

OBESITY, TRABECULAR MICROARCHITECTURE AND CORTICAL BONE STRENGTH
IN LATE ADOLESCENT FEMALES

By

HANNAH LEIGH GOFF

(Under the Direction of Richard D. Lewis)

ABSTRACT

The influence of excess body fat on bone strength is unclear. This study determined if adiposity influences bone strength in white females. Participants, 18-19 years of age, were grouped into obese (n = 12) and normal-weight (n = 12). Bone strength was assessed using MRI and DXA. Obesity was associated with lower tibia and radius trabecular bone measures (all $P < 0.03$), and after adjustment for fat-free soft-tissue, both trabecular (radius and lumbar spine) and cortical bone (tibia, radius, femur) outcomes were lower in obese versus normal weight participants. Significant inverse correlations existed between measures of obesity and MRI and DXA bone outcomes, supporting the hypothesis that obesity is associated with lower bone strength in late adolescent females.

Index words: Obesity, bone, trabecular, cortical, MRI

OBESITY, TRABECULAR MICROARCHITECTURE AND CORTICAL BONE STRENGTH
IN LATE ADOLESCENT FEMALES

by

HANNAH LEIGH GOFF

BS, Virginia Polytechnic Institute and State University, 2010

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment
of the Requirements for the Degree

MASTERS OF SCIENCE

ATHENS, GEORGIA

2012

© 2012

Hannah L Goff

All Rights Reserved

OBESITY, TRABECULAR MICROARCHITECTURE AND CORTICAL BONE STRENGTH
IN LATE ADOLESCENT FEMALES

by

HANNAH LEIGH GOFF

Major Professor: Richard D. Lewis

Committee: Norman K. Pollock
Clifton A. Baile

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2012

ACKNOWLEDGEMENTS

Mom – Thank you for your unconditional love, infinite trust, endless support and weekly cards.

From BST to VT to UGA, thanks for your enthusiasm and encouragement - I love you!

Michael – Thank you for listening to my rambling, reading my horrible rough drafts and giving me advice, even though you didn't know what I was talking about half of the time. I love you for always making me laugh and telling me to not take everything so seriously.

Dr. Lewis – Thank you for giving me this awesome project! I would not have had this unique experience anywhere else. Your constant guidance and “teaching moments” have exceeded my expectations for graduate school.

Jessica – I could not have completed this study without your expert advice and guidance. Thank you for encouragement, friendship and 4:00 snack breaks.

Lewis Lab – Thank you for your constant willingness to help with anything and everything.

From Lewis lab lunches to end of the year potlucks, you all have become part of my extended family.

Alexis – Thank you for keeping me sane, being my co-chef and roommate and becoming one of my best friends!

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iv
LIST OