measurement of BMI is a useful tool in clinical settings for overweight and obesity screenings and in epidemiological studies for investigating the health risks in population groups;[164-166] however, it does not provide a true measure of adiposity. Finally, limited information is available regarding the effects of both adiposity and race on bone development thus leaving one to question whether adiposity and bone relationships can be generalized to all races.[158]

Another limitation of the studies summarized above is that aBMD, BMC and bone area, determined by DXA, were the primary bone outcome variables reported and not the structural or geometrical properties of bone. In order to better ascertain the independent influence of excess fat mass on the developing skeleton, 3-dimensional bone imaging techniques should be employed. Recently, we reported that excess weight in the form of fat mass does not provide additional benefits to material and geometric measurements of bone strength, relative to

prevailing loads, in predominately white late adolescent females, aged 18-19 years.[20] Using pQCT, we found in high-fat (≥32 percentage body fat) females that polar strength strain index, an estimate of torsional bone strenth,[66] was lower that normal-fat (<32 percentage body fat) females at cortical bone sites of the tibia (8.7%) and radius (8.0%). In another study in males and females, aged 13-21 years, Janicka et al[30] found total fat mass to be negatively associated with bone structure, assessed by 3-dimensional imaging, at axial and appendicular sites, after accounting for muscle forces. Why bone strength and structure are not adapting appropriately to the excess weight of fat mass relative to the prevailing loads is unknown.

Race, ethnicity and the skeleton

Although differences in fracture risk between different racial/ethnic groups have been investigated and noted in adults, our understanding of the basis for these differences is still incomplete and even more so in children. Most osteoporotic-fracture prevalance and bone strength investigations have focused on differences between black and white American populations, while data are more limited when comparisons are made among other racial/ethnic ethnic groups. Thus, the following sections will discuss primarily previous investigations regarding bone health among blacks and whites.

Racial differences in fracture risk

Reports have indicated that the prevalence of fracture risk, particularly at the hip, is higher in whites than blacks in both sexes.[167-169] For example, Jacobsen and colleagues[167] examined data from the Health Care Financing Administration and Department on Veterans Affairs from 1984 through 1987 and identified all those with a hip fracture aged 65 and over. From a sample of 150,000, 79% occurred in women and 93 % occurred in whites.[167] After adjustment for age, the annual rate for hip fracture was 8.07 per 1000 and 3.06 per 1000 in white

and black women, respectively, and was 4.28 and 2.38 in white and black men, respectively.[167] Moreover, the same research group[169] further estimated the annual rate of vertebral fractures. The annual age-adjusted rates of vertebral fracture were higher for whites compared to blacks in both sex groups: 17.1 and 3.7 per 10,000 in white and black women, respectively, and 9.9 and 2.5 per 10,000 in white and black men, respectively. In a separate study, Barrett and colleagues[170] estimated the actual risk of fracture for each of the four race-sex groups (black vs. white; male vs. female) using Medicare data from 1986 through 1990. Their analysis estimated the actual risk of hip fracture to be 16.3 and 5.3 % in white and black women, respectively, and 5.5 and 2.6 % in white and black men, respectively.

From the aforementioned studies,[167-169] it is evident that osteoporotic-related fractures occur at a lower rate in blacks versus whites. This trend could be attributed to data showing that adult blacks have higher aBMD than whites and other racial/ethnic groups, including Asian, Hispanic and Native Americans.[171-173] These differences in unadjusted aBMD may be partly justified by differences in bone size and body size among the races.[172, 174] Thus, when bone size and body sizes are taken into account, many racial differences in aBMD diminish or disappear.[175, 176] Recent data have shown that approximately 1.5 million black women have low aBMD and are at-risk for developing osteoporosis.[177, 178] Despite the lower prevalence of osteoporosis compared to white women, black women who sustain a hip fracture have higher morbidity and nearly twice the rate of mortality.[168, 179-181] Futher investigations are necessary to determine whether racial/ethnic bone health differences are large enough to warrant racial/ethnic screening and treatment recommendations.

Childhood racial differences in bone strength

Much of our knowledge regarding bone mineral acquisition and skeletal growth is based on data acquired in white children, with limited information available in blacks. Contrary to the perception that black children, similar to adults, have greater bone strength than whites is not convincing. For example, when comparing non-Hispanic whites and blacks of comparable age, body weight, height, fat-free mass, and sexual maturation level, total body aBMD is the only measurement site (apart from lumbar spine and proximal femur) that is higher in non-Hispanic blacks than whites.[182-184]

Most childhood reports indicating racial differences in aBMD and BMC, like those conducted in adults, are questionable since bone size and body size were not adequately taken into consideration, and when they are taken into account, are adjusted for by statistical methods using height, weight or body mass index. Failure to match child and adolescent participants on age, sex, body size, and pubertal maturation have, to some extent, misinformed us about the structural differences in bone strength between racial and ethnic groups.[185] Only two childhood studies assessing racial differences in bone strength have matched on bone size and body size dependent variables. For example, Gilsanz and colleagues[186] matched for age and sexual maturation in black (n=75) and white (n=75) females between the ages of 2 and 20 years to determine differences in vertebral volumetric BMD (vBMD) at various stages of sexual development. The vBMD, measured by quantitative computed tomography (QCT), did not differ between black and white girls before puberty but differed significantly between them in late puberty. In a separate study,[187] the same group of investigators matched black (n=80) and white (n=80) subjects, aged 8 to18 years, for age, gender, weight, height, and sexual maturation in order to investigate differential effects of race on the skeleton. Using QCT, the investigators

found that blacks had higher vertebral bone density and femoral cross-sectional area, but only in subjects at Tanner stage-5 for sexual maturation. The investigators, however, did not observe any racial differences at the cross-sectional area of the vertebral body and at the cortical bone area and density of the midshaft of the femur. These two studies using 3-dimensional bone assessments indicate that blacks versus whites, particularly during the late stages of puberty, indeed have vBMD advantages but only at the lumbar spine. Whether blacks versus whites during late adolescence commence to display bone strength advantages at other skeletal sites, weight-bearing versus non-weight-bearing, remain to be determined.

It has been suggested that adiposity may augment the relationship between race and bone strength in both adults and children. In postmenopausal women, Castro et al.[143] demonstrated that for each unit increase in BMI, the odds ratio for having poor aBMD were lower for non-Hispanic white women, while non-Hispanic black women had slightly higher odds for poor aBMD. In children, Afghani and colleagues[158] observed total abdominal mass, assessed by QCT, was negatively associated with BMC, assessed by DXA. There were differential effects of race, however, with regard to BMC relationships with the subcutaneous fat and visceral fat of the abdomen. For instance, they reported an inverse correlation between subcutaneous abdominal adipose tissue and BMC in white children, but not in black children.[158] In contrast, they also found an inverse association between intra-abdominal adipose tissue and BMC in blacks, but not in white children.[158] Whether black versus white children and adolescents are more or equally susceptible to fractures because of potentially negative consequences of excess adiposity on bone strength is unknown. To date, no studies have examined the interrelationships between adiposity levels, race and bone strength in children and adolescents.

Summary

- Bone is a dynamic tissue that continually adapts to functional needs to produce a structure that is strong enough to prevent fractures in most activities.
- Bone modeling, seen in early childhood up to early adulthood, is the process in which bones become larger and denser; hence, osteoblastic activity exceeds osteoclastic activity. Bone remodeling begins to take over in adulthood, where bone mineral undergoes constant and equal removal of old bone and renewal with newly formed bone.
- Fat has significant roles with respect to bone metabolism and may have positive and/or negative effects on skeletal health.
- Predictions of bone strength require 3-dimensional bone assessments of both the material (e.g., mineral density) and geometric (e.g., size and shape) properties of bone.
- Based on DXA-derived measures of BMC and aBMD, it is unclear if overweight is detrimental to bone health in children and adolescents.
- Black children have higher total body, hip and spine aBMD than whites.
- Vertebral vBMD and femoral cross-sectional area, assessed by QCT, are also higher in black versus white children, but only in more developmentally mature children.
- Three-dimensional bone imaging analyses between races are limited, particularly at non-loading skeletal sites.
- Overweight prevalence and severity have increased dramatically, particularly in black female children and adolescents.
- To date, no studies have examined the relationships between adiposity, race and bone strength measured by 3-dimensional imaging techniques.

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CHAPTER 3

IS ADIPOSITY ADVANTAGEOUS FOR BONE STRENGTH? A PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY STUDY IN LATE ADOLESCENT FEMALES [1]

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ABSTRACT

Whereas excess adiposity is presumed to be advantageous for the skeleton, studies investigating relationships between bone strength and fat during youth have been equivocal.

Relationships of percent body fat (%fat) and bone strength indices were assessed in late-adolescent females, taking into consideration surrogates of muscle force (i.e., muscle cross-sectional area, MCSA and bone length). Bone measurements in normal- and high-fat groups were also compared. Females (N=115, aged 18.2±0.4 years) participated in this cross-sectional study. Fat-free soft tissue (FFST), fat mass (FM) and %fat were measured using dual energy X-ray absorptiometry. Tibial and radial peripheral quantitative computed tomography measurements were taken at the 4% (trabecular bone), 20% (cortical bone) and 66% (for measurement of MCSA) sites from the distal metaphyses. Percent fat was inversely related to radial cortical bone area, total bone cross-sectional area (CSA), cortical bone mineral content (BMC), periosteal circumference, and strength-strain index (SSI) [20% site; all $P<0.05$]. After controlling for MCSA and limb-length, negative relationships remained between %fat and radial measurements and were also observed at the tibia (20% site). Unadjusted bone measures were not different between groups. After controlling for MCSA, the high- vs. normal-fat group had lower bone measures at the 20% site (cortical bone area and cortical BMC at the tibia, total bone CSA at the radius and SSI at both the tibia and radius; all $P<0.05$). Excess weight in the form of fat mass does not provide additional benefits, and may potentially be negative, for adolescent bone.

KEY WORDS: pQCT, Late adolescent, Bone strength, Body composition, Obesity

INTRODUCTION

Childhood and adolescence are critical stages for developing optimal bone strength. The majority of bone acquisition occurs between 12 to 18 years of age, when there is a convergence of genetic, hormonal and environmental influences interacting to enhance skeletal mineralization, expansion and linear growth (1). Any disorder or condition that alters bone formation or enhances bone resorption during the maturational period will lead to suboptimal skeletal development and presumably a greater risk of osteoporotic fracture later in life (2, 3). Given that the prevalence of child and adolescent overweight has increased almost three-fold since the early 1970's (4, 5) combined with recent evidence suggesting that being overweight may contribute to skeletal fractures in children and adolescents (6-8), it is vital to understand the effects of excess fat mass on bone development.

Studies that have investigated skeletal strength using dual energy X-ray absorptiometry (DXA) in overweight youth have shown mixed results. Some reports indicate that overweight children and adolescents have higher bone mass relative to height, maturation and/or fat-free soft-tissue mass compared to non-overweight peers (9-11) or that fat mass is a positive contributor to bone mass (12, 13). Others conclude that pediatric overweight is linked to lower bone mass (14, 15) or that the extra weight from fat mass had no influence on bone mass (11).

Although it is possible that the inconsistencies in these studies can be attributed to the statistical evaluation and presentation of either adjusted (e.g., for body size, sex, maturity) or unadjusted bone mass data (16, 17), it is also likely that small changes in bone size or shape can lead to significant changes in bone strength, independent of changes in bone mass (18). Ideally, predicting bone strength (or ultimately, a bone's failure load) requires knowledge of both the material (e.g., mineral density) and geometric (e.g., size and shape) properties of bone (19, 20).

Whereas DXA-derived outcomes reflect only a 2-dimensional view of bone and do not represent true density or bone geometry, peripheral quantitative computed tomography (pQCT) is a 3-dimensional imaging technique that measures size, shape and mineral density of bone and has shown to predict failure load at the radius more accurately than DXA (21, 22). Notably, pQCT depicts an image of the cross-sectional area of muscle (MCSA) bordering the bone, which is considered an acceptable surrogate of muscle strength (23, 24). Since the rate of bone formation during growth is highly influenced by mechanical loading generated by muscle forces (25), it has been proposed to not only consider the absolute bone measurements but also these measures relative to surrogates of muscle strength and bone length (17, 23, 26).

To our knowledge, no studies have assessed the relationships between body fatness and distinct skeletal compartments (i.e., trabecular and cortical) using pQCT, while taking into account the muscle-bone relationship. The study cohort was selected to minimize the influence of differences in age, sex and maturational status. The primary objective of this study was to examine the relationships of percent body fat and pQCT-derived tibial and radial measurements in late adolescent females (i.e., post-maturation), before and after controlling for mechanical loading effects (e.g., MCSA and bone length for each respective site). The second objective was to compare these tibial and radial bone measurements between two groups defined as having normal and high levels of body fat.

SUBJECTS AND METHODS

Study Participants

Late adolescent females (N=115), aged 18 to 19 years, enrolled in their first semester at The University of Georgia and who had participated in the Fighting Osteoporosis in College Using Soy intervention study, served as participants for this investigation. This age group was

selected to minimize any influence of sexual maturation on the bone outcome variables. Thus, all participants must have reported normal menstruation (e.g., ≥ 4 menstrual periods in the last 6 months) for inclusion in the study. Participants were excluded if they reported significant weight loss or gain in the past 6 months (± 10% initial body weight), participation in NCAA Division I athletics, diagnosis of eating disorders, present illness or chronic disease, and use of medications or herbal supplements known to affect body weight, body fat or bone metabolism. Procedures were approved by the Institutional Review Board for Human Subjects at The University of Georgia, and all participants provided written consent.

Participants were divided into 2 groups on the basis of their percent body fat: normal-fat (<32% body fat; *n*=93) and high-fat (≥32% body fat; *n*=22). These classifications were selected based on levels of body fat associated with cardiovascular risk factors (27, 28). By grouping participants by body fat percent, we exclude the possibility of misclassification of those with high levels of body fat that may have otherwise been classified as normal weight if BMI had been used for the grouping procedure. Participant ethnicity (Hispanic or Latino/Non-Hispanic or Latino) and race (American Indian or Alaska Native, Asian, Black or African-American, Native Hawaiian or other Pacific Islander, White, or any combination of the above) were classified using the National Institutes of Health Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research (29). Within the normal-fat group, 78 participants were White, 10 were Asian, 3 were Hispanic, and 2 were Black; whereas, in the high-fat group, 20 participants were White and 2 were Hispanic. When race was included as a covariate in the analyses of bone outcomes between normal- and high-fat groups, it did not have a significant effect; therefore, the Asian, Hispanic and Black participants were included in all analyses.

Anthropometry

Height and body weight measurements were collected by a trained laboratory technician. Participants were measured for height and weighed in light indoor clothing following the removal of shoes. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Novel Products Inc., Rockton, IL). Body weight was measured to the nearest 0.1 kg using an electronic scale (Seca Bella 840, Columbia, MD). Prior to testing each week, the scale was checked for accuracy using known weights. Recalibration of the scale was not required during the testing sessions. Limb lengths were measured with anthropometric tape (Rosscraft, Inc) to the nearest 0.10 mm at the tibia (the distal edge of the medial malleolus to the tibial plateau) and forearm (distance between the ulnar styloid process and olecranon).

Body composition

Body composition variables [fat mass (kg), fat-free soft tissue mass (FFST; kg), and percentage body fat (%fat)] were measured using DXA (Delphi A; S/N 70467; Hologic Inc., Bedford, MA). The same technician analyzed all scans using Hologic Whole Body Analysis software, version 11.2. Quality assurance for fat mass, FFST and %fat measured by DXA was carried out by calibration against a three-step soft tissue wedge (Hologic anthropomorphic spine phantom, model DPA/QDR-1; SN 9374) composed of different thickness levels of aluminum and lucite, calibrated against stearic acid (100% fat) and water (8.6% fat). In our laboratory, a coefficient of variation of 0.36% was observed from 648 scans of the spine phantom over a 3-year period. Based on a one-way random effects model, single measure intra-class coefficients (ICC) were calculated in 5 females, aged 18 to 30 years, scanned twice in our lab during a 7-day period for fat mass, FFST and %fat (all $R \geq 0.87$).

Peripheral quantitative computed tomography

Peripheral QCT (Stratec XCT-2000; Stratec Medizintechnic GmbH, Pforzheim, Germany) measurements were taken of the nondominant tibia and radius. Tibial measures were taken at the 4% and 20% sites of the total tibial length from the distal metaphysis and represent areas high in trabecular and cortical bone, respectively. Measurements were also assessed at the 4% and 20% sites of the forearm length, proximal to the distal radial metaphysis. Each scan was acquired with a 0.4-mm voxel and at a slice thickness of 2.4-mm. The positioning of the two cross-sectional measurements from the tibia and radius were determined in a scout view using their medial endplate as an anatomic marker and automatically set by the software at 4% or 20% sites. Image processing and calculation of the various bone indices and MCSAs were determined using the Stratec software (version 5.50*d*). Total and trabecular volumetric BMD (Tot BMD and Trab BMD, mg/cm^3) and total bone cross-sectional area (Tot area, mm^2) were calculated for tibia and radius 4% sites using contour mode 2 and peel mode 2. The following variables were assessed at the tibia and radius 20% sites: cortical volumetric BMD (Cort BMD, mg/cm^3), cortical bone area (Cort area, mm^2), total bone cross-sectional area, cortical bone mineral content (Cort BMC, mg), cortical thickness (Cort thk, mm), periosteal circumference (Peri circ, mm), endosteal circumference (Endo circ, mm), and polar strength-strain index (SSI, mm^3). Cortical bone variables for both 20% sites were assessed using cort mode 1 and the default threshold of 710 mg/cm^3. The SSI was analyzed with cort mode 1 and a threshold of 280 mg/cm^3.

A third measurement was taken at the 66% site of both the tibia and radius to assess MCSA (mm^2), an estimate of muscle strength. The proximal two-thirds site was chosen because in this region the muscle has the highest circumference and cross-sectional area (30, 31). The MCSA was determined by placing a region of interest within the subcutaneous fat tissue.

Contour mode 3 with a threshold of 34 mg/cm^3 and peel mode 1 was used to obtain the "area of muscle plus bone" (i.e., muscle + tibia + fibula or muscle + radius + ulna). Next, the analysis was performed with contour mode 1, threshold of 280 mg/cm^3 and peel mode 1 to determine the "area of bone" (i.e., tibia + fibula or radius + ulna). The MCSA is finally determined by subtracting the "area of bone" from the "area of muscle plus bone".

All pQCT measures were performed and analyzed by one trained operator. The pQCT operator scanned the phantom daily to maintain quality assurance. Test-retest measurements were performed in 5 females, aged 18 to 24 years, to determine reliability of the pQCT in our laboratory. The one-way random effects model, ICCs for all pQCT measurements were calculated to be $R \leq 0.97$.

Physical Activity

Information on physical activity for the past week was collected using the interviewer-administered 7-day recall questionnaire (32), which has been validated in females within this age group (33). Participants reported the amount of time spent sleeping as well as time spent performing moderate, hard and very hard activities during the previous week. Light physical activity was calculated from the remaining time. From this questionnaire, each participant's average daily energy expenditure (kcal/day) was estimated.

Dietary Intake

Three-day diet records were used to estimate average intakes of daily energy, macronutrient, calcium, and vitamin D intake per day. The 3 days included 2 weekdays and 1 weekend day. The 3-day diet records were analyzed by Food Processor for Windows version 8.0 (ESHA Research, Salem, OR). In our laboratory, the reliability of diet records was investigated in a previous study of females 6 to 10 years of age (n=10) who completed 3-day diet records

twice over a 2-week period. In that investigation, one-way random effects model, ICCs were computed for 3-day energy intake and 3-day calcium intake and found to be R = 0.47 and calcium R = 0.71, respectively.

Statistical analyses

Data were analyzed using SPSS version 11.0.2 (Chicago, IL) for the Mac OS X. Normal distribution and homogeneity of variances were confirmed by Shapiro-Wilks *W* and Levene's tests, respectively. Pearson's bivariate correlations were used to examine the associations of %fat, fat mass and FFST with various bone response variables. Partial Pearson's correlation coefficients were also computed between these same outcome variables, with control for MCSA and limb length. A $P < 0.05$ was considered statistically significant.

Group differences for anthropometric, body composition, physical activity, dietary intake, and unadjusted bone response variables were determined using unpaired (i.e., independent samples) two-tailed *t*-tests if data were distributed normally and Mann-Whitney U tests, otherwise. Descriptive statistics for raw variables are presented as mean ± SD if not stated otherwise. Group differences for categorical variables (e.g., oral contraceptive use) were tested using X^2 tests. An *F*-test was performed to test the assumption of homogeneity of regression slopes with regard to the interaction between the independent variables (i.e., adiposity groups) and the covariate (i.e., MCSA). Since there was no interaction, analysis of covariance was used to compare the differences in bone response variables between normal-fat and high-fat groups after adjusting for MCSA differences. Estimated means of bone variables in the adjusted analyses are reported in the form of mean ± SE. Statistically significant differences are reported if $P < 0.05$.

RESULTS

Participant characteristics

Mean age, weight, height, BMI-for-age, BMI percentile, total FFST and fat mass, %fat, tibial and forearm length and MCSA, oral contraceptive use, and unadjusted bone variables of the participants are provided in **Table 3.1**. Age and height values were not statistically different between adiposity groups, however body weight, BMI-for-age, BMI-for-age percentiles, fat mass, and %fat were significantly higher in the high-fat versus the normal-fat group (all $P < 0.05$). Total FFST mass as well as tibial and forearm length were not different between groups. The MCSA at the tibia, but not at the forearm, was significantly higher in the high-fat group compared with the normal-fat group ($P < 0.05$). No significant difference was found between groups in the percent of women reporting oral contraceptive use. Among the bone variables, no significant tibial or radial differences were seen at any site between adiposity groups.

Bivariate correlations between body composition and bone measurements

Percent body fat was not associated with Tot BMD, Trab BMD or Tot area at the 4% site of the tibia and radius (**Table 3.2**). In contrast, at the 20% site of the radius, but not the tibia, %fat was negatively correlated with Cort area, Tot area, Cort BMC, Peri circ, and SSI (all $P < 0.05$). Positive relationships were observed between total fat mass and Tot area at the 4% site of the tibia and between total fat mass and Cort area ($P = 0.054$), Tot area ($P = 0.010$), Peri circ ($P = 0.009$), Endo circ ($P = 0.041$), and SSI ($P = 0.009$) at the 20% site of the tibia. Positive associations were also found between total FFST mass and Tot area of the 4% site as well as Cort area, Tot area, Cort BMC, Peri circ, Endo circ, and SSI of the 20% sites of both the tibia and radius (all $P < 0.05$). An inverse relationship, however, was observed between total FFST mass and Cort BMD of the tibia ($P = 0.013$).

Partial correlations between body composition and bone measurements

Consistent with the bivariate correlations, after adjustment for MCSA and limb length, no significant relationships were found between %fat and Tot BMD, Trab BMD and Tot area of the 4% sites of the tibia and radius (**Table 3.3**). At the 20% site, significant negative associations were found between %fat and Cort area, Tot area, Cort BMC, Peri circ, and SSI of tibia and radius (all $P < 0.05$). An inverse relationship was also found between %fat and Cort thk at the tibia at the 20% site ($P = 0.052$). Total fat mass was not associated with Tot BMD, Trab BMD and Tot area of the 4% sites of the tibia and radius. However, significant negative correlations were found between total fat mass and Cort area and Cort BMC at the tibia and radius (all $P < 0.05$). Positive relationships were found between total FFST mass and Tot area at the 4% site and between FFST mass and Tot area, Peri circ, Endo circ, and SSI of the tibia and radius at the 20% site (all $P < 0.05$). Positive associations were also found at the 20% site for tibial Cort area and Cort BMC (both $P < 0.05$). Negative relationships were observed at the 20% site between FFST and Cort BMD at the tibia ($P = 0.051$) and at the radius ($P = 0.035$) as well as Cort thk at the radius only ($P = 0.006$).

Comparisons between normal- and high-fat groups

Adjusted bone measurements

Table 3.4 summarizes group-specific means for each bone variable based upon an analysis of covariance that controls for differences in MCSA. After controlling for MCSA, the high-fat compared to the normal-fat group had lower bone measures at the 20% site: Cort area ($P = 0.015$), Cort BMC ($P = 0.029$) and Peri circ ($P = 0.059$) at the tibia, Tot area at the radius ($P = 0.046$) and SSI at both the tibia ($P = 0.039$) and radius ($P = 0.051$).

Physical activity

No significant differences were observed between adiposity groups in reported hours of sleep, light, moderate, hard, and very hard activities. The high-fat group, however, had significantly higher total daily energy expenditure compared to the normal-fat group (2357 ± 345 versus 2019 ± 269 kcals/day; $P = 0.010$).

Dietary Intake

Energy intakes for the normal-fat and high-fat groups were 1810 ± 426 and 1713 ± 507 kcals/day, respectively ($P = 0.360$). Mean intakes for all macronutrients and micronutrients were not different between groups. Both the normal-fat and high-fat groups met the U.S. Recommended Dietary Allowance for carbohydrate and protein but reported low intakes of calcium (720 ± 324 versus 626 ± 286 mg, respectively) and vitamin D (98 ± 104 versus 83 ± 56 IU, respectively). Seventy-four percent of the normal-fat (69/93) and 91% of the high-fat (20/22) group consumed less than two-thirds of the adequate intake (AI) for calcium, whereas 76% of the normal-fat (71/93) and 82% of the high-fat group (18/22) consumed less than two-thirds of the AI for vitamin D ($P > 0.01$ for both).

DISCUSSION

One of the key findings from this study was that percent body fat was inversely related to pQCT-derived bone measurements assessed at a predominantly cortical site of the radius (Cort area, Tot area, Cort BMC, Peri Circ, and SSI) in late adolescent females. When taking into account MCSA and limb length, negative relationships not only remained between percent body fat and radial measurements, but were also observed at the tibia (cortical site). When participants were compared by level of adiposity, we found that tibial and radial bone measurements were not different between groups. Given that both the high-fat and normal-fat groups had no significant

differences in total FFST mass, it was interesting to find that the additional 9 kilograms of fat mass in the high-fat group provided no advantage with respect to pQCT-derived bone measurements at the tibia and radius. Consistent with the correlational data, after correcting for MCSA differences, the high-fat group had significantly lower tibial Cort area, Cort BMC and SSI, as well as radial Tot area and SSI compared to the normal-fat group. Collectively, our data suggest that contrary to the idea that extra body weight is advantageous for the skeleton, excess weight in the form of fat mass does not provide additional benefits and may potentially be negative for adolescent bone.

The few studies that have examined the relationships between overweight and measures of bone, assessed by DXA, demonstrate conflicting results. Overweight youth, ranging from 3 to 19 years of age, have been reported to either have higher lumbar spine or total body bone mass relative to height, maturation and/or fat-free soft-tissue mass (9-11) or lower bone mass when corrected for their body weight (14, 15). Considering the limitations associated with DXA's 2-dimensional bone measurements in children and adolescents of different age, sex, body size, body composition, and sexual maturation, adjusted DXA bone outcomes can be difficult to interpret, and may explain some of the discrepancies among these studies (9-11). A novel aspect of this study involved the evaluation of pQCT-derived bone measurements relative to limb-specific muscle strength and bone length, a technique that is gaining recognition within the framework of pediatric bone health (17, 23, 26). Since the rate of bone formation is highly influenced by the mechanical stimulation from muscle forces during growth (25, 34), evaluating indices of bone strength relative to muscle strength and bone length has been recommended (34-36).

From pre-adolescence to young adulthood, fat mass has been shown to be positively correlated with unadjusted DXA-assessed bone measurements at weight-bearing skeletal sites (13, 37). Thus, it was not surprising in our study that fat mass was positively related to unadjusted bone measurements at the tibia (weight-bearing site), but not at the radius (non-weight-bearing site). Since we observed negative associations between radial bone measurements and percent body fat, one could speculate that obesity increases risk for fractures at non-weight-bearing skeletal sites, such as the forearm, given that the mechanical force during a fall is proportional to body weight. This may explain why studies have reported higher forearm fractures in overweight compared to normal weight children and adolescents (38-40). Because of the tibia's weight-bearing location, it is also reasonable to expect that the extra weight from fat mass could lead to a greater muscle contraction and eventually, greater bone strength compared to the radius. However, when interpreting the relationships between percent body fat as well as fat mass with bone measurements at the tibia relative to MCSA and tibial length, negative associations were observed. Therefore, our data suggest that high levels of body fat negatively influence bone independent of its weight-bearing effects, and areas consisting predominantly of cortical bone seem to be affected more than trabecular bone. Whereas additional studies are needed to confirm our interpretation, our findings are consistent with Janicka et al.,(41) who showed that fat mass was negatively correlated with CT-derived measures of bone strength in adolescent males, after accounting for surrogates of muscle force.

Analyses from cellular and molecular studies suggest that the mechanisms involving bone and fat are intricate by nature, since both adipocytes and osteoblasts originate from mesenchymal stem cells in bone marrow. Factors regulating lipid metabolism may also have a significant effect on bone formation. Extra weight in the form of fat mass has not only been

shown to stimulate bone growth via direct mechanical actions from increased load (42), but also through increased production of the hormones insulin, estrogen and leptin, all of which have demonstrated increases in markers of bone formation when administered in vivo (43-47). Alternatively, excess adipose tissue has also been shown to hinder bone growth, in vitro, by enhancing the role of oxidized lipids in accelerating atherogenesis, thus activating calcifying vascular cells and inhibiting osteoblastic differentiation (48). Moreover, bone marrow adipogenesis increases with conditions that induce bone loss, such as estrogen depletion (49), disuse and hindlimb unloading (50, 51). Future work should continue to explore these potential mechanisms to enhance our knowledge of fat and bone relationships.

Figure 3.1 shows a visual representation of the overall effect by which smaller bone dimensions and lesser bone material, as was observed in the high- vs. the normal-fat group, had on the tibial and radial strength-strain index, an estimate of torsional bone strength (52). It is assumed here that MCSA provides an approximate assessment of muscle strength and therefore is a surrogate measure of the loads to which the tibial and radial bones are exposed. Why bone strength in the high-fat group was not appropriately adapted to the prevailing loads is unknown; however, modifiable factors such as physical activity and diet not only play an important role in obesity progression but also significantly impact bone strength. It is possible that higher proportions of fat mass could be a marker for reduced physical activity. However, when information regarding physical activity was collected in this study, no significant differences were found for physical activity levels between groups. The high- and normal-fat groups reported moderate amounts of physical activity (27.2 versus 29.4 minutes/day, respectively), lower than the US-recommendations for this age group (i.e., ~ 60 minutes/day of moderate intensity activity) (53). Specific types of high-impact exercise have been documented as having a

positive effect on bone mineral accrual, particularly during growth (54-56). It is unlikely that reported current activity levels explain the group differences in bone strength. However, we did not collect information on the types of activities performed by the participants and therefore, it is uncertain the degree to which participants engaged in high-impact physical activities.

With regard to dietary intake, the groups reported no significant differences in energy, macronutrient and micronutrient intakes. Mean calcium intakes in both groups were low and less than the US-recommended AI; however, a higher percentage of individuals in the high-fat vs. the normal-fat group consumed less than 2/3 AI for calcium. ANCOVA was used to assess whether dietary calcium had an effect on the adjusted bone outcomes between groups. After controlling for dietary calcium in addition to MCSA, there was no significant effect from calcium on the adjusted bone outcomes. The mean intakes for vitamin D were also low in both groups and less than the AI. Because cutaneous synthesis of vitamin D has a greater influence than dietary vitamin D on serum 25-hydroxyvitamin D [25(OH)D] levels, it would have been preferable to measure circulating concentrations of serum 25(OH)D to better understand the influences of vitamin D status on bone in these subjects. Since overweight and obese individuals tend to have lower levels of circulating vitamin D, possibly due to vitamin D being partially sequestered in the adipose tissue (57), vitamin D could be a mediating factor in the relationship between excess fatness and bone.

Some limitations in our study must be acknowledged. First, it is important to note that the analysis of muscle strength is complex and the use of MCSA does not reflect the functional status of the entire muscle system, including muscle length, contraction velocity, structure, and coordination (58). Whether the data generated using this technique can be used to predict bone health and risk of skeletal fractures must be validated by subsequent prospective studies.

Secondly, the present study utilized baseline data from a randomized nutrition intervention trial and was not specifically designed to examine adiposity and bone relationships. As a result, the sample was relatively homogenous with respect to BMI. If BMI-for-age percentiles were used in the overweight classification scheme like other investigators have employed (7-10), few participants would have been classified as at-risk of overweight or overweight (10 of 22 participants in the high-fat group exceeded the 85th BMI-for-age percentile and one participant exceeded the 95th BMI-for-age percentile). The homogeneity of this sample with respect to body fatness may partly explain why there were no significant differences in total body FFST among the high- and normal-fat groups. The high-fat subjects did have greater MCSA of the tibia and this may be related to the weight-bearing effect at this skeletal site, since no significant differences existed at the radius (non-loading site). An advantage of this sample was that the degree of variability in factors known to influence bone, such as sex, age and maturational status were minimized. All participants were healthy females, between the ages of 18 and 19 years and reported having regular menstrual cycles.

While the clinical significance of bone strength lies in the occurrence of fractures, our study provides important insight into the obesity and bone strength relationship using pQCT. Specifically, our results suggest that extra weight in the form of fat mass does not provide additional benefits to material and geometric properties of bone strength in late adolescent females. However, prospective research is needed to confirm a cause and effect relationship that considers physical inactivity, metabolic diseases and environmental influences.

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Table 3.1

Characteristics of the participants [1]

	Total sample	Normal-fat [2] (<32 % body fat)	High-fat [2] (≥32 % body fat)
N	115	93	22
Age (yrs)	18.2 ± 0.4	18.2 ± 0.4	18.4 ± 0.5
Weight (kg)	60.1 ± 7.7	58.3 ± 6.2	67.7 ± 8.5 [3]
Height (cm)	164.0 ± 6.0	164.0 ± 6.2	164.6 ± 5.3
BMI-for-age (kg/m^2)	22.3 ± 2.5	21.7 ± 1.9	25.0 ± 2.8 [3]
BMI percentile	56.3 ± 21.9	51.3 ± 20.7	75.9 ± 16.0 [3]
FFST mass (kg) [4]	41.3 ± 4.4	40.9 ± 4.3	42.7 ± 4.7
Fat mass (kg)	17.7 ± 4.7	16.1 ± 2.8	24.6 ± 4.6 [3]
Fat mass (%)	28.8 ± 4.3	27.1 ± 2.9	35.3 ± 3.0 [3]
Tibial length (mm)	372.6 ± 20.8	372.9 ± 21.2	372.1 ± 19.0
Forearm length (mm)	257.4 ± 14.2	257.2 ± 14.5	257.8 ± 13.2
Tibial MCSA (mm^2) [5]	7060 ± 1239	6889 ± 1069	7777 ± 1608 [3]
Forearm MCSA (mm^2)	2489 ± 358	2472 ± 317	2547 ± 497
Oral contraceptive use (%) [6]	40.7 ± 0.5	37.9 ± 0.5	52.3 ± 0.5
Bone variables (*4% site*) [7]			
Tot BMD (mg/cm^3)			
Tibia	313.3 ± 38.6	314.1 ± 37.9	310.2 ± 41.8
Radius	348.8 ± 56.5	348.3 ± 57.4	350.7 ± 53.8
Trab BMD (mg/cm^3)			
Tibia	254.3 ± 26.1	255.0 ± 26.2	251.2 ± 26.2
Radius	212.5 ± 31.5	213.7 ± 31.4	207.5 ± 32.1
Tot area (mm^2)			
Tibia	926.1 ± 107.3	921.7 ± 104.3	944.7 ± 119.9
Radius	271.8 ± 44.8	273.1 ± 41.5	266.5 ± 57.8

Bone variables (*20% site*) [7]			
Cort BMD (mg/cm^3)			
Tibia	1174 ± 16.2	1173 ± 15.7	1176 ± 18.4
Radius	1193 ± 20.5	1193 ± 18.1	1192 ± 29.1
Cort area (mm^2)			
Tibia	190.7 ± 22.7	191.0 ± 22.1	189.2 ± 25.7
Radius	68.7 ± 8.3	69.0 ± 8.2	67.4 ± 8.8
Tot area (mm^2)			
Tibia	332.7 ± 47.6	333.0 ± 48.5	331.5 ± 44.8
Radius	94.2 ± 14.0	95.0 ± 14.0	90.8 ± 13.9
Cort BMC (mg)			
Tibia	223.7 ± 26.8	224.1 ± 26.0	222.5 ± 30.7
Radius	81.9 ± 10.0	82.3 ± 9.8	80.4 ± 11.0
Cort thk (mm)			
Tibia	3.59 ± 0.5	3.60 ± 0.4	3.58 ± 0.5
Radius	2.65 ± 0.2	2.64 ± 0.2	2.67 ± 0.3
Peri circ (mm)			
Tibia	64.5 ± 4.6	64.5 ± 4.6	64.4 ± 4.5
Radius	34.2 ± 2.8	34.3 ± 2.8	33.7 ± 2.6
Endo circ (mm)			
Tibia	41.9 ± 5.7	41.9 ± 5.7	41.9 ± 5.6
Radius	17.8 ± 3.4	18.0 ± 3.4	16.9 ± 3.1
SSI (mm^3)			
Tibia	1268 ± 244	1270 ± 246	1261 ± 239
Radius	217.9 ± 42.2	220.3 ± 42.1	208.0 ± 41.8

[1] Values are means ± SD.

[2] Cutpoints used to denote normal fat and high fat were determined using cardiovascular risk factors (27, 28).

[3] Tests of significance between groups are based on 2-tailed independent *t*-tests. Significantly different from normal weight, $P \leq 0.05$.

[4] FFST, fat-free soft-tissue

[5] MCSA, muscle cross-sectional area

[6] Tests of significance between groups for oral contraceptive use are based on the X^2 test.

[7] Bone variables measured by peripheral quantitative computed tomography at the 4% (trabecular) and the 20% (cortical) site: Tot BMD = total volumetric BMD, Trab BMD = trabecular volumetric BMD, Tot area = total cross-sectional area of bone, Cort BMD = cortical volumetric BMD, Cort area = cortical bone area, Cort BMC = cortical bone mineral content, Cort thk = cortical thickness, Peri circ = periosteal circumference, Endo circ = Endosteal circumference, SSI = strength strain index

Table 3.2

Bivariate correlations of bone outcomes at the tibia and radius with percent body fat, fat mass, and fat-free soft tissue

	% Body Fat		Fat Mass		Fat-Free Soft Tissue	
	r	*P*	*r*	*P*	*r*	*P*
4% site						
Tot BMD (mg/cm^3)						
Tibia	-0.015	0.873	0.009	0.926	0.042	0.657
Radius	-0.032	0.738	-0.003	0.979	0.041	0.663
Trab BMD (mg/cm^3)						
Tibia	0.027	0.774	0.031	0.743	0.053	0.573
Radius	-0.003	0.976	-0.020	0.835	0.008	0.935
Tot area (mm^2)						
Tibia	0.053	0.572	0.286	0.002	0.619	0.000
Radius	-0.089	0.346	0.082	0.384	0.416	0.000
20% site						
Cort BMD (mg/cm^3)						
Tibia	0.002	0.984	-0.078	0.407	-0.232	0.013
Radius	-0.031	0.741	-0.088	0.347	-0.136	0.146
Cort area (mm^2)						
Tibia	-0.087	0.355	0.180	0.054	0.609	0.000
Radius	-0.191	0.041	0.030	0.750	0.519	0.000
Tot area (mm^2)						
Tibia	-0.016	0.862	0.239	0.010	0.643	0.000
Radius	-0.187	0.045	0.041	0.660	0.542	0.000
Cort BMC (mg)						
Tibia	-0.086	0.361	0.169	0.070	0.576	0.000
Radius	-0.193	0.039	0.018	0.848	0.496	0.000
Cort thk (mm)						
Tibia	-0.102	0.278	0.004	0.966	0.184	0.049
Radius	-0.062	0.509	0.016	0.862	0.181	0.053

Peri circ (mm)						
Tibia	-0.015	0.877	0.243	0.009	0.645	0.000
Radius	-0.199	0.033	-0.015	0.875	0.422	0.020
Endo circ (mm)						
Tibia	0.035	0.713	0.190	0.041	0.428	0.000
Radius	-0.094	0.320	0.063	0.505	0.380	0.000
SSI (mm^3)						
Tibia	-0.026	0.783	0.244	0.009	0.668	0.000
Radius	-0.196	0.035	0.032	0.734	0.535	0.000

Bone variables measured by peripheral quantitative computed tomography at the 4% (trabecular) and the 20% (cortical) site: Tot BMD = total volumetric BMD, Trab BMD = trabecular volumetric BMD, Tot area = total cross-sectional area of bone, Cort BMD = cortical volumetric BMD, Cort area = cortical bone area, Cort BMC = cortical bone mineral content, Cort thk = cortical thickness, Peri circ = periosteal circumference, Endo circ = Endosteal circumference, SSI = strength strain index.

Pearson's bivariate correlations were used to examine associations between %fat, fat mass and FFST with bone response variables in this sample (N=115). Statistically significant coefficients ($P \leq 0.05$).

Table 3.3

Partial correlations of bone outcomes at the tibia and radius with percent body fat, fat mass, and fat-free soft tissue

	% Body Fat		Fat Mass		Fat-Free Soft Tissue	
	r	*P*	*r*	*P*	*r*	*P*
4% site						
Tot BMD (mg/cm^3)						
Tibia	-0.066	0.488	-0.062	0.514	0.002	0.983
Radius	-0.034	0.722	-0.062	0.513	-0.159	0.092
Trab BMD (mg/cm^3)						
Tibia	-0.011	0.906	-0.012	0.901	0.061	0.523
Radius	0.006	0.953	-0.034	0.721	-0.073	0.441
Tot area (mm^2)						
Tibia	-0.085	0.369	0.041	0.668	0.438	0.000
Radius	-0.110	0.245	-0.029	0.764	0.285	0.002
20% site						
Cort BMD (mg/cm^3)						
Tibia	0.063	0.508	0.023	0.811	-0.184	0.051
Radius	-0.027	0.777	-0.087	0.363	-0.198	0.035
Cort area (mm^2)						
Tibia	-0.335	0.000	-0.209	0.026	0.367	0.000
Radius	-0.280	0.003	-0.228	0.015	0.135	0.154
Tot area (mm^2)						
Tibia	-0.229	0.015	-0.115	0.226	0.398	0.000
Radius	-0.238	0.011	-0.138	0.145	0.330	0.000
Cort BMC (mg)						
Tibia	-0.317	0.001	-0.201	0.033	0.329	0.000
Radius	-0.281	0.003	-0.240	0.010	0.098	0.302
Cort thk (mm)						
Tibia	-0.183	0.052	-0.133	0.159	0.093	0.326
Radius	-0.089	0.347	-0.151	0.112	-0.256	0.006

Peri circ (mm)						
Tibia	-0.232	0.013	-0.118	0.213	0.394	0.000
Radius	-0.237	0.011	-0.165	0.081	0.218	0.020
Endo circ (mm)						
Tibia	-0.075	0.431	-0.019	0.841	0.230	0.014
Radius	-0.103	0.276	-0.001	0.991	0.365	0.000
SSI (mm^3)						
Tibia	-0.261	0.005	-0.136	0.150	0.421	0.000
Radius	-0.249	0.008	-0.149	0.116	0.319	0.001

Bone variables measured by peripheral quantitative computed tomography at the 4% (trabecular) and the 20% (cortical) site: Tot BMD = total volumetric BMD, Trab BMD = trabecular volumetric BMD, Tot area = total cross-sectional area of bone, Cort BMD = cortical volumetric BMD, Cort area = cortical bone area, Cort BMC = cortical bone mineral content, Cort thk = cortical thickness, Peri circ = periosteal circumference, Endo circ = Endosteal circumference, SSI = strength strain index.

Partial Pearson's correlations were used to examine associations between %fat, fat mass and FFST with bone response variables, controlling for muscle cross-sectional area and limb length in this sample (N=115). Statistically significant coefficients ($P \leq 0.05$).

Table 3.4

Bone measurements of the tibia and radius after adjustment for muscle cross-sectional area in normal-fat and high-fat adolescent females

Bone variable [1]	Normal-fat [2,3] (<32% body fat)	High-fat [2,3] (≥32% body fat)	*P-value* [4]
4% site			
Tot BMD (mg/cm^3)			
Tibia	315.1 ± 4.0	305.8 ± 8.4	0.326
Radius	349.0 ± 5.7	348.1 ± 11.7	0.948
Trab BMD (mg/cm^3)			
Tibia	255.7 ± 2.7	248.4 ± 5.7	0.255
Radius	214.0 ± 3.2	206.2 ± 9.3	0.295
Tot area (mm^2)			
Tibia	926.7 ± 10.7	923.7 ± 22.5	0.907
Radius	273.6 ± 4.5	264.4 ± 9.3	0.377
20% site			
Cort BMD (mg/cm^3)			
Tibia	1173 ± 1.7	1178 ± 3.5	0.206
Radius	1193 ± 2.1	1192 ± 4.4	0.768
Cort area (mm^2)			
Tibia	192.9 ± 2.0	181.3 ± 4.2	0.015
Radius	69.1 ± 0.7	66.5 ± 1.4	0.080
Tot area (mm^2)			
Tibia	336.4 ± 4.4	317.1 ± 9.2	0.063
Radius	95.3 ± 1.2	89.6 ± 2.6	0.046
Cort BMC (mg)			
Tibia	226.1 ± 2.4	213.7 ± 5.0	0.029
Radius	82.6 ± 0.8	79.3 ± 1.7	0.077
Cort thk (mm)			
Tibia	3.61 ± 0.05	3.51 ± 0.09	0.355
Radius	2.65 ± 0.02	2.66 ± 0.04	0.795

Peri circ (mm)			
Tibia	64.9 ± 0.4	63.0 ± 0.9	0.059
Radius	34.3 ± 0.3	33.5 ± 0.5	0.128
Endo circ (mm)			
Tibia	42.1 ± 0.6	41.0 ± 1.2	0.393
Radius	18.1 ± 0.3	16.8 ± 0.7	0.096
SSI (mm^3)			
Tibia	1288 ± 21.7	1181 ± 45.8	0.039
Radius	221.1 ± 3.7	204.2 ± 7.7	0.051

[1] Bone variables measured by peripheral quantitative computed tomography at the 4% (trabecular) and the 20% (cortical) site: Tot BMD = total volumetric BMD, Trab BMD = trabecular volumetric BMD, Tot area = total cross-sectional area of bone, Cort BMD = cortical volumetric BMD, Cort area = cortical bone area, Cort BMC = cortical bone mineral content, Cort thk = cortical thickness, Peri circ = periosteal circumference, Endo circ = Endosteal circumference, SSI = strength strain index

[2] Cutpoints used to denote normal fat (n = 93) and high fat (n = 22) by percent body fat were determined using cardiovascular risk factors (27, 28).

[3] Values are means ± SE adjusted for muscle cross-sectional area (66% site).

[4] Tests of significance between groups are based on group main effect by ANCOVA.

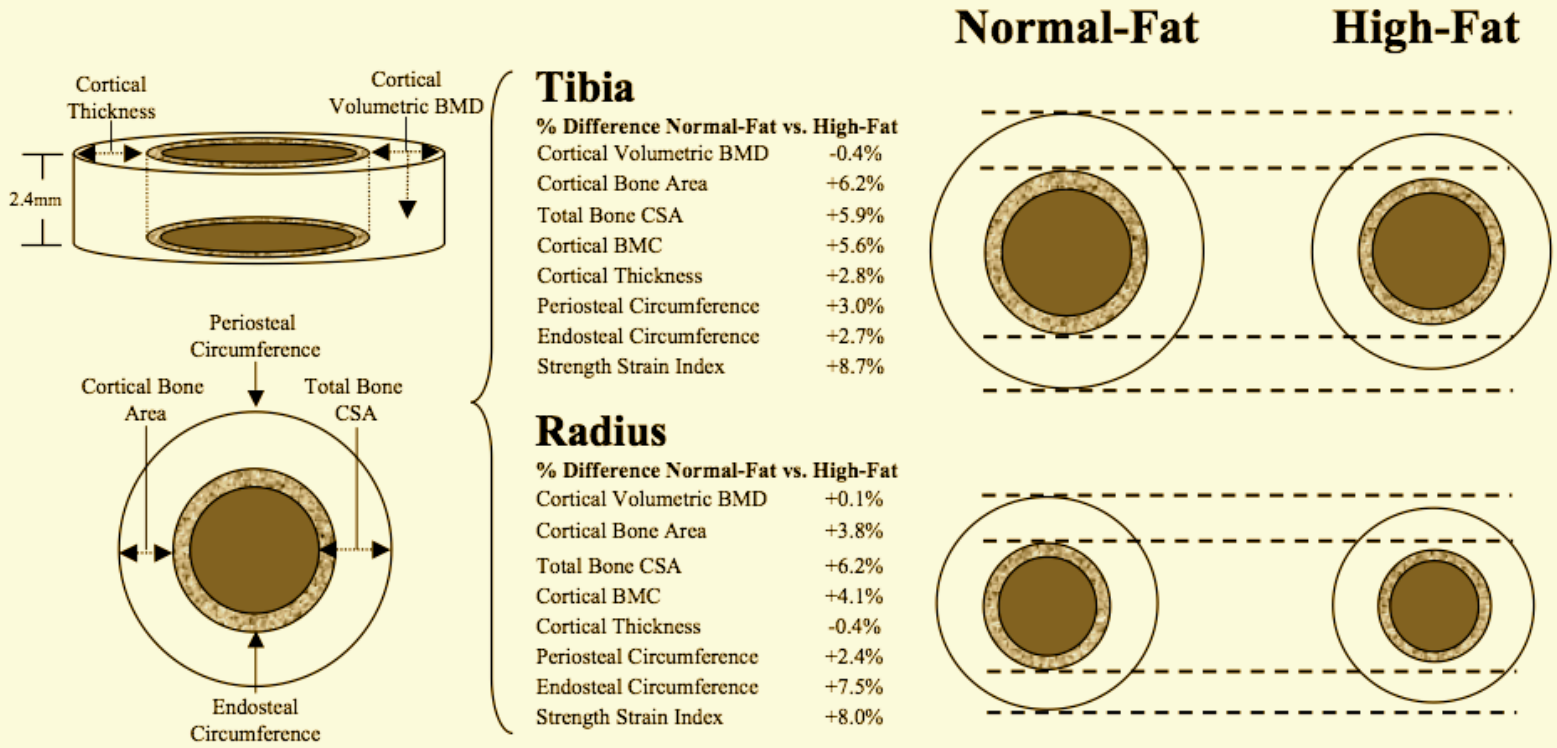

Figure 3.1 Schematic representation of the average magnitude of difference [A-B/((A+B)/2)*100] at the 20% site of the tibia and radius, after controlling for muscle cross-sectional area, in normal-fat (n=93) vs. high-fat (n=22) adolescent females. The outer white circles represent cortical bone, the textured circles represent trabecular bone and the gray circles represent the medullary cavity.

CHAPTER 4

DO RACIAL DIFFERENCES EXIST IN BONE STRUCTURE AND STRENGTH IN LATE ADOLESCENT FEMALES? [1]

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[1]Pollock, N.K., Laing, E.M., Taylor, R.G., Baile, C.A., Hamrick, M.W., Lewis, R.D. 2008. To be submitted to *The Journal of Clinical Endocrinology and Metabolism*.

ABSTRACT

Racial differences in bone strength, using 3-dimensional imaging, at non-weight bearing skeletal sites have not been investigated. The purpose of the study was to determine whether there are racial differences in bone strength measurements, assessed by pQCT, at the tibia (weight-bearing skeletal region) and radius (non-weight-bearing skeletal region) in black (*n*=48) and white (*n*=98) females in late adolescence. Because of the importance of body size on bone, 25 whites and 25 blacks were individually matched on age, height, FFST mass, and weight. Body composition [FFST mass, fat mass and %fat] was measured using DXA. Tibial and radial measurements were assessed at the 4%, 20% and 66% sites from the distal metaphyses, which reflect trabecular bone, cortical bone and MCSA, respectively. Limb lengths were also measured at the tibia and radius. Due to racial differences in muscle size and body segment lengths, the data were statistically adjusted for differences in MCSA and limb length at each respective bone site. Blacks vs. whites had greater tibial and radial length. MCSA differences were not observed between groups at the radius; however, at the tibia, blacks vs. whites had smaller MCSA. In the unadjusted data, blacks vs. whites had higher total volumetric BMD (tibia and radius), cortical BMD (Cort BMD; tibia only), cortical cross sectional area (tibia only), cortical BMC (tibia only), cortical thickness (tibia only), polar strength-strain index (tibia only) and lower trabecular BMD (tibia only), total cross sectional area (tibia and radius at 4% site only), and Cort BMD (radius only). After adjustments, the racial differences in most bone measurements remained. However, bone length differences between groups at the radius explained the greater radial total bone cross-sectional area (20% site) in the blacks versus whites and the higher radial cortical vBMD in the whites versus blacks. The results create a bone strength profile reflecting stronger bone at the tibia in young black versus white females, possibly accounting for the lower fracture

rates in older blacks at weight-bearing sites (hip and spine). However, at a non-weight bearing site such as the radius, racial differences are less evident.

KEY WORDS: Race, Ethnicity, African American, Bone structure, Bone geometry, bone strength, quantitative computed tomography

INTRODUCTION

Although osteoporotic fractures occur at twice the rate in white compared to black females,[1-4] recent reports have suggested that approximately 1.5 million black women have poor bone health and are at-risk for skeletal fractures.[5, 6] The higher osteoporotic fracture rates in white compared to black females may be related to a racial dimorphism in skeletal strength that is thought to emerge during growth. However, the actual existence of racial differences in bone strength has been a controversial issue, primarily due to the methodology employed, the skeletal sites measured and the statistical adjustments of data.

The postulation that young blacks may have stronger bones than whites was generated from dual energy X-ray absorptiometry (DXA) studies, which focused on the material surrogates of bone strength (i.e., aBMD and BMC).[11-15] In these studies, young blacks were found to have higher areal bone mineral density (aBMD) and bone mineral content (BMC) at the lumbar spine, hip, forearm, and total body than whites, which seems to be evident from prepuberty[11, 12] to late puberty.[13-15] However, racial differences in aBMD and BMC, at the lumbar spine, hip and total body, but not at the forearm, were reduced or eliminated when statistical adjustments were made for differences in bone size and body size.[11-17]

Bone strength during growth is the result of changes in both the material (e.g., mineral density) and geometric (e.g., size and shape) properties of cancellous (trabecular) and compact (cortical) bone.[7-10] DXA can measure material surrogates of bone strength; however, because of its two-dimensional methodology, DXA lacks the ability to assess structural components related to bone strength. Furthermore, it is incapable of differentiating trabecular from cortical bone. Greater accuracy in predicting bone strength requires three-dimensional bone assessments of both the material and geometric properties of trabecular and cortical bone.[7-10] Quantitative

computed tomography (QCT) and peripheral QCT (pQCT) have the capability to measure, in three dimensions, surrogates of material and geometric bone strength at trabecular and cortical bone, independently.[19, 20]

The use of three-dimensional methodology to investigate racial differences in skeletal strength during growth is limited because, currently, only two studies have been conducted.[21, 22] Gilsanz and colleagues[21] used QCT to assess lumbar spine volumetric BMD (vBMD) in black and white females between the ages of 2 and 20 years, matched for age and sexual maturation. In the overall analyses, groups did not differ in lumbar spine vBMD; however, among those in late puberty, the black females were found to have 23% greater lumbar spine vBMD than white females. In the second QCT investigation,[24] the same group of investigators matched black and white males and females, aged 8 to 18 years, for age, sex, weight, height, and sexual maturation to assess racial differences in vBMD and cross-sectional area at the lumbar spine and femoral midshaft. At the lumbar spine, blacks were found to have higher vBMD than whites, but a similar cross-sectional area. Conversely, at the femur, no differences were found in vBMD; however, the black females had greater cross-sectional area than their white counterparts, which the authors attributed to their longer femoral bone length. [24] While these studies provide valuable insight to racial differences in bone strength at weight-bearing skeletal regions, they lacked information pertaining to non-weight bearing skeletal sites such as the radius. This is important since an increase in distal forearm fractures in children and adolescents have been observed over the past few decades.[23]

The purpose of this investigation was to determine whether there are racial differences in material and geometric properties of bone strength, assessed by pQCT, at cortical and trabecular sites of the tibia (weight-bearing skeletal region) and radius (non-weight-bearing skeletal region)

in black and white females in late adolescence. To minimize any influences of age, body size and sexual maturation on bone outcome variables, 25 whites and 25 blacks were individually matched on age, height, fat-free soft tissue mass, and weight. We further examined whether racial differences in bone measures at the tibia and radius could be explained by differences in bone length and muscle cross-sectional area for each site measured.

SUBJECTS AND METHODS

Study Participants

The baseline data from white (*n*=98; aged 18 to 19 years) and black (*n*=48; aged 18 to 22 years) female students at The University of Georgia, who originally participated in two separate studies [Fighting Osteoporosis in College Using Soy (white subjects)[24] in the winter of 2005-2006 and the Health and Bone Study (black subjects) in the winter of 2007-2008], were used for matching in this investigation. Because of the importance of body size on bone, 25 black females were individually matched with 25 white females on age (± 2%), height (± 1%), fat-free soft tissue mass (FFST; ± 2%), and weight (± 7%). All participants must have reported normal menstruation (e.g., ≥ 4 menstrual periods in the last 6 months) for inclusion in the study. Participants were excluded if they reported significant weight loss or gain in the past 6 months (± 10% initial body weight), participation in NCAA Division I athletics, diagnosis of eating disorders, present illness or chronic disease, and use of medications or herbal supplements known to affect body weight, body fat or bone metabolism. Participant ethnicity (Hispanic or Latino/Non-Hispanic or Latino) and race (American Indian or Alaska Native, Asian, Black or African-American, Native Hawaiian or other Pacific Islander, White, or any combination of the above) were classified using the National Institutes of Health Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research.[25] Procedures were

approved by the Institutional Review Board for Human Subjects at The University of Georgia, and all participants provided written consent.

Anthropometry

Height and body weight measurements were collected by a trained laboratory technician. Participants were measured for height and weighed in light indoor clothing following the removal of shoes. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Novel Products Inc., Rockton, IL). Body weight was measured to the nearest 0.1 kg using an electronic scale (Seca Bella 840, Columbia, MD). Prior to testing each week, the scale was checked for accuracy using known weights. Recalibration of the scale was not required during the testing sessions. Limb lengths were measured with anthropometric tape (Rosscraft, Inc) to the nearest 0.10 mm at the tibia (the distal edge of the medial malleolus to the tibial plateau) and forearm (distance between the ulnar styloid process and olecranon).

Body composition

Body composition variables [fat mass (kg), FFST (kg) and percentage body fat (%fat)] were measured using DXA (Delphi A; S/N 70467; Hologic Inc., Bedford, MA). The same technician analyzed all scans using Hologic Whole Body Analysis software, version 11.2. Quality assurance for fat mass, FFST and %fat measured by DXA was carried out by calibration against a three-step soft tissue wedge (Hologic anthropomorphic spine phantom, model DPA/QDR-1; SN 9374) composed of different thickness levels of aluminum and lucite, calibrated against stearic acid (100% fat) and water (8.6% fat). In our laboratory, a coefficient of variation of 0.36% was observed from 648 scans of the spine phantom over a 3-year period. Based on a one-way random effects model, single measure intra-class coefficients (ICC) were

calculated in 5 females, aged 18 to 30 years, scanned twice in our lab during a 7-day period for fat mass, FFST and %fat (all $R \geq 0.87$).

Peripheral quantitative computed tomography

Peripheral QCT (Stratec XCT-2000; Stratec Medizintechnic GmbH, Pforzheim, Germany) measurements were taken of the nondominant tibia and radius. Tibial measures were taken at the 4% and 20% sites of the total tibial length from the distal metaphysis and represent areas high in trabecular and cortical bone, respectively. Measurements were also assessed at the 4% and 20% sites of the forearm length, proximal to the distal radial metaphysis. Each scan was acquired with a 0.4-mm voxel and at a slice thickness of 2.4-mm. The positioning of the two cross-sectional measurements from the tibia and radius were determined in a scout view using their medial endplate as an anatomic marker and automatically set by the software at 4% or 20% sites. Image processing and calculation of the various bone measures and MCSAs were determined using the Stratec software (version 5.50*d*). Total and trabecular vBMD (mg/cm^3) and total bone cross-sectional area (mm^2) were calculated for tibia and radius 4% sites using contour mode 2 and peel mode 2. The following variables were assessed at the tibia and radius 20% sites: cortical vBMD (mg/cm^3), cortical bone area (mm^2), total bone cross-sectional area, cortical BMC (mg), cortical thickness (mm), periosteal circumference (mm), endosteal circumference (mm), and polar strength-strain index (mm^3). Cortical bone variables for both 20% sites were assessed using cort mode 1 and the default threshold of 710 mg/cm^3. The polar strength-strain index was analyzed at cort mode 1 and a threshold of 280 mg/cm^3.

A third measurement was taken at the 66% site of both the tibia and radius to assess MCSA (mm^2), an estimate of muscle strength. The proximal two-thirds site was chosen because in this region the muscle has the highest circumference and cross-sectional area.[26, 27] The MCSA

was determined by placing a region of interest within the subcutaneous fat tissue. Contour mode 3 with a threshold of 34 mg/cm^3 and peel mode 1 were used to obtain the "area of muscle plus bone" (i.e., muscle + tibia + fibula or muscle + radius + ulna). Next, the analysis was performed with contour mode 1, threshold of 280 mg/cm^3 and peel mode 1 to determine the "area of bone" (i.e., tibia + fibula or radius + ulna). The MCSA is finally determined by subtracting the "area of bone" from the "area of muscle plus bone".

All pQCT measures were assessed and analyzed by the same trained operator. The pQCT operator scanned the phantom daily to maintain quality assurance. Test-retest measurements were performed in 5 females, aged 18 to 24 years, to determine reliability of the pQCT in our laboratory. The one-way random effects model, ICCs for all pQCT measurements were calculated to be $R \leq 0.97$.

Statistical analyses

Data were analyzed using SPSS version 11.0.2 (Chicago, IL) for the Mac OS X. Normal distribution and homogeneity of variances were confirmed by Shapiro-Wilks *W* and Levene's tests, respectively. Racial differences for anthropometric, body composition and unadjusted bone response variables were determined using paired-samples *t* test if data were normally distributed and Wilcoxon matched-pairs signed-rank tests, otherwise. Descriptive statistics for raw variables are presented as mean ± SD unless stated otherwise. An *F*-test was performed to test the assumption of homogeneity of regression slopes with regard to the interaction between the paired sample (i.e., white and black females) variables and the covariates (i.e., tibial length, forearm length, tibial MCSA and radial MCSA). Since there was no interaction, repeated measures analysis of covariance was used to compare the differences of means for bone response variables for the paired samples after adjusting for limb length and MCSA differences. Estimated means of

bone variables in the adjusted analyses are reported in the form of mean ± SE. Statistically significant differences are reported if $P < 0.05$.

RESULTS

Anthropometric and unadjusted bone measurements

Mean age, weight, height, BMI-for-age, total FFST and fat mass, %fat, tibial and forearm length, MCSA, and unadjusted bone variables of the participants are provided in **Table 4.1**. By design, no differences were found in mean age, height, weight, and FFST values between white and black females. BMI-for-age, fat mass and %fat also were not statistically different between groups; however, tibial and radial lengths were significantly greater in the black versus the white group ($P = 0.001$ and $P < 0.001$, respectively). The MCSA at the tibia ($P < 0.001$), but not at the radius ($P = 0.876$), was significantly greater in the white group compared with the black group.

At the 4% site, significant differences were observed where blacks > whites in total vBMD at the tibia ($P < 0.001$) and radius ($P < 0.001$), and whites > blacks in trabecular vBMD at the tibia ($P = 0.023$) and total cross-sectional area at the tibia ($P < 0.001$) and radius ($P = 0.014$). There were no differences between the groups in trabecular vBMD at the radius ($P = 0.115$).

For the 20% site, blacks had greater values than whites for tibial cortical vBMD ($P = 0.016$), cortical bone cross-sectional area ($P = 0.005$), cortical BMC ($P = 0.002$), cortical thickness ($P = 0.012$), and polar strength-strain index ($P = 0.035$), as well as in radial total cross-sectional area ($P = 0.037$). Cortical vBMD at the radius, however, was significantly greater in the white versus the black females ($P = 0.016$). There were no group differences at the tibia in total cross-sectional area, periosteal circumference or endosteal circumference, or at the radius in

cortical bone cross-sectional area, cortical BMC, cortical thickness, periosteal circumference, endosteal circumference, or polar strength-strain index.

Adjusted bone measurements

Adjusted tibial bone measures, controlling for differences in MCSA and tibial length, as well as adjusted radial bone outcomes, controlling for differences in forearm length at the radial site are summarized by group in **Table 4.2**. After adjusting for MCSA and tibial length differences at the 4% and 20% sites of the tibia, the adjusted bone measurements were similar to the unadjusted bone measures in Table 1 (all $P < 0.05$).

After adjusting for differences in forearm length at the 4% and 20% sites of the radius, the adjusted bone results were similar to the unadjusted bone observations in Table 1 (all $P < 0.05$). However, cortical vBMD ($P = 0.107$) and total cross-sectional bone area ($P = 0.557$) differences between groups were no longer observed once correcting for limb length differences at the 20% site of the radius.

DISCUSSION

The objective of this study was to determine whether there are racial differences in material and geometric properties of bone strength, assessed by pQCT, at cortical and trabecular sites of the tibia (weight-bearing skeletal region) and radius (non-weight-bearing skeletal region) in black and white females in late adolescence. At the tibial and radial trabecular bone sites, we found that black versus white females had greater total vBMD at the tibia and radius; whereas, the whites had larger total cross-sectional areas at the tibia and radius and also had greater trabecular vBMD at the tibia only compared to the black females. The analyses at the tibial cortical bone site revealed that black females had greater bone strength parameters (cortical vBMD, cortical bone cross-sectional area, cortical BMC, cortical thickness, and polar strength-

strain index) in comparison to the whites. At the radial cortical bone site, the blacks versus whites had a greater total bone cross-sectional area; whereas, the whites versus blacks had higher cortical vBMD. However, after controlling for radial limb length, differences in total bone cross-sectional area and cortical vBMD were no longer seen between groups. Our data suggest that at a weight-bearing site (tibia), but not at a weight-bearing site (radius), differences in cortical bone strength are evident between black and white females. However, racial differences are less evident at predominately trabecular bone sites in both the tibia and radius.

Our finding that blacks had greater cortical bone geometric parameters at the tibia is somewhat consistent with previous work by Gilsanz et al.[22] They found that black children, age 8 to 18 years, had greater total bone cross-sectional area at the weight-bearing femoral midshaft than whites. They did not, however, detect racial differences in femoral cortical vBMD;[22] whereas, in our study, the tibial cortical vBMD was higher in the black versus white females. At the lumbar spine, a predominately trabecular and weight-bearing bone site, blacks have been shown to have higher vBMD than whites,[21, 22] which is reflective of our trabecular bone observations at the tibia. In additional Gilsanz et al.[22] analyses, concerning the geometrical aspects of bone strength, racial differences in bone cross-sectional area were not found of the lumbar spine, although the white females in our investigation had larger bone cross-sectional areas at weight-bearing and non-weight-bearing sites than their counterparts. Despite the similarities and differences between the presented data and the aforementioned studies,[21, 22] direct comparisons must be interpreted cautiously because of two important reasons. First, we studied black and white females within a narrow age range (18-19 years of age) and fully sexually mature in order to minimize any confounding effects of sexual maturation on bone outcome variables. Estrogen is thought to have a biphasic role in both sexes; lower levels stimulate

growth, possibly by stimulating growth hormone and insulin-like growth factor-I, and higher levels reduce longitudinal growth and lead to closure of the epiphyseal growth plates.[28-30] Since prior investigations involved wide ranges of age (2-20 years of age) and sex, the large variances in sex hormones may have manipulated the bone outcome variables.[21, 22] Second and more importantly, our findings at the tibia and radius may reflect the complex nature of mechanical stimuli at various sites of the skeleton, as animal studies have observed considerable heterogeneity in the response of bone to mechanical stimuli, not only among different skeletal sites but also among different regions of the same bone.[31, 32] Further work is necessary to ascertain both the hormonal effects of pubertal timing and various types of mechanical loading, independently and combined, on bone development at various skeletal sites.

Whereas blacks seem to have bone strength advantages over whites at weight-bearing skeletal sites such as the lumbar spine and midshaft of the femur,[21, 22] we are the first to account that this may not be the case at a non-weight bearing skeletal site, particularly at the radius. Although the material (e.g., vBMD) and geometric (e.g., size and shape) properties of bone contribute to overall skeletal strength, it has been suggested that bone's mechanical integrity depends mainly on the size, cross-sectional area and internal architecture; whereas, material properties vary less, and thus, may not be as important.[33-35] It has been reported that increasing the periosteal diameter will reduce the vBMD without affecting bone-bending strength.[34, 36-38] Since the white females in our study had a larger total cross-sectional area at the 4% site of the radius (indicative of a larger periosteal diameter) than blacks, they may have greater fracture protection at a site that is often fractured in children and adolescents. Even though distal forearm fractures are increasing in children and adolescents, the increase, however, seems to be independent of race.[23]

Since bones perceive loading-induced strains and adapt their structure to increase its overall strength via changes in size and shape to the prevalent loading environment,[39-41] the use of site-specific surrogates of muscle force and limb length have been employed when comparing bone strength differences in populations of different body sizes.[34, 42, 43] In this study, we wanted to determine whether racial differences in bone strength could be explained by differences in muscle size and limb length at each respective site. After the statistical adjustments at each respective bone site, the racial differences in most bone measurements remained. However, forearm length differences between groups at the radius explained the greater radial total bone cross-sectional area (20% site) in the blacks versus whites and the higher radial cortical vBMD in the whites versus blacks. These findings suggest that factors other than site-specific muscle and skeletal size are important with respect to bone strength at weight-bearing skeletal sites. It is thought that estrogen secretion during growth may reduce the bone remodeling threshold on the endocortical surface and thereby sensitize bone next to marrow to the effect of weight-bearing loads,[44] resulting in an greater storage of bone mineral and an increase in bone area. Although we did not collect menstrual start age of the study participants, other studies have found black females to enter sexual maturation earlier than whites.[45, 46] Thus, it possible that the stronger cortical bone observed in blacks versus whites at the tibia could be related to the influence of longer estrogen exposure between the onset of pubertal maturation and their initial bone measurement. Continued monitoring is essential to determine the potential estrogen-related effect on weight-bearing cortical bone differences in white and black females.

In addition to not having menstrual start age of the participants, other limitations of the study were that we did not collect information on pre- and postpubertal weight-bearing activities and biochemical measures of bone-related hormones. Furthermore, our study utilized cross-

sectional data and thus was not specifically designed to examine the longitudinal effects of race and bone development. It is also important to note that the analysis of muscle loading effects is complex and the use of total FFST and MCSA does not reflect the functional status of the entire muscle system, including muscle length, contraction velocity, structure, and coordination.[47] Whether the data generated using this technique can be used to predict bone health and risk of skeletal fractures must be validated by subsequent prospective studies.

An advantage of our sample was that we minimized the degree of variability by controlling for factors known to influence bone, such as sex, age and maturational status. Furthermore, we individually matched the groups on age, height, FFST, and weight because of the disparities in bone and body size between whites and blacks.[48] We also analyzed the effects of race on bone strength, independent of site-specific muscle loads and skeletal size. In addition, our study utilized pQCT, which enabled us to examine separate details of both trabecular and cortical bone geometry and strength parameters in the appendicular skeleton.

In conclusion, these results create a bone strength profile reflecting stronger bone at the weight-bearing tibia in young adult black versus white females, possibly accounting for the lower fracture rates in older black females at weight-bearing skeletal sites (hip and spine). However, at a non-weight bearing site such as the radius, racial differences are less evident. Further research is warranted to determine the factors responsible for the racial differences at the weight-bearing, but not at the non-weight-bearing, skeletal sites.

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Table 4.1

Characteristics of the participants [1]

	White	Black
n	25	25
Age (yrs)	18.2 ± 0.4	18.5 ± 0.5
Weight (kg)	63.5 ± 8.8	63.7 ± 9.1
Height (cm)	163.8 ± 6.8	162.9 ± 6.7
BMI-for-age (kg/m^2)	23.7 ± 3.2	24.1 ± 3.7
FFST mass (kg) [3]	43.5 ± 4.8	44.0 ± 5.4
Fat mass (kg)	18.9 ± 5.4	19.8 ± 6.0
Fat mass (%)	28.9 ± 4.8	29.5 ± 5.5
Tibial length (mm)	372.0 ± 21.5	387.2 ± 26.2 [2]
Forearm length (mm)	256.6 ± 14.8	267.5 ± 12.8 [2]
Tibial MCSA (mm^2) [4]	7636 ± 1487 [2]	6292 ± 1082
Forearm MCSA (mm^2)	2743 ± 360	2728 ± 513
Bone variables (*4% site*) [5]		
Tot vBMD (mg/cm^3)		
Tibia	313.9 ± 38.3	394.1 ± 72.7 [2]
Radius	343.6 ± 58.9	407.3 ± 74.9 [2]
Trab vBMD (mg/cm^3)		
Tibia	254.5 ± 26.2 [2]	228.6 ± 44.1
Radius	208.8 ± 38.5	225.2 ± 32.1
Tot area (mm^2)		
Tibia	947.0 ± 119.4 [2]	732.4 ± 190.4
Radius	285.3 ± 47.1 [2]	254.1 ± 47.1

Bone variables (*20% site*) [5]		
Cort vBMD (mg/cm^3)		
Tibia	1171 ± 18.1	1188 ± 21.3 [2]
Radius	1195 ± 18.0 [2]	1174 ± 45.0
Cort area (mm^2)		
Tibia	196.9 ± 27.8	215.2 ± 27.6 [2]
Radius	71.4 ± 10.0	73.4 ± 9.2
Tot area (mm^2)		
Tibia	346.3 ± 45.8	360.0 ± 53.6
Radius	98.6 ± 16.2	106.5 ± 19.0 [2]
Cort BMC (mg)		
Tibia	230.5 ± 32.3	255.7 ± 31.3 [2]
Radius	85.3 ± 11.8	86.2 ± 10.9
Cort thk (mm)		
Tibia	3.63 ± 0.5	3.95 ± 0.5 [2]
Radius	2.68 ± 0.3	2.63 ± 0.3
Peri circ (mm)		
Tibia	65.8 ± 4.3	67.1 ± 4.9
Radius	34.5 ± 3.8	36.5 ± 3.2
Endo circ (mm)		
Tibia	43.0 ± 5.2	42.2 ± 5.7
Radius	18.8 ± 4.9	19.9 ± 4.3
pSSI (mm^3)		
Tibia	1335 ± 259	1460 ± 300 [2]
Radius	232.0 ± 50.7	243.1 ± 57.2

[1] Values are means ± SD.

[2] Tests of significance between groups are based on 2-tailed independent *t*-tests.

Significant difference between groups, $P \leq 0.05$.

[3] FFST, fat-free soft-tissue

[4] MCSA, muscle cross-sectional area

[5] Bone variables measured by peripheral quantitative computed tomography at the 4% (trabecular) and the 20% (cortical) site: Tot vBMD = total volumetric BMD, Trab vBMD = trabecular volumetric BMD, Tot area = total bone cross-sectional area, Cort vBMD = cortical volumetric BMD, Cort area = cortical bone area, Cort BMC = cortical bone mineral content, Cort thk = cortical thickness, Peri circ = periosteal circumference, Endo circ = Endosteal circumference, pSSI = polar strength-strain index

Table 4.2

Adjusted bone measurements of the tibia and radius in white and black late adolescent females

Bone variable [1]	White (n = 25)	Black (n = 25)	*P-value* [4]
4% site			
Tot vBMD (mg/cm^3)			
Tibia[2]	309.9 ± 12.8	398.1 ± 12.8	0.001
Radius[3]	342.0 ± 14.1	408.9 ± 14.1	0.002
Trab vBMD (mg/cm^3)			
Tibia	254.3 ± 8.0	228.8 ± 8.0	0.043
Radius	206.8 ± 7.4	227.2 ± 7.4	0.065
Tot area (mm^2)			
Tibia	950.8 ± 33.9	728.6 ± 33.9	0.001
Radius	291.0 ± 9.4	264.4 ± 9.4	0.003
20% site			
Cort vBMD (mg/cm^3)			
Tibia	1172 ± 4.2	1187 ± 4.2	0.023
Radius	1193 ± 7.1	1176 ± 7.1	0.107
Cort area (mm^2)			
Tibia	191.9 ± 5.1	220.2 ± 5.1	0.001
Radius	72.3 ± 2.0	72.5 ± 2.0	0.950
Tot area (mm^2)			
Tibia	343.7 ± 8.7	362.2 ± 8.7	0.172
Radius	101.1 ± 3.5	104.1 ± 3.5	0.557
Cort BMC (mg)			
Tibia	225.0 ± 5.9	261.2 ± 5.9	0.001
Radius	86.2 ± 2.3	85.2 ± 2.3	0.774

Cort thk (mm)			
Tibia	3.54 ± 0.10	4.04 ± 0.10	0.002
Radius	2.68 ± 0.07	2.63 ± 0.07	0.648
Peri circ (mm)			
Tibia	65.6 ± 0.8	67.3 ± 0.8	0.175
Radius	34.7 ± 0.7	36.2 ± 0.7	0.171
Endo circ (mm)			
Tibia	43.4 ± 1.1	41.9 ± 1.1	0.377
Radius	19.4 ± 0.9	19.3 ± 0.9	0.938
pSSI (mm^3)			
Tibia	1304 ± 50.2	1491 ± 50.2	0.019
Radius	238.0 ± 10.9	237.1 ± 10.9	0.953

[1] Bone variables measured by peripheral quantitative computed tomography at the 4% (trabecular) and the 20% (cortical) site: Tot vBMD = total volumetric BMD, Trab vBMD = trabecular volumetric BMD, Tot area = total bone cross-sectional area, Cort vBMD = cortical volumetric BMD, Cort area = cortical bone area, Cort BMC = cortical bone mineral content, Cort thk = cortical thickness, Peri circ = periosteal circumference, Endo circ = Endosteal circumference, pSSI = polar strength-strain index

[2] Tibial bone values are means ± SE adjusted for muscle cross-sectional area (66% site) and limb length.

[3] Radial bone values are means ± SE adjusted for limb length.

[4] Tests of significance between groups are based on group main effect using ANCOVA.

CHAPTER 5

ADIPOSITY AND BONE STRENGTH IN AFRICAN AMERICAN FEMALES[1]

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[1]Pollock, N.K., Laing, E.M., Baile, C.A., Hamrick, M.W., Lewis, R.D. 2008. To be submitted to *The American Journal of Clinical Nutrition*.

ABSTRACT

Obesity has been shown to have a negative impact on bone strength in late adolescent white females, but little is known for the same in blacks. The purpose of this investigation was to examine the relationships between total fat mass and measures of tibial and radial bone strength in young black ($N = 48$; aged 19.2 ± 1.2 years) females. Bone measurements in the normal- and high-fat groups were also compared. Fat-free soft tissue (FFST) mass, fat mass and percentage body fat were measured using dual energy X-ray absorptiometry. Tibial and radial bone measurements were assessed by peripheral quantitative computed tomography at the 4% (trabecular bone), 20% (cortical bone) and 66% (muscle cross-sectional area; MCSA) sites from the distal metaphyses. Partial correlations were performed to determine associations between total fat mass and the bone outcome variables, controlling for height, limb length, FFST mass, and MCSA. Significant inverse relations were found between total fat mass and cortical bone area (radius only), total cross-sectional area (radius only), cortical BMC (tibia only), periosteal circumference (radius only), and polar strength-strain index (tibia only) at the 20% site ($P < 0.05$). When black participants were compared by amount of adiposity, no significant differences were observed in tibial and radial bone measurements between groups, even though the high-fat group was carrying significantly greater soft-tissue loads (14-kg fat mass and 4-kg of FFST mass) than the normal-fat group. After controlling for FFST mass differences, the high-fat group had significantly lower tibial cortical bone measures than did the normal-fat group: cortical bone area ($P = 0.053$, $\eta^2 = 0.083$), total bone cross-sectional area ($P = 0.013$, $\eta^2 = 0.133$), cortical BMC ($P = 0.040$, $\eta^2 = 0.092$), periosteal circumference ($P = 0.012$, $\eta^2 = 0.136$), and polar strength-strain index ($P = 0.004$, $\eta^2 = 0.175$). Consistent with our adiposity and bone strength analyses in a predominately white sample of late adolescent females, our findings in black females entering

adulthood also suggest that excess adiposity levels may adversely influence the overall strength of cortical bone at appendicular skeletal sites.

KEY WORDS: African American, Black, Obesity, Bone structure, Bone geometry, Bone strength, Quantitative computed tomography

INTRODUCTION

High body weight in children and adolescents has typically been associated with increased bone strength. Overweight youth have been reported to either have higher lumbar spine or total body bone mineral content (BMC) relative to height, maturation and/or fat-free soft-tissue mass.[1-3] Leonard et al.[2] observed in children and adolescents that being overweight, based on BMI-for-age percentiles, was a predictive factor for higher lumbar spine areal bone mineral density (aBMD) corrected for height. Ellis et al.[1] placed children into three groups by percent body fat (< 25%, 25-30% and > 30% body fat) and found that the > 30% body fat group had significantly higher total body BMC relative to height than the other groups.

This paradigm that a higher body weight is associated with improved bone strength has recently been questioned.[4] Goulding and colleagues[5, 6] observed that overweight children and adolescents had lower lumbar spine and total body BMC and bone area relative to weight compared to those with normal BMI-for-age percentiles. Moreover, the same group of investigators found that a high body weight, independent of total body lean mass, contributed to fracture risk in children and adolescents who had fractured their forearms repeatedly.[7] In another fracture study of females, four to 15 years of age, those who sustained a fracture were more overweight and had a smaller forearm cross-sectional area compared to girls who did not experience a fracture.[8]

The discrepancies in the above studies were most likely related to the use of different statistical approaches presenting either adjusted (e.g., for body size, sex, maturity) or unadjusted bone mineral data, and/or the use of dual-energy X-ray absorptiometry (DXA) to assess bone strength.[1-3, 5-13] Bone strength during growth is the result of changes in both the material (e.g., mineral density) and geometric (e.g., size and shape) properties of cancellous (trabecular) and

compact (cortical) bone.[12, 14-16] While DXA can measure material surrogates of bone strength, this two-dimensional methodology lacks the ability to assess structural components related to bone strength. Furthermore, it is incapable of differentiating trabecular from cortical bone. Greater accuracy in predicting bone strength requires three-dimensional bone imaging techniques,[12, 14-16] such as quantitative computed tomography (QCT) and peripheral QCT (pQCT), which both have the capability to measure material and geometric aspects of bone strength and to differentiate between trabecular and cortical bone.[17, 18]

We recently reported in late adolescent females that excess weight in the form of fat mass does not provide additional weight-loading benefits to material and geometric properties of bone strength, assessed by pQCT.[19] The strength strain index, an estimate of torsional bone strength calculated from material and geometric measurements, was found to be lower at cortical bone sites of the tibia (8.7%) and radius (8.0%) in the high-fat (≥ 32% body fat) group compared to the normal-fat (< 32% body fat) group.[19] In another study, which included males, aged 13 to 21 years, Janicka et al.[20] found that total fat mass was negatively associated with bone cross-sectional area, assessed by QCT, at axial and appendicular skeletal sites, after accounting for surrogates of loading forces. Why bone strength and structure are not adapting appropriately to the excess weight of fat mass is unknown.

A limitation of the aforementioned studies was that the research participants were predominately white children and adolescents. US obesity rates are highest in young African American females. The National Health and Nutrition Examination Survey, 2003-2006,[21] estimated that the overweight prevalence among black females, aged 12 to 19 years, was almost double the rate for white females (28% versus 15%). If obesity has a negative impact on skeletal strength, fractures in this population could be a public health issue. Currently, only one study has

investigated bone and fat relations in black children, independent of other races or ethnicities. Afghani and Goran,[22] found an inverse association between visceral adipose tissue, assessed by QCT, and total body BMC, measured by DXA. Although this finding proposes a potential concern for bone health in a population experiencing high rates of obesity, further work is warranted.

In our previous investigation,[19] we determined relationships between adiposity and bone strength measurements, using pQCT, in 115 late adolescent females, however, only 2 of the participants were black. As a result, we sought to investigate in a larger sample of young African American females the relations between total fat mass and pQCT-assessed trabecular and cortical bone measurements within the tibia and radius. Since height, limb lengths and surrogates of muscle loads [e.g., total body fat-free soft tissue (FFST) mass and/or muscle cross-sectional area (MCSA)] may confound total fat mass and bone outcome variables,[23-26] we elected to observe these fat and bone relationships independent of the following variables: height, limb lengths for each respective bone site, FFST mass, and MCSA for each respective bone site. The second objective was to compare tibial and radial bone measurements between two adiposity groups defined as having normal and high percentages of body fat, before and after controlling for any differences in the same confounding variables (i.e., height, limb lengths for each respective bone site, FFST mass, and MCSA for each respective bone site).

SUBJECTS AND METHODS

Study Participants

African American females students ($N = 48$; aged 18 to 22 years) attending The University of Georgia (UGA), and who participated in the UGA Health and Bone Study, were used in this investigation. We elected to include only black females within this age range in order

to minimize any confounding effects from race, sex and pubertal maturation on bone outcome variables. Black or African American race was determined by self-report, in accordance with the National Institutes of Health Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research. [27] All participants must have reported normal menstruation (e.g., ≥ 4 menstrual periods in the last 6 months) for inclusion in the study. Participants were excluded if they reported significant weight loss or gain in the past 6 months (± 10% initial body weight), previous participation in competitive high school sports and/or NCAA Division I athletics, diagnosis of eating disorders, present illness or chronic disease, and use of medications or herbal supplements known to affect body weight, body fat or bone metabolism. Procedures were approved by the Institutional Review Board for Human Subjects at UGA, and all participants provided written consent.

Participants were divided into 2 groups on the basis of their percent body fat: normal fat (< 32% body fat; $n = 33$) and high fat (≥ 32% body fat; $n = 15$). These classifications were selected based on levels of body fat associated with cardiovascular risk factors.[28, 29] By grouping the black females by body fat percent, we exclude the possibility of misclassification of those with high levels of body fat that may have otherwise been classified as normal weight if BMI had been used for the grouping procedure.

Determination of the sample size needed for this investigation was based on inverse relations observed between adiposity and bone strength measurements in prepubertal black children[22] and postpubertal white females.[19] From these two studies, we expected relationships between adiposity and bone strength measures to have effect sizes in the range of -0.30 to - 0.50, which would require 27 to 50 participants. It is noteworthy that with a sample size of 48 and

observed *r* values in the range of -0.32 to -0.41, we had over 82% power at the 0.05 level of significance.

Anthropometry

Height and body weight measurements were collected by a trained laboratory technician. Participants were measured for height and weighed in light indoor clothing following the removal of shoes. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Novel Products Inc., Rockton, IL). Body weight was measured to the nearest 0.1 kg using an electronic scale (Seca Bella 840, Columbia, MD). Prior to testing each week, the scale was checked for accuracy using known weights. Recalibration of the scale was not required during the testing sessions. Limb lengths were measured with anthropometric tape (Rosscraft, Inc) to the nearest 0.10 mm at the tibia (the distal edge of the medial malleolus to the tibial plateau) and forearm (distance between the ulnar styloid process and olecranon).

Body composition

Body composition variables [fat mass (kg), FFST mass (kg) and percentage body fat (%fat)] were measured using DXA (Delphi A; S/N 70467; Hologic Inc., Bedford, MA). The same technician analyzed all scans using Hologic Whole Body Analysis software, version 11.2. Quality assurance for fat mass, FFST mass and %fat measured by DXA was carried out by calibration against a three-step soft tissue wedge (Hologic anthropomorphic spine phantom, model DPA/QDR-1; SN 9374) composed of different thickness levels of aluminum and lucite, calibrated against stearic acid (100% fat) and water (8.6% fat). In our laboratory, a coefficient of variation of 0.36% was observed from 648 scans of the spine phantom over a 3-year period. Based on a one-way random effects model, single measure intra-class coefficients (ICC) were

calculated in 5 females, aged 18 to 30 years, scanned twice in our lab during a 7-day period for fat mass, FFST mass and %fat (all $R \geq 0.87$).

Peripheral quantitative computed tomography

Peripheral QCT (Stratec XCT-2000; Stratec Medizintechnic GmbH, Pforzheim, Germany) measurements were taken of the nondominant tibia and radius. Tibial measures were taken at the 4% and 20% sites of the total tibial length from the distal metaphysis and represent areas high in trabecular and cortical bone, respectively. Measurements were also assessed at the 4% and 20% sites of the forearm length, proximal to the distal radial metaphysis. Each scan was acquired with a 0.4-mm voxel and at a slice thickness of 2.4-mm. The positioning of the two cross-sectional measurements from the tibia and radius were determined in a scout view using their medial endplate as an anatomic marker and automatically set by the software at 4% or 20% sites. Image processing and calculation of the various bone measures and MCSAs were determined using the Stratec software (version 5.50*d*). Total and trabecular vBMD (mg/cm^3) and total bone cross-sectional area (mm^2) were calculated for tibia and radius 4% sites using contour mode 2 and peel mode 2. The following variables were assessed at the tibia and radius 20% sites: cortical vBMD (mg/cm^3), cortical bone area (mm^2), total bone cross-sectional area, cortical BMC (mg), cortical thickness (mm), periosteal circumference (mm), endosteal circumference (mm), and polar strength-strain index (mm^3). Cortical bone variables for both 20% sites were assessed using cort mode 1 and the default threshold of 710 mg/cm^3. The polar strength-strain index was analyzed at cort mode 1 and a threshold of 280 mg/cm^3.

A third measurement was taken at the 66% site of both the tibia and radius to assess MCSA (mm^2), an estimate of muscle strength. The proximal two-thirds site was chosen because in this region the muscle has the highest circumference and cross-sectional area.[30, 31] The MCSA

was determined by placing a region of interest within the subcutaneous fat tissue. Contour mode 3 with a threshold of 34 mg/cm^3 and peel mode 1 was used to obtain the "area of muscle plus bone" (i.e., muscle + tibia + fibula or muscle + radius + ulna). Next, the analysis was performed with contour mode 1, threshold of 280 mg/cm^3 and peel mode 1 to determine the "area of bone" (i.e., tibia + fibula or radius + ulna). The MCSA is finally determined by subtracting the "area of bone" from the "area of muscle plus bone".

All pQCT measures were assessed and analyzed by the same trained operator. The pQCT operator scanned the phantom daily to maintain quality assurance. Test-retest measurements were performed in 5 females, aged 18 to 24 years, to determine reliability of the pQCT in our laboratory. The one-way random effects model, ICCs for all pQCT measurements were calculated to be $R \leq 0.97$.

Statistical analyses

All analyses were performed using SPSS version 11.0.2 (Chicago, IL) for the Mac OS X. Normal distribution and homogeneity of variances were confirmed by Shapiro-Wilks *W* and Levene's tests, respectively. Partial Pearson's correlation coefficients were performed to determine the associations of total fat mass with the bone outcome variables, controlling for height, limb length, total FFST mass, and MCSA. A $P < 0.05$ was considered statistically significant.

Group differences for anthropometric, body composition and unadjusted bone response variables were determined using unpaired (i.e., independent samples) two-tailed *t*-tests if data were distributed normally and Mann-Whitney *U* tests, otherwise. Descriptive statistics for raw variables are presented as mean ± SD if not stated otherwise. An *F*-test was performed to test the assumption of homogeneity of regression slopes with regard to the interaction between the

independent variables (i.e., adiposity groups) and the potential covariates (i.e., height, limb lengths for each respective bone site, FFST mass, and MCSA for each respective bone site). Since there was no interaction, analysis of covariance was used to compare the differences in bone response variables between normal-fat and high-fat groups after adjusting for FFST mass differences. Estimated means of bone variables in the adjusted analyses are reported in the form of mean ± SE. Statistically significant differences are reported if $P < 0.05$. Medium and large effects are designated by partial eta-squared (η^2) ≥ 0.06 and 0.14, respectively.

RESULTS

Participant characteristics

Mean age, weight, height, BMI, total FFST and fat mass, %fat, tibial and forearm lengths and MCSA, and bone variables of the participants are provided in **Table 5.1**. Age and height were not statistically different between adiposity groups. The high-fat group, however, was found to have statistically higher body weight, BMI, FFST mass, and %fat than the normal-fat group (all $P < 0.05$). There were no significant differences in tibial and forearm lengths and MCSA between the groups. Among the bone variables, no significant tibial or radial differences were found at any site between the adiposity groups.

Partial correlations between total fat mass and bone measurements

Table 5.2 shows partial correlations (adjusting for height, limb length, FFST mass, and MCSA) between total fat mass and bone variables. At the 4 and 20% sites of the tibia and radius, total fat mass was inversely related to total vBMD, trabecular vBMD, total cross-sectional area (4% site only), cortical vBMD, cortical thickness, or endosteal circumference. At the 20% site, total fat mass was observed to have significant inverse relationships with radial cortical bone area, total cross-sectional area and periosteal circumference, and with tibial cortical BMC and

polar strength-strain index (all $P < 0.05$). Negative relations were also found between total fat mass and cortical bone area ($P = 0.063$), total cross-sectional area ($P = 0.118$) and periosteal circumference ($P = 0.118$) at the tibia as well as cortical BMC ($P = 0.127$) and polar strength-strain index ($P = 0.077$) at the radius.

Adjusted bone measurement comparisons between normal- and high-fat groups

Group-specific means for each bone variable based on an analysis of covariance that controls for differences in FFST mass are summarized in **Table 5.3**. After controlling for FFST mass, the high-fat group had lower tibial cortical (20% site) bone measures than did the normal-fat group: cortical bone area ($P = 0.053$, $\eta^2 = 0.083$), total bone cross-sectional area ($P = 0.013$, $\eta^2 = 0.133$), cortical BMC ($P = 0.040$, $\eta^2 = 0.092$), periosteal circumference ($P = 0.012$, $\eta^2 = 0.136$), and polar strength-strain index ($P = 0.004$, $\eta^2 = 0.175$).

DISCUSSION

In this study of late adolescent African American females, we found that total body fat mass was negatively correlated with cortical, but not trabecular, bone structure and strength indices within the tibia and radius. These adverse associations were independent of confounders to total body fat mass (i.e., height and total FFST mass) and site-specific bone measurements (i.e., limb length and MCSA). When black participants were compared by degree of adiposity, we observed no significant differences in tibial and radial bone measurements between groups, even though the high-fat group was carrying significantly greater soft-tissue loads (14-kg fat mass and 4-kg of FFST mass) than the normal-fat group. After correcting for FFST mass differences, the high-fat group had significantly lower cortical bone area, total bone cross-sectional area, cortical BMC, periosteal circumference, and polar strength-strain index at the tibial site than did the normal-fat group. Consistent with our adiposity and bone strength analyses

in a predominately white sample of late adolescent females,[19] this investigation in black females entering adulthood also suggests that excess adiposity levels may adversely influence the overall strength of cortical bone at appendicular skeletal sites.

It has recently been proposed that race may modify the relationship between adiposity and bone strength. In postmenopausal women, Castro et al.[32] demonstrated that for each unit increase in BMI, the odds ratio for having poor aBMD were lower for white women, while black women had slightly higher odds for poor aBMD. In prepubertal children, Afghani and Goran[22] observed total abdominal mass, assessed by QCT, was negatively associated with bone mineral, measured using DXA. There were differential effects of race, however, with regard to the bone mineral relationships with the subcutaneous fat and visceral fat of the abdomen. For instance, an inverse correlation between subcutaneous abdominal adipose tissue and bone mineral was observed in white, but not in black children.[22] Conversely, an inverse association between visceral abdominal adipose tissue and bone mineral was found in black, but not in white children.[22] The mechanism for these racial differences is unclear; however, it was suggested by the authors that insulin may play a more prominent role in fat and bone metabolism in the black children, whereas leptin may be the more dominating factor in the white children.[22] In contrast to Afghani and Goran,[22] the relationships between adiposity and bone strength measurements at the cortical and trabecular bone sites were markedly similar between our current findings in young black females with our analogous work in white females.[19] As a result, if obesity does negatively impact skeletal strength, it could be serious public health issue, independent of race.

As reported in our previous investigation,[19] it appears as though geometric measures indicative of cortical, but not trabecular, bone strength at both the tibia and radius are unfavorably affected with increasing fat mass. Human studies have shown that an increase in

cortical thickness and area is evident when muscular strength and parallel biomechanical usage increase, particular during puberty.[33-36] Animal work has also indicated that skeletal unloading due to immobilization or inactivity is associated with a conversion of stromal cells to adipocytes rather than osteocytes, leading to reduced bone formation.[37] It is possible that our findings regarding the inverse relationships between adiposity and cortical bone geometry measures may be a consequence of inadequate physical activity associated with excess body fat; however, we did not collect any information on the types and frequencies of activities performed from early to late puberty or any weight history documentations from the participants. Therefore, we are unable to identify which participants engaged in high impact activities or gained excessive weight due to reduced physical activity during adolescence.

Analyses from cellular and molecular studies suggest that the mechanisms involving bone and fat are intricate by nature, since both adipocytes and osteoblasts originate from mesenchymal stem cells in bone marrow. Factors regulating lipid metabolism may also have a significant effect on bone formation. Extra weight in the form of fat mass has not only been shown to stimulate bone growth via direct mechanical actions from increased load,[38] but also through increased production of the hormones insulin, estrogen and leptin, all of which have demonstrated increases in markers of bone formation when administered in vivo.[39-43] Alternatively, excess adipose tissue has also been shown to hinder bone growth, in vitro, by enhancing the role of oxidized lipids in accelerating atherogenesis, thus activating calcifying vascular cells and inhibiting osteoblastic differentiation.[44] Moreover, bone marrow adipogenesis increases with conditions that induce bone loss, such as estrogen depletion,[45] disuse and hindlimb unloading.[37, 46] Future work should continue to explore these potential mechanisms to enhance our knowledge of fat and bone relationships.

A novel aspect of this study is that we analyzed the relationships of fat mass and bone strength, independent of total muscle loads and skeletal size. In adults and children, it is thought that excess adiposity, via increased loading, appears to be a protective factor against skeletal fractures. This relationship seems plausible since extra weight from fat mass can potentially increase the load that the skeleton must endure. Our controlled adiposity group analyses show, however, that the negative contribution of adipose tissue offsets its potential benefit as a mechanical load. Although prior studies have used total fat mass to assess its relationship with bone strength,[11, 47] the study designs generally did not account for the large effects from muscle loads and skeletal size on the bone measurements; and, thus, their conclusions may have been confounded.[26, 48] If the relationship between fat mass and measures of bone strength were simply a loading phenomenon, the impact of total fat mass to bone strength would be equal to the contribution of total FFST mass to bone strength. However, this is not the case since unequal contributions of total FFST mass and total fat mass to bone strength measurements have been reported.[19, 20] Further investigations are needed to delineate the negative contribution of adipose tissue that seems to offset its benefit as a weight-bearing load.

To our knowledge, this was the first study to investigate the independent role of total fat mass and relationships with pQCT-derived bone measurements taken at trabecular and cortical bone sites of the tibia and radius in young black females. In addition to not having historical information on weight-bearing activities and weight status history, another limitation in our study was that we did not collect biochemical measures of specific adipose- and bone-related hormones. Furthermore, our study utilized cross-sectional data and thus was not specifically designed to examine the longitudinal effects of adiposity and bone development. It is also important to note that the analysis of muscle loading effects is complex and the use of total FFST

mass and MCSA does not reflect the functional status of the entire muscle system, including muscle length, contraction velocity, structure, and coordination.[49] Whether the data generated using these surrogates of muscle loading effects can be used to predict bone health and risk of skeletal fractures must be validated by subsequent prospective studies. On the other hand, an advantage of our sample was that the degree of variability in factors known to influence bone, such as race, sex, age, and maturational status were minimized. Another strength of our study was that we utilized pQCT, a three-dimensional technique, to measure material and geometrical aspects of bone strength; whereas, most prior bone and fat investigations employed the use of DXA, a two-dimensional modality that only provides information about the material properties of bone. Peripheral QCT further enabled us to examine separate details of the trabecular and cortical bone at weight-loaded and non-weight-loaded skeletal sites in order to examine more distinct bone relationships with total adiposity.

In summary, our study provides important insight into obesity and bone strength relations in African American females entering adulthood. Overall, our results suggest that increasing adiposity levels may adversely influence cortical bone strength within the tibia and radius, independent of confounders known to influence both adiposity and bone outcome variables. Thus, the negative contribution of adipose tissue seems to offset its potential benefit as a mechanical load. Although our study suggests a concern for bone health in a population experiencing high rates of obesity, prospective research is needed to confirm a cause and effect relationship that considers physical inactivity, metabolic diseases and environmental influences.

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Table 5.1

Characteristics of the participants [1]

	Total sample (N = 48)	Normal-fat group [2] (n = 33)	High-fat group [2] (n = 15)
Age (yrs)	19.2 ± 1.2	19.3 ± 1.3	19.0 ± 1.1
Weight (kg)	65.2 ± 14.9	59.6 ± 8.9	77.6 ± 18.1[3]
Height (cm)	163.4 ± 7.3	163.3 ± 6.2	163.9 ± 6.2
Body mass index (kg/m^2)	24.4 ± 5.3	22.4 ± 3.1	28.9 ± 6.4 [3]
FFST mass (kg) [4]	44.2 ± 6.7	42.9 ± 5.8	47.1 ± 7.8[3]
Fat mass (kg)	20.8 ± 9.5	16.3 ± 3.9	30.8 ± 10.6[3]
Fat mass (%)	29.8 ± 6.9	26.2 ± 3.4	37.7 ± 4.3[3]
Tibial length (mm)	388.1 ± 28.5	384.5 ± 29.4	395.9 ± 25.8
Forearm length (mm)	269.1 ± 13.5	268.3 ± 13.9	270.9 ± 13.1
Tibial MCSA (mm^2) [5]	6209 ± 1069	6127 ± 1127	6379 ± 954
Forearm MCSA (mm^2)	2699 ± 548	2657 ± 569	2781 ± 515
Bone variables (*4% site*) [6]			
Tot vBMD (mg/cm^3)			
Tibia	391.1 ± 68.7	384.2 ± 62.8	406.4 ± 79.9
Radius	414.0 ± 97.8	405.7 ± 108.9	431.6 ± 68.5
Trab vBMD (mg/cm^3)			
Tibia	234.0 ± 40.9	234.1 ± 39.9	234.0 ± 44.2
Radius	224.7 ± 32.8	221.1 ± 33.1	232.4 ± 31.9
Tot area (mm^2)			
Tibia	748.8 ± 184.0	755.8 ± 177.9	734.1 ± 200.9
Radius	260.9 ± 59.8	262.1 ± 62.9	258.4 ± 54.6

Bone variables (*20% site*) [6]			
Cort vBMD (mg/cm^3)			
Tibia	1191 ± 20.8	1193 ± 18.6	1187 ± 25.1
Radius	1181 ± 42.0	1180 ± 39.3	1185 ± 47.9
Cort area (mm^2)			
Tibia	214.6 ± 30.9	214.1 ± 26.2	215.7 ± 40.4
Radius	74.2 ± 9.6	73.6 ± 9.7	75.4 ± 9.6
Tot area (mm^2)			
Tibia	359.4 ± 52.9	361.9 ± 51.0	354.1 ± 58.3
Radius	107.6 ± 20.4	107.8 ± 21.7	107.4 ± 17.9
Cort BMC (mg)			
Tibia	255.4 ± 35.2	255.3 ± 29.9	255.7 ± 45.7
Radius	87.7 ± 11.5	86.8 ± 11.3	89.4 ± 12.0
Cort thk (mm)			
Tibia	3.94 ± 0.5	3.91 ± 0.4	4.00 ± 0.7
Radius	2.64 ± 0.3	2.61 ± 0.3	2.69 ± 0.3
Peri circ (mm)			
Tibia	67.0 ± 4.8	67.3 ± 4.7	66.5 ± 5.3
Radius	36.6 ± 3.3	36.6 ± 3.5	36.6 ± 3.0
Endo circ (mm)			
Tibia	42.3 ± 5.6	42.7 ± 5.6	41.3 ± 5.7
Radius	20.1 ± 4.3	20.2 ± 4.5	19.7 ± 3.9
pSSI (mm^3)			
Tibia	1454 ± 292	1469 ± 283	1422 ± 319
Radius	246.1 ± 62.0	242.2 ± 62.4	254.4 ± 62.3

[1] Values are means ± SD.

[2] Cutoffs used to denote normal fat (<32% body fat) and high fat (≥32% body fat) were determined with cardiovascular risk factors.[27, 28]

[3] Significantly different from normal-fat group, $P \leq 0.05$ (2-tailed independent *t* tests).

[4] FFST, fat-free soft-tissue

[5] MCSA, muscle cross-sectional area

[6] Bone variables measured by peripheral quantitative computed tomography at the 4% (trabecular) and the 20% (cortical) sites: Tot vBMD = total volumetric BMD, Trab vBMD = trabecular volumetric BMD, Tot area = total bone cross-sectional area, Cort vBMD = cortical volumetric BMD, Cort area = cortical bone area, Cort BMC = cortical bone mineral content, Cort thk = cortical thickness, Peri circ = periosteal circumference, Endo circ = Endosteal circumference, pSSI = polar strength-strain index

Table 5.2

Partial correlations of bone outcomes at the tibia and radius with total fat mass

Bone Variable	*r*	*P*
4% site		
Tot vBMD (mg/cm^3)		
Tibia	-0.092	0.576
Radius	-0.113	0.486
Trab vBMD (mg/cm^3)		
Tibia	0.114	0.489
Radius	0.016	0.921
Tot area (mm^2)		
Tibia	0.032	0.846
Radius	-0.101	0.537
20% site		
Cort vBMD (mg/cm^3)		
Tibia	-0.079	0.631
Radius	0.172	0.289
Cort area (mm^2)		
Tibia	-0.300	0.063
Radius	-0.337	0.033
Tot area (mm^2)		
Tibia	-0.255	0.118
Radius	-0.370	0.019

Cort BMC (mg)		
Tibia	-0.323	0.045
Radius	-0.246	0.127
Cort thk (mm)		
Tibia	-0.169	0.303
Radius	-0.018	0.911
Peri circ (mm)		
Tibia	-0.254	0.118
Radius	-0.359	0.023
Endo circ (mm)		
Tibia	-0.098	0.552
Radius	-0.224	0.164
pSSI (mm^3)		
Tibia	-0.408	0.010
Radius	-0.282	0.077

Bone variables were measured by peripheral quantitative computed tomography at the 4% (trabecular) and the 20% (cortical) sites. Tot BMD = total volumetric BMD, Trab BMD = trabecular volumetric BMD, Tot area = total bone cross-sectional area, Cort BMD = cortical volumetric BMD, Cort area = cortical bone area, Cort BMC = cortical bone mineral content, Cort thk = cortical thickness, Peri circ = periosteal circumference, Endo circ = Endosteal circumference, pSSI = polar strength-strain index. Partial Pearson's correlations were used to examine associations between total fat mass and bone variables, controlling for height, limb length, FFST mass and muscle cross-sectional area in this sample ($N = 48$). Statistically significant coefficients ($P \leq 0.05$).

Table 5.3

Bone measurements of the tibia and radius in normal- and high-fat late adolescent black females after adjustment for total fat-free soft tissue mass[1]

Bone variable	Normal-fat group [2] (n = 33)	High-fat group [2] (n = 15)	*P-value* [3]	(η^2) [4]
4% site				
Tot vBMD (mg/cm^3)				
Tibia	386.6 ± 12.2	401.7 ± 18.2	0.503	0.010
Radius	403.8 ± 17.7	435.6 ± 26.2	0.330	0.022
Trab vBMD (mg/cm^3)				
Tibia	235.3 ± 7.4	231.2 ± 10.9	0.763	0.002
Radius	222.2 ± 5.9	230.3 ± 8.7	0.451	0.013
Tot area (mm^2)				
Tibia	767.1 ± 31.9	709.9 ± 47.3	0.330	0.022
Radius	267.8 ± 9.6	246.3 ± 14.3	0.228	0.033
20% site				
Cort vBMD (mg/cm^3)				
Tibia	1191 ± 3.6	1190 ± 5.3	0.760	0.002
Radius	1178 ± 7.5	1189.2 ± 11.1	0.432	0.014
Cort area (mm^2)				
Tibia	218.8 ± 3.6	205.7 ± 5.4	0.053	0.083
Radius	75.0 ± 1.2	72.5 ± 1.8	0.289	0.026
Tot area (mm^2)				
Tibia	369.4 ± 6.7	337.9 ± 9.9	0.013	0.133
Radius	110.4 ± 2.9	101.9 ± 4.3	0.114	0.056

Cort BMC (mg)				
Tibia	260.6 ± 4.2	244.4 ± 6.2	0.040	0.092
Radius	88.3 ± 1.6	86.3 ± 2.4	0.432	0.014
Cort thk (mm)				
Tibia	3.95 ± 0.09	3.91 ± 0.13	0.802	0.001
Radius	2.62 ± 0.06	2.66 ± 0.09	0.697	0.003
Peri circ (mm)				
Tibia	68.0 ± 0.6	65.0 ± 0.9	0.012	0.136
Radius	37.1 ± 0.4	35.7 ± 0.7	0.130	0.051
Endo circ (mm)				
Tibia	43.1 ± 0.9	40.5 ± 1.4	0.127	0.052
Radius	20.6 ± 0.7	19.0 ± 1.1	0.233	0.032
pSSI (mm^3)				
Tibia	1514 ± 21.7	1326 ± 50.2	0.004	0.175
Radius	250.2 ± 8.6	237.3 ± 12.8	0.413	0.015

Bone variables measured by peripheral quantitative computed tomography at the 4% (trabecular) and the 20% (cortical) sites: Tot vBMD = total volumetric BMD, Trab vBMD = trabecular volumetric BMD, Tot area = total bone cross-sectional area, Cort vBMD = cortical volumetric BMD, Cort area = cortical bone area, Cort BMC = cortical bone mineral content, Cort thk = cortical thickness, Peri circ = periosteal circumference, Endo circ = Endosteal circumference, pSSI = polar strength-strain index. Values are means ± SE adjusted for fat-free soft tissue mass.

[2] Cutoffs used to denote normal fat (<32% body fat) and high fat (≥32% body fat) were determined with cardiovascular risk factors.[27, 28]

[3] Tests of significance between groups are based on group main effect using ANCOVA.

[4] Medium and large effects are designated by partial eta-squared (η^2) ≥ 0.06 and 0.14, respectively.

CHAPTER 6

SUMMARY AND CONCLUSION

This work was conducted to determine the relationships between measures of adiposity and bone strength, using peripheral quantitative computed tomography (pQCT), and whether these relationships vary by race. Results from the study presented in Chapter 3 demonstrate that excess weight in the form of fat mass does not provide additional weight-loading benefits, and may potentially be negative for bone in predominately white late adolescent females (N = 115, aged 18.2 ± 0.4 years). In this study, percent body fat (%fat) was inversely related to radial cortical bone area, cortical bone cross-sectional area (CSA), cortical bone mineral content (BMC), periosteal circumference, and polar strength-strain index (SSI) [20% site; all $P<0.05$]. After controlling for muscle cross-sectional area (MCSA) and limb-length, negative relationships remained between %fat and radial measurements and were also observed at the tibia (20% site). Bone measurements in normal- (< 32% body fat) and high- (≥ 32% body fat) fat groups were also compared. Unadjusted bone measures were not different between groups. After controlling for MCSA, the high- vs. normal-fat group had lower bone measures at the 20% site (cortical bone area and cortical BMC at the tibia, total bone CSA at the radius and SSI at both the tibia and radius; all $P<0.05$). Given that both the high-fat and normal-fat groups had no significant differences in total fat-free soft tissue (FFST) mass, it was interesting to find that the additional 9 kilograms of fat mass in the high-fat group provided no advantage with respect to pQCT-derived bone measurements at the tibia and radius.

The study presented in Chapter 4 was conducted to determine whether there are racial differences in bone structure and strength measurements, assessed by pQCT, at cortical and trabecular sites of the tibia (weight-bearing skeletal region) and radius (non-weight-bearing skeletal region) in black and white females, 18 to19 years of age. To minimize any influences of age, body size and sexual maturation on bone outcome variables, 25 whites and 25 blacks were individually matched on age, height, fat-free soft tissue mass, and weight. We further examined whether racial differences in bone measures at the tibia and radius could be explained by differences in MCSA and bone length for each respective site. In the unadjusted data, blacks versus whites had higher total volumetric bone mineral density (vBMD; tibia and radius), cortical vBMD (tibia only), cortical bone CSA (tibia only), cortical BMC (tibia only), cortical thickness (tibia only), and lower trabecular vBMD (tibia only), total bone CSA (tibia and radius at 4% site only), and cortical vBMD (radius only). After adjustments for muscle and bone length parameters at each respective bone site, the racial differences in most bone measurements remained. However, bone length differences between groups at the radius explained the greater radial total bone cross-sectional area (20% site) in the blacks versus whites and the higher radial cortical vBMD in the whites versus blacks. Collectively, these results create a bone strength profile reflecting a stronger bone at the weight-bearing tibia in young adult black vs. white females, possibly accounting for the lower fracture rates in older black females at weight-bearing skeletal sites (hip and spine). However, at a non-weight bearing site such as the radius, racial differences are less evident.

In Chapter 3 we determined relationships between adiposity and bone strength measurements, using pQCT, in 115 late adolescent females, however, only 2 of the participants

were black. As a result, we sought to investigate in a larger sample of young African American females the relations between total fat mass and pQCT-assessed trabecular and cortical bone measurements within the tibia and radius (Chapter 5). Since height, limb lengths and surrogates of muscle loads [e.g., total body fat-free soft tissue (FFST) mass and/or MCSA] may confound total fat mass and bone outcome variables, we elected to observe these fat and bone relationships independent of the following variables: height, limb lengths for each respective bone site, FFST mass, and MCSA for each respective bone site. The second objective was to compare tibial and radial bone measurements between two adiposity groups defined as having normal and high percentages of body fat, before and after controlling for any differences in the same confounding variables (i.e., height, limb lengths for each respective bone site, FFST mass, and MCSA for each respective bone site). Consistent with our adiposity and bone strength analyses in a predominately white sample of late adolescent females, our findings in black females entering adulthood also suggest that excess adiposity levels may adversely influence the overall strength of cortical bone at appendicular skeletal sites.

The results of the studies in Chapters 3 through 5 provide insight into the interrelationships between bone, fat and race. However, many questions remain to be answered. Given that these studies are cross-sectional, prospective studies are needed to examine the effects of fat gain on bone development. Important participant information relative to both bone and fat, such as pre- and postpubertal weight-bearing activities, weight status history and biochemical measures of specific adipose- and bone-related hormones, should also be included in future studies. The use of 3-dimensional imaging in bone investigations will certainly advance our understanding of both the material and geometric properties of bone strength; however, there are currently no published studies showing an association between fracture risk and pQCT

measurements in children. Thus, work in this area needs to be conducted. Furthermore, it is important to note that the analysis of muscle loading effects is complex and the use of total FFST and MCSA does not reflect the functional status of the entire muscle system, including muscle length, contraction velocity, structure, and coordination. Whether the data generated using these surrogates of muscle loading effects can be used to predict bone health and risk of skeletal fractures must be validated by subsequent prospective studies.

Investigations such as these are important for gaining valuable information about the complex relationship between fat and bone during skeletal development, particularly in young black females. Overall, the findings provide evidence that adipose tissue does not seem to benefit bone structure and strength, independent of race, total FFST mass, height, and surrogates of site-specific muscle loading effects. However, continued research is necessary to determine the mechanism(s) behind this phenomenon, and ultimately, the levels of fat in proportion to total body mass that become a potential threat to skeletal health.

APPENDICES I

Soy, Bone and Health in College Females Study Questionnaires

TELEPHONE SCREENING QUESTIONNAIRE
CONSENT FORMS
3-DAY DIET RECORDS
7-DAY PHYSICAL ACTIVITY RECALL
ANTHROPOMETRIC DATA RECORDING SHEET
ADDITIONAL FOCUS QUESTIONS
MENSTRUAL CYCLE QUESTIONNAIRE

APPENDIX I-A

Telephone Screening Questionnaire

Soy, Bone and Health in College Females

Telephone Screening Questionnaire

This interview should only take approximately ten minutes:

Date: _____ Time: _____ Screen completed by: _____

Participant's name: _____

Address: _____

Zip Code: _____

Daytime Phone Number: _____

How did you hear about the study?_____

---

1. Ethnicity/Race:_____ (Asian Non-Oriental, Asian-Oriental, Black, Caucasian, Hispanic, Mixed, Native American, Others: _____)

2. Age _____years DOB: _____/_____/_____
mm dd yy

3. What year are you in school? _____

4. Height ____ft____ in

5. Weight_____lbs

6. Have you lost or gained weight in the past 3 months? **YES** or **NO**; *circle one*
If yes, how much? _____lbs

7. Are you a student athlete? **YES** or **NO;** *circle one*
*if the answer to #7 is **YES**, please tell the potential volunteer that she does not qualify for the study.*

8. How physically active are you? _____ mins/day, _____day/week

9. Number of periods in last 6 months_____

10. Are you taking any medications? **YES** or **NO** ; *circle one*

If yes, what medication(s)? _____
(*check specifically for birth control pills, Adderall, Ritalin, and steroid medications*)

How long have you been taking the above medication(s)?_____

11. Do you have any food allergies?_____
(*check specifically for soy, milk and chocolate*) - *if the answer to #11 includes allergies to soy, milk or choclate, please tell the potential volunteer that she does not qualify for the study.*

12. Do you have any of the following diseases or conditions:
*if the answer to any of the questions from 12a-12h is **YES**, please stop and tell the potential volunteer that she does not qualify for the study.*

a) Diabetes	YES_____	NO_____
b) Thyroid Disease	YES_____	NO_____
c) Soy Allergies	YES_____	NO_____
d) Pregnancy	YES_____	NO_____
e) Gall bladder Disease	YES_____	NO_____
f) Kidney Disease	YES_____	NO_____
g) Osteoporosis	YES_____	NO_____
h) Psychological Illness	YES_____	NO_____

13. Are you taking an herbal or dietary supplement? **YES** or **NO;** *circle one*

If yes, what supplement(s), how much and how often? _____
_____

If yes, would you be willing to stop taking the supplement? **YES** or **NO;**
circle one

14. Are you a vegetarian? **YES** or **NO;** *circle one*

15. Are you soy food consumer? **YES** or **NO;** *circle one*

How many servings per week? _____
*If the answer is **YES** to #15, ask if she would be willing to limit her soy intake to less than one serving per week for the 16-week duration of the study. If the reply is yes, proceed to the next question. If the reply is no, please tell the potential volunteer that she does not qualify for the study.*

16. In this study, all participants must provide blood and urine samples 3 times (at the start, at 8 weeks, and at 16 weeks), and each testing session could last up to 1 ½-2 ½ hours. Are you willing to do this? **YES** or **NO;** *circle one*

17. If selected to participate, what mornings during the week would you be available to come to the UGA Bone and Body Composition Lab, located in Dawson Hall, for testing?
M____ T____ W_____ Th_____ F_____ S _____

Morning testing will begin as early as 7 am, will involve a fasting blood draw and will take approximately 1 hour and 30 minutes.

"That's the end of our telephone screening. We will review this and determine your eligibility for the study. We will get back to you with in a week to let you know the status of your eligibility. Do you have any additional questions for me?"

Make sure the potential volunteer has contact numbers for future questions.

APPENDIX I-B

Consent Forms

Soy, Bone and Health in College Females
Consent Form

I agree to participate in the research study entitled "Soy, Bone and Health in College Females," which is being conducted by Dr. Richard Lewis, Department of Foods and Nutrition, The University of Georgia. Dr. Richard Lewis can be reached at (706) 542-4901.

The purpose of this study is to determine the effect of isoflavone-rich soy protein on bone parameters in college-age women. In order to make this study a valid one, some information about my participation will be withheld until after the study. Upon completion of the final testing procedures at the 16-week testing session, the Investigator will inform me about this information.

The following points have been explained to me:

1) The reason for this research is to determine if isoflavone-rich soy protein, eaten daily as a food for 16 weeks will safely promote bone health in college-aged women. The benefits that I may expect from my participation are to receive payments of $25 for completing each of three visits to the UGA Clinical and Sports Nutrition Lab for measurements that have been explained to me. The meal supplement will be provided without cost to me. I will also learn about my bone density, diet and activity habits without cost. However, if abnormalities are found in bone density measurements, I will be notified and advised to contact my physician.

2) The procedures are as follows:
(Single Center Trials)
I will be one of approximately 120 participants to be asked to participate in this trial. To qualify for the study, I must:

- Be an incoming freshman at UGA;
- Not be underweight based on my body weight and height;
- Have had regular menses for the past 6 months;
- Be non-vegan (consumes animal foods);
- Be in good health (e.g. no evidence of thyroid, gall bladder, kidney, or liver disease; no history of psychological illness; no history of bone fractures; no established osteoporosis; no diabetes);
- Not be on any dietary, exercise, or drug treatment for high blood cholesterol;
- Agree not to begin any dietary, drug, or exercise treatment during the 16-week study period unless it is medically necessary;
- Must agree to come to the Clinical and Sports Nutrition Laboratory at the University of Georgia for blood work, bone density testing and to complete several questionnaires;
- Must agree to not eat any food after dinner in the evening before coming to the laboratory for blood tests.

- During my visit the following procedures will be done:
    - My weight, height, and vital signs will be taken;
    - A second void urine sample will be collected at baseline, 8-weeks, and 16-weeks;
    - A blood sample (up to 50 mL) will be taken from an arm vein;
    - The total amount of bone in my body will be measured with a bone-scanning machine that will take approximately 20 minutes. The measurement will be within the normal ranges based on young adult women;
    - I will complete the following questionnaires:
        - Three-day Diet Record to estimate my energy intake;
        - A Soy Food Questionnaire to document my soy intake;
        - A Menstrual Cycle Questionnaire to document my menstrual activity;
        - A Physical Activity Questionnaire to document my physical activity;
        - A Satiety Questionnaire to document my satiety after eating the meal supplement;
        - A Beck Depression Inventory to document my feelings throughout the study.

If any of the results from the blood test are not within the normal ranges, I will not be enrolled in the study. If I qualify for the study, I will receive a 8-week supply of meal supplements. I will be asked to drink the daily meal supplement with my first meal of the day for a period of 16 weeks. I will be educated by the Investigator on how to replace part or all of my food choices with this meal supplement, in order to eat the same number of calories that I would usually eat at breakfast during these 16 weeks.

I will return to the Laboratory in 8 weeks to pick up a new supply of meal supplements. All the supplements will be provided free of charge. Bone measurements will be repeated on me at my 16-week visit, and clinical blood chemistries will be repeated on me at my 8-week and 16-week visits. I will complete the soy food and physical activity questionnaires and the Lifestyle/Health Questionnaire during my 8 and 16-week visits. I will complete a 3-Day Food Diary 3 times during the course of the study. I also understand that the placebo supplement will not contain any soy protein or isoflavones. I will have no choice on the supplement I receive. I will be assigned randomly to receive either the placebo or soy and I will not be told which supplement I am receiving during the study.

3) The following discomforts and risks have been explained in detail to me:

Other studies with soy isoflavones lasting 6 months did not show any adverse effects, but the long-term effect is not known. The amount of isoflavones to be used in this study is less than 1/4 of that used in these studies. The total bone scan takes approximately 20 minutes. I will be exposed to 9.3 μSv of radiation during each measurement. The radiation exposure of an adult chest X-ray is approximately 500 to 800 μSv. However, any illness or injury not related to the study is not the responsibility of the investigator or the University of Georgia. If I am pregnant or could possibly

become pregnant, I realize that the radiation involved in this study could be harmful to a fetus. I have no intentions of becoming pregnant during this study. If I do become pregnant, I will notify the Investigator.

No risk is expected, but I may experience some discomfort or stress when my blood is drawn. The risks of drawing blood from my arm include the unlikely possibilities of a small bruise or localized infection, bleeding, and fainting. These risks will be reduced in the following ways: my blood will be drawn only by a qualified and experienced phlebotomist who will follow standard sterile techniques, who will observe me after the needle is withdrawn, and who will apply pressure to the blood-draw site. In the event that I have any health problems associated with the blood draws, my insurance or I will be responsible for any related medical expenses. My blood will not be tested for HIV-AIDS. I understand that these questions and blood tests are not for diagnostic purposes. If I have questions about my test results I should see a physician.

Less than 1% of American adults have allergies to soy protein. Allergy symptoms are those associated with most food allergies such as abdominal discomfort (nausea, diarrhea, or constipation), and/or a mild skin rash. Allergy symptoms cease with discontinuation of soy products. I may withdraw from the study at any time if I experience food allergy symptoms.

The risks of participating in this study are minimal and the data will increase our knowledge on how safe and effective soy protein and isoflavones are in increasing bone health in college-age women. Therefore, the benefits of the study are believed to equal or outweigh the very minimal risk.

If I have questions, Dr. Lewis, the research coordinator, or graduate student will be available to talk with me.

4) The results of this participation will be confidential, and will not be released in any individually identifiable form without my prior consent, unless otherwise required by law. The data generated from my tests will be stored on the computers in the lab room 279, Dawson Hall. All data will be associated with a code number and not my name. There will be a list of participant's names and corresponding code numbers, but no data with that list. My data will be destroyed on or before September 1, 2010. The information collected will be used for research purposes.

I have been informed that there may be unknown risks/discomforts involved, and that I will receive any new information discovered during the course of the study concerning significant treatment findings that may affect my willingness to continue to participate.

In the event of injury resulting from this research, the University of Georgia and/or the laboratory of Dr. Richard Lewis are not able to offer financial compensation or to absorb the costs of medical treatment. However, necessary facilities, emergency treatment and professional services will be available to research participants, just as they are to the community in general. My signature below acknowledges my voluntary participation

in this research project. Such participation does not release the investigator(s), institution(s), sponsor(s), or granting agency(ies) from their professional and ethical responsibility to me.

My participation is voluntary and I may refuse to participate or may discontinue my participation AT ANY TIME, without penalty, loss of benefits, or change in my present or future care. The investigator has the right to withdraw me from the study at any time. My withdrawal from the study may be for reasons related solely to me (e.g. not following study-related directions from the Investigator; a serious adverse event reaction) or because the entire study has been terminated. The Sponsor has the right to terminate the study or the Investigator's participation in the study at any time.

The investigator or his designee has answered all of my questions. If I have additional questions during the course of this study about the research or my rights as a research participant, I may address them to the University of Georgia Review Board for Human Subject Research Office at (706) 542-3199. In the event of a research-related injury or if any other problems arise, I may contact Richard D. Lewis, Ph.D., at 706-542-4901.

I HAVE READ THE INFORMATION PROVIDED ABOVE (OR HAVE HAD IT READ TO ME) AND HAD MY QUESTIONS ANSWERED TO MY SATIFACTION. I VOLUNTARILY AGREE TO PARTICIPTATE IN THIS STUDY. I WILL RECEIVE A COPY OF THIS CONSENT FORM.

_____ _____

Signature of Investigator Date Signature of Participant Date

Richard D. Lewis
706-542-4901
rlewis@fcs.uga.edu

PLEASE SIGN BOTH COPIES OF THIS FORM. KEEP ONE AND RETURN THE OTHER TO THE INVESTIGATOR.

Additional questions or problems regarding your rights as a research participant should be addressed to The Chairperson, Institutional Review Board, University of Georgia, 612 Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706) 542-3199; E-Mail Address IRB@uga.edu

Soy, Bone and Health in College Females

Consent Form for the Use of the Hologic Delphi A
X-Ray Bone Densitometer and XCT 2000 pQCT

Are you pregnant or do you think you might be pregnant? YES NO

*If yes, please do not participate in this study using the Delphi A bone densitometer and the XCT 2000 pQCT.

I, _____, am hereby giving my consent to be used for research conducted by Dr. Richard D. Lewis, University of Georgia, Foods and Nutrition Department, 279 Dawson Hall.

I understand that by giving my consent I am agreeing to be scanned on the Hologic Bone Delphi A X-Ray Densitometer and on the XCT 2000 peripheral Quantitative Computer Tomography machine. Both of these instruments use a low dose x-ray to determine bone mineral density and body composition

I understand that the Hologic Delphi A X-Ray Bone Densitometer uses a very low level of x-ray and that under most operating conditions, the entrance dose to the patient is 0.5mRem-10mRem. This equals about 3% to 30% of the exposure of a standard chest x-ray and is of no danger to me.

I understand that the XCT 2000 pQCT uses a very low level of x-ray and that under most operating conditions, the maximum entrance dose to the patient is less than 1 mRem.

I understand that The University of Georgia is responsible for my safety during my participation in this study. However, any illness or injury not related to this study is not the responsibility of the investigator or the University of Georgia.

I understand that my participation is entirely voluntary. I can withdraw my consent at any time without penalty and have the results of my participation returned to me, removed from records or destroyed.

_____ _____

Signature of Investigator Date Signature of Participant Date

APPENDIX I-C

3-Day Diet Records

Soy, Bone and Health in College Females

3-DAY DIET RECORD

DIRECTIONS FOR KEEPING A 3-DAY DIET DIARY

Please write down everything you eat (meals, snacks, beverages) for three days on these forms. Please select **TWO WEEKDAYS AND ONE WEEKEND DAY.** Use as much space as you need.

1. **Write down the date and day at the top of the form.**

2. **Write down the first foods you ate for that day. Write down:**

   a. The time of day you ate the food(s).

   b. Each food that you ate.

   c. How the food was prepared (baked, boiled, fried, microwaved).

   d. How much you ate (cup, 1/2 cup, pieces, tablespoons, teaspoons).

3. **It is important to describe each food you eat in detail.**
   For example:

   Write down brand names for each food you ate if you know them.

   Write down the type of milk (whole, 2%, or skim) and bread (white, wheat, etc).

   Write down if the food was fresh, frozen, or canned.

   If you ate a casserole or a salad, write down the foods/ingredients there were in it and the amounts.

   If you add things like butter, jelly, sugar, honey, or cream to foods or beverages, please write them down with the amounts used.

4. **Do you drink whole _____, 2% _____, 1% _____, or skim _____ milk?**

5. **Do you use white _____ or whole-wheat _____ bread?**

6. **What is the complete name and brand of bread that you eat most often?**

   _____

7. **About how many glasses of water do you drink each day? _____**

DAY 1 OF THE DIET DIARY

ID: _____CHECKED BY: _____

DATE: _____DAY OF THE WEEK: _____

Did you drink a calcium-fortified beverage today (e.g. Calcium-fortified orange juice) or eat a calcium-fortified food (e.g. Total breakfast cereal)? Yes No

If yes, list all the calcium-fortified beverages/foods, with the BRAND name, and how much you consumed:

_____

_____

_____

Write down everything you eat, beginning with the first thing you have for breakfast. Be sure to include very detailed information such as how the food was prepared, how much you ate, and the brand names.

Time Eaten	Foods Eaten	Preparation Methods	Amount (cup, 1/2 cup, piece, etc)

Please continue on the back of page if necessary.

DAY 2 OF THE DIET DIARY

ID: _____CHECKED BY: _____

DATE: _____DAY OF THE WEEK: _____

Did you drink a calcium-fortified beverage today (e.g. Calcium-fortified orange juice) or eat a calcium-fortified food (e.g. Total breakfast cereal)? Yes No

If yes, list all the calcium-fortified beverages/foods, with the BRAND name, and how much you consumed:

_____

_____

_____

Write down everything you eat, beginning with the first thing you have for breakfast. Be sure to include very detailed information such as how the food was prepared, how much you ate, and the brand names.

Time Eaten	Foods Eaten	Preparation Methods	Amount (cup, 1/2 cup, piece, etc)

Please continue on the back of page if necessary.

DAY 3 OF THE DIET DIARY

ID: _____CHECKED BY: _____

DATE: _____DAY OF THE WEEK: _____

Did you drink a calcium-fortified beverage today (e.g. Calcium-fortified orange juice) or eat a calcium-fortified food (e.g. Total breakfast cereal)? Yes No

If yes, list all the calcium-fortified beverages/foods, with the BRAND name, and how much you consumed:

_____

_____

_____

Write down everything you eat, beginning with the first thing you have for breakfast. Be sure to include very detailed information such as how the food was prepared, how much you ate, and the brand names.

Time Eaten	Foods Eaten	Preparation Methods	Amount (cup, 1/2 cup, piece, etc)

Please continue on the back of page if necessary.

APPENDIX I-D

7-Day Physical Activity Recall

Subject Code No. _____

Date _____

Soy, Bone and Health in College Females

7-DAY PHYSICAL ACTIVITY RECALL QUESTIONNAIRE

1. On the average, how many hours did you sleep each night during the last 5 weekday nights (Sunday-Thursday)? Record to nearest quarter-hour.

   Hours: _____ Minutes: _____
2. On the average, how many hours did you sleep each night last Friday and Saturday nights?

   Hours: _____ Minutes: _____
3. First let's consider moderate activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these moderate activities or others like them? Please tell me to the nearest half-hour.

   Hours: _____ Minutes: _____
4. Last Saturday and Sunday, how many hours did you spend on moderate activities and what did you do? (Can you think of any other sport, job, or household activities that would fit in this category?

   Hours: _____ Minutes: _____
5. Now let's look at hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these hard activities or others like them? Please tell me to the nearest half-hour.

   Hours: _____ Minutes: _____
6. Last Saturday and Sunday, how many hours did you spend on hard activities and what did you do? (Can you think of any other sport, job, or household activities that would fit in this category?)

   Hours: _____ Minutes: _____
7. Now let's look at very hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these very hard activities or others like them? Please tell me to the nearest half-hour.

   Hours: _____ Minutes: _____
8. Last Saturday and Sunday, how many hours did you spend on very hard activities and what did you do? (Can you think of other sport, job, or household activities that would fit in this category?)

   Hours: _____ Minutes: _____

Physical Activity List

Moderate Activities

Occupational Tasks:

- Delivering mail or patrolling on foot
- House painting
- Truck driving (making deliveries – lifting and carrying light objects)

Household activities:

- Raking the lawn
- Sweeping and mopping
- Mowing the lawn with a power mower
- Cleaning windows

Sports Activities (Actual playing time)

- Volleyball
- Ping pong
- Brisk walking for pleasure or to work (3 mph or 20 min/mile)
- Golf-walking and pulling or carrying clubs
- Calisthenic exercises

Hard Activities

Occupational Tasks:

- Heavy carpentry
- Construction work – doing physical labor

Household Tasks:

- Scrubbing floors

Sports Activities (Actual playing time):

- Doubles tennis
- Disco, Square, or Folk dancing

Very Hard Activity

Occupational Tasks:

- Very Hard physical labor – digging or chopping with heavy tools
- Carrying heavy loads, such as bricks or lumber

Sports Activities (Actual playing time):

1. Jogging or swimming
2. Singles tennis
3. Racquetball
4. Soccer
5. Aerobics
6. Stair climbing
7. Weight training
8. Gymnastics

7-DAY PHYSICAL ACTIVITY RECALL

	Activity	Time Spent
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		

Worksheet for Calculating Daily Energy Expenditure

1.	Add up all the hours of sleep and naps you had.	
2.	Multiply the total number of hours of sleep and naps (line 1) by 1.	X 1 =
3.	Add up the total number of hours spent in moderate activity.	
4.	Multiply the hours spent in moderate activity (line 3) by 4.	X 4 =
5.	Add up the total number of hours spent in hard activity.	
6.	Multiply the hours spend in hard activity (line 5) by 6.	X 6 =
7.	Add up the total number of hours spent in very hard activity.	
8.	Multiply the hours spent in very hard activity (line 7) by 10.	X 10 =
9.	Add up the figures in lines 1, 3, 5, and 7. (1 + 3 + 5 + 7) =	
10.	Hours spent in light activity is equal to 24 hours minus the hours in lines 1, 3, 5, and 7. 24 - (1 + 3 + 5 + 7) =	
11.	Multiply the figure in line 10 by 1.5.	X 1.5 =
12.	Add up the figures in lines 2, 4, 6, 8, and 11. (2 + 4 + 6 + 8 + 11) =	
13.	The figure you arrived at in line 12 is the total kilocalories per kilogram of body weight expended per day. (kcal • kg^{-1} • day^{-1}) =	
14.	To calculate the total number of calories you expended in one day, multiply your total body weight in kilograms)weight in pounds ÷ 2.2046 = kilograms) by the figure in line 13. Body weight (kg) X kcal • kg^{-1} • day^{-1} = total calories expended =	

The following are some average kcal • kg^{-1} • day^{-1} for individuals of different ages:

17-19 years	*20-29 years*	*30-39 years*
male = 44	male = 40	male = 38
female = 35	female = 35	female = 33
40-49 years	*50-59 years*	*60-69 years*
male = 37	male = 36	male = 34
female = 31	female = 30	female = 29

APPENDIX I-E

Anthropometric Data Recording Sheet

Soy, Bone and Health in College Females

Participant Information Sheet

Anthropometrics/DXA/PQCT

Subject ID: _____ Visit Date:_____

Race/Ethnicity: _____

DOB:_____Month _____ Day _____ Year _____

Weight (kg): _____ _____ _____
Measure 1 Measure 2 Average of 1 and 2

Height (cm): _____ _____ _____
Measure 1 Measure 2 Average of 1 and 2

BMI: _____

Body fat (%): _____

DXA operator use	PQCT operator use
❑ WB ❑ Hip ❑ AP Spine Scan date: _____ Completed by: _____ initials of operator	**Non-Dominant Limb: R L** circle one ❑ Arm Length _____ and MCSA _____ ❑ Leg Length _____ and MCSA _____ Scan date: _____ Completed by: _____ initials of operator

[Return a copy of this completed info sheet to the study coordinator.]

APPENDIX I-F

Additional Focus Questions

Soy, Bone and Health in College Females

ID#_____

Additional FOCUS Questions

1) Have you ever experienced a fracture in your lifetime?
    a. No

    b. Yes: # of fractures = _____

        1) If yes, at what age did you experience fracture(s)?

        2) In what type of event did the fracture take place?

        3) How was the fracture treated? (casting, medication, rest, etc.)

2) Did you start any new medications since the beginning of the FOCUS study?

        1) If yes, what medications are you taking and when did you start?

3) If you are taking a birth control medication, how much estrogen and or progesterone is in the medication.

4) What type of shake do you believe you are taking?

    a. Placebo
    b. Soy

APPENDIX I-G

Menstrual Cycle Questionnaire

Participant ID# _____
Date: _____ V# _____

Soy, Bone and Health in College Females

Menstrual Cycle Questionnaire

1. Do you have regular menstrual cycles? YES_____ NO_____

2. What is the usual length of time between cycles in days?

   Circle one 25-28 29-32 33-37

3. Menstrual flow is? (Circle one) Light Moderate Heavy

4. Would you consider yourself to have had PMS in the past 3 months?

   YES_____ NO_____

APPENDICES II

UGA Health and Bone Study Questionnaires

TELEPHONE SCREENING QUESTIONNAIRE
CONSENT FORMS
ANTHROPOMETRIC DATA RECORDING SHEET
HEALTH HISTORY QUESTIONNAIRE

APPENDIX II-A

Telephone Screening Questionnaire

UGA Health and Bone Study

Telephone Screening Questionnaire

*** This cover sheet is to be separated from
the rest of the collected information ***

This interview should only take approximately ten minutes:

Date: _____ Time: _____ Screen completed by: _____

Participant's name: _____

Address: _____

Zip Code: _____

Daytime Phone Number: _____

Study Code _____

UGA Health and Bone Study

Telephone Screening Questionnaire

This interview should only take approximately ten minutes:

Study Code _____

1. Age _____years DOB: _____/_____/_____
mm dd yy

2. Do you attend school? **YES** or **NO**; *circle one*

3. Height ____ft____ in

4. Weight_____lbs

5. Have you lost or gained weight in the past 3 months? **YES** or **NO**; *circle one*
If yes, how much? _____lbs

6. Are you a collegiate student athlete? **YES** or **NO;** *circle one*
*if the answer to #6 is **YES**, please tell the potential volunteer that she does not qualify for the study.*

7. How physically active are you? _____ mins/day, _____days/week

8. Have you ever been pregnant? **YES** or **NO;** *circle one*
*if the answer to #8 is **YES**, please tell the potential volunteer that she does not qualify for the study.*

9. Number of menstrual periods in last 6 months_____

What was the date of your last menstrual period?_____

10. Are you taking any medications? **YES** or **NO** ; *circle one*

If yes, what medication(s)? _____
check specifically for birth control pills, Insulin, Adderall, Ritalin, and steroid medications

How long have you been taking the above medication(s)? _____

11. Have you acquired any of the following diseases or conditions since 2005?

a) Diabetes YES_____NO_____
If yes, please indicate Type I_____ or Type II____
b) High Blood Pressure YES_____NO_____
c) High Cholesterol YES_____NO_____
d) Cerebral Palsy YES_____NO_____
e) Rheumatoid Arthritis YES_____NO_____
f) Growth Disorder YES_____NO_____
g) Thyroid Disease YES_____NO_____
h) Pregnancy YES_____NO_____
i) Gall bladder Disease YES_____NO_____
j) Kidney Disease YES_____NO_____
k) Osteoporosis YES_____NO_____
l) Psychological Illness YES_____NO_____

12. Are you taking an herbal or dietary supplement? **YES** or **NO;** *circle one*

If yes, what supplement(s), how much and how often?
_____

13. What mornings during the week would you be available to come to the UGA Bone and Body Composition Lab, located in Dawson Hall, for testing?
M____ T____ W_____ Th_____ F_____ S _____

Morning testing will begin as early as 7 am, will involve a fasting blood draw, bone and body composition measurements and will take approximately 1 hour and 30 minutes. We encourage you to read the consent form (ask if they prefer the form emailed or mailed) prior to your first laboratory visit to minimize any discomforts associated with fasting.

"This is the end of our telephone screening. Do you have any additional questions for me?" Make sure the potential volunteer has contact numbers for future questions.

APPENDIX II-B

Consent Forms

UGA Health and Bone Study
Consent Form

I agree to participate in the research study entitled "UGA Health and Bone Study," which is being conducted by Dr. Richard Lewis, Department of Foods and Nutrition, The University of Georgia. Dr. Richard Lewis can be reached at (706) 542-4901.

The purpose of this study is to determine if body composition may have implications in bone structure and strength.

The following points have been explained to me:

1) The reason for this project is to examine relationships among bone strength, body fatness, and race in a baseline sample of 18-19 year-old females. A secondary aim is to determine if serum vitamin D status in those participants with risk factors of the metabolic syndrome is associated with bone strength. The benefits that I may expect from my participation are to learn about my bone density, diet and activity habits. However, if abnormalities are found in bone density measurements, I will be notified and advised to contact my physician. I will also receive a payment of $25 for completing all testing procedures conducted at the UGA Bone and Body Composition Laboratory.

2) The procedures are as follows:
(Single Center Trials)
I will be one of approximately 160 participants to be asked to participate in this study. To qualify for the study, I must:

- Be non-Hispanic white or non-Hispanic black female;
- Be post-pubertal, aged 18-19 years;
- Not be participating or have participated in competitive high school sports during senior year or in NCAA Division I college sports;
- Have had no significant weight loss or gain of >5% in the past three months or >10% in the past six months;
- Have had regular menses for the past 6 months;
- Have no known bone disease or disease known to influence bone metabolism (e.g., cerebral palsy, juvenile rheumatoid arthritis), growth disorders, pregnancies, hyperglycemia, hypertension, or hyperlipidemia;
- Must agree to come to the Bone and Body Composition Laboratory at the University of Georgia for blood work, bone density testing and to complete several questionnaires.
- During my visit the following procedures will be done:
    - A blood sample (10 mL) will be taken from an arm vein and a snack will be provided afterward;
    - My height, body weight, and blood pressure will be taken;
    - My body composition and bone measurements will be assessed which will take approximately 50 minutes;
    - I will complete the following questionnaires:

- GSEL Food Frequency Questionnaire to estimate daily dietary intake;
- A Seven-Day Physical Activity Recall Questionnaire to assess typical physical activity levels
- A Health History Questionnaire to document surgery, medication, fracture and other lifestyle historical events

3) The following risks and discomforts have been explained in detail to me:

The body composition and bone scans take approximately 50 minutes. I will be exposed to a total of 65.61 μSv of radiation during these body composition and bone measurements. This amount of radiation is minimal since the radiation exposure of an adult chest X-ray is approximately 500 to 800 μSv. However, any illness or injury not related to the study is not the responsibility of the investigator or the University of Georgia. If I am pregnant or could possibly become pregnant, I realize that the radiation involved in this study could be harmful to a fetus. Prior to testing, if I am not sure about whether I am pregnant, I will notify the Investigator(s) and they will provide a pregnancy test to take in the privacy of my own home. If I am pregnant, I may maintain confidentiality by electing not to notify the Investigator, however I will voluntarily withdraw from the study. If I am pregnant and decide to notify the Investigator, I understand that I would be told that I cannot participate in the study, and I will receive information about and a referral to the UGA Health Center's Women's Clinic.

No risk is expected, but I may experience some discomfort or stress when my blood is drawn. The risks of drawing blood from my arm include the unlikely possibilities of a small bruise or localized infection, bleeding, and fainting. These risks will be reduced in the following ways: my blood will be drawn only by a qualified and experienced phlebotomist who will follow standard sterile techniques, who will observe me after the needle is withdrawn, and who will apply pressure to the blood-draw site. In the event that I have any health problems associated with the blood draws, my insurance or I will be responsible for any related medical expenses. My blood will not be tested for HIV-AIDS. I understand that these questions and blood tests are not for diagnostic purposes. If I have questions about my test results I should see a physician.

The risks of participating in this study are minimal and the data will increase our knowledge on the associations between bone strength, body fatness, and race. Therefore, the benefits of the study are believed to equal or outweigh the very minimal risk.

If I have questions, Dr. Lewis, the research coordinator, or graduate student will be available to talk with me.

4) The results of this participation will be confidential, and will not be released in any individually identifiable form without my prior consent, unless otherwise required by law. The data generated from my tests will be stored on the computers in the lab room 279, Dawson Hall. All data will be associated with a code number and not my name. There

will be a list of participant's names and corresponding code numbers, but no data with that list. My data will be destroyed on or before September 1, 2010. The information collected will be used for research purposes.

I have been informed that there may be unknown risks/discomforts involved, and that I will receive any new information discovered during the course of the study concerning significant treatment findings that may affect my willingness to continue to participate.

In the event of injury resulting from this research, The University of Georgia and/or the laboratory of Dr. Richard Lewis are not able to offer financial compensation or to absorb the costs of medical treatment. However, necessary facilities, emergency treatment and professional services will be available to research participants, just as they are to the community in general. My signature below acknowledges my voluntary participation in this research project. Such participation does not release the investigator(s), institution(s), sponsor(s), or granting agency(ies) from their professional and ethical responsibility to me.

My participation is voluntary and I may refuse to participate or may discontinue my participation AT ANY TIME, without penalty, loss of benefits, or change in my present or future care. The investigator has the right to withdraw me from the study at any time. My withdrawal from the study may be for reasons related solely to me (e.g. not following study-related directions from the Investigator; a serious adverse event reaction) or because the entire study has been terminated. The Sponsor has the right to terminate the study or the Investigator's participation in the study at any time.

The investigator or his designee has answered all of my questions. If I have additional questions during the course of this study about the research or my rights as a research participant, I may address them to The University of Georgia Review Board for Human Subject Research Office at (706) 542-3199. In the event of a research-related injury or if any other problems arise, I may contact Richard D. Lewis, Ph.D., at 706-542-4901.

I HAVE READ THE INFORMATION PROVIDED ABOVE (OR HAVE HAD IT READ TO ME) AND HAD MY QUESTIONS ANSWERED TO MY SATIFACTION. I VOLUNTARILY AGREE TO PARTICIPTATE IN THIS STUDY. I WILL RECEIVE A COPY OF THIS CONSENT FORM.

_____ _____

Signature of Investigator Date Signature of Participant Date

Richard D. Lewis
706-542-4901
rlewis@fcs.uga.edu

PLEASE SIGN BOTH COPIES OF THIS FORM. KEEP ONE AND RETURN THE OTHER TO THE INVESTIGATOR.

Additional questions or problems regarding your rights as a research participant should be addressed to The Chairperson, Institutional Review Board, University of Georgia, 612 Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706) 542-3199; E-Mail Address IRB@uga.edu

Pregnancy Test Instructions: How do I use this EPT Pregnancy Test?

When to Use: The EPT pregnancy test can be used as early as 4 days before an expected period. However, because pregnancy hormone levels are lower the earlier you test, results are less reliable. For a result that's accurate, use EPT from the day you expect your period.

1. Read EPT package instructions.
2. Remove the Pregnancy test stick from its foil packet just prior to use.
3. Remove the cap to expose the absorbent tip.
4. Hold the test stick by its thumb grip. Point the absorbent tip downward.
5. Place the absorbent tip in the urine flow for just 5 seconds, or dip the absorbent tip into a clean container of urine for just 20 seconds. Keep the absorbent tip pointing downwards.
6. Place the test stick on a flat surface with the windows facing up for at least 2 minutes. (If you wish, replace the cap to cover the absorbent tip.) You may notice a light color moving across the windows.

Important: To avoid affecting the test result, wait at least 2 minutes before lifting the stick.

How to read the results of a home pregnancy test: Wait 2-3 minutes to read the result. A line will appear in the square window as a control to show that you have done the test correctly. Be sure to read the result before 10 minutes have passed.

Pregnant
A (+) sign in the round window indicates that you are pregnant. Please see your doctor to discuss your pregnancy and the next steps. Early prenatal care is important to ensure the health of you and your baby. Please notify the project coordinator that you are voluntarily withdrawing from the study.

Not Pregnant
A (-) sign in the round window indicates that you are not pregnant. If your period does not start please see your doctor.

Important:
If no line appears in the control window, the test result is invalid. Do not read the result. Call the project coordinator to request an additional test kit: 706-542-4918

Questions:
Call the project coordinator with any questions or concerns: 706-542-4918

EPT Pregnancy Test


UGA Health and Bone Study

Consent Form for the Use of the Hologic Delphi A
X-Ray Bone Densitometer and XCT 2000 pQCT

Are you pregnant or do you think you might be pregnant? YES NO

*If yes, please do not participate in this study using the Delphi A bone densitometer and the XCT 2000 pQCT.

I, _____, am hereby giving my consent to be used for research conducted by Dr. Richard D. Lewis, University of Georgia, Foods and Nutrition Department, 279 Dawson Hall.

I understand that by giving my consent I am agreeing to be scanned on the Hologic Bone Delphi A X-Ray Densitometer and on the XCT 2000 peripheral Quantitative Computer Tomography machine. Both of these instruments use a low dose x-ray to determine bone mineral density and body composition

I understand that the Hologic Delphi A X-Ray Bone Densitometer uses a very low level of x-ray and that under most operating conditions, the entrance dose to the patient is 0.5mRem-10mRem. This equals about 3% to 30% of the exposure of a standard chest x-ray and is of no danger to me.

I understand that the XCT 2000 pQCT uses a very low level of x-ray and that under most operating conditions, the maximum entrance dose to the patient is less than 1 mRem.

I understand that The University of Georgia is responsible for my safety during my participation in this study. However, any illness or injury not related to this study is not the responsibility of the investigator or the University of Georgia.

I understand that my participation is entirely voluntary. I can withdraw my consent at any time without penalty and have the results of my participation returned to me, removed from records or destroyed.

_____ _____

Signature of Investigator Date Signature of Participant Date

APPENDIX II-C

Anthropometric Data Recording Sheet

UGA Soy, Bone and Health in College Females

Participant Information Sheet

Anthropometrics/DXA/PQCT

Subject ID: _____ Visit Date:_____

Race/Ethnicity: _____

DOB:_____Month _____ Day _____ Year _____

Weight (kg): _____ _____ _____
Measure 1 Measure 2 Average of 1 and 2

Height (cm): _____ _____ _____
Measure 1 Measure 2 Average of 1 and 2

BMI: _____

Body fat (%): _____

DXA operator use	PQCT operator use
❑ WB ❑ Hip ❑ AP Spine Scan date: _____ Completed by: _____ initials of operator	**Non-Dominant Limb: R L** circle one ❑ Arm Length _____ and MCSA _____ ❑ Leg Length _____ and MCSA _____ Scan date: _____ Completed by: _____ initials of operator

[Return a copy of this completed info sheet to the study coordinator.]

APPENDIX II-D

Health History Questionnaires

UGA Health and Bone Study

Health History Questionnaire

Subject ID#_____
Interviewer _____
Date _____

Surgery/Medication/Fracture History

1. Please list major medical procedures, surgeries and/or injuries in your lifetime and related medications. Give the time of the procedure or injury and/or the frequency and duration of medication.

2. Have you ever gone through an extended period of time where you were bedridden or immobilized? YES or NO; *circle one*
    - If yes, how old were you and how long did this immobilization last?
    - Briefly explain the circumstances.

3. Are you currently taking any medications either prescribed by a doctor or over-the-counter (self-prescribed)? YES or NO; *circle one*
    - If yes, what medications?

4. Has any member of your family been diagnosed with any medical condition related to obesity or osteoporosis? YES or NO; *circle one*

5. Have you ever experienced a skeletal fracture in your lifetime? YES or NO; *circle one*
    - If yes, at what age did you experience a fracture?
    - In what type of circumstance did the fracture take place?
    - How was the fracture treated (casting, medication, rest, etc.)?

Other History

1. How would you rate your present health? ____Poor____Good____Fair____Excellent

2. Do you currently smoke cigarettes? ____ YES or NO; *circle one*

    a. If yes, on the average, about how many cigarettes a day do you smoke?

    ____1-5, ____6-14, ____15-24, ____25-35, ____35 or more

3. If you used to smoke but do not smoke now, how long did you smoke? _____years.

4. At what age did you start your menstrual cycles? _____

5. Are your menstrual cycles regular? YES or NO; *circle one*

    a. If not, how long have they been irregular? _____

6. Have you ever used birth control pills? YES or NO; *circle one*

    a. How old were you when you began using birth control pills? _____

    b. How long have you been using them? _____

7. What periods of time did you stop using birth control pills? (Please give dates, if applicable)

8. Are you on any nutritional supplements? _____

9. Are you currently dieting, or on a special type of weight loss program? YES or NO; *circle one*

    a. If yes, what program are you following? _____

10. Do you have any health problems that limit your physical activity? _____

11. How many hours, on average, do you spend watching TV, or on the computer? _____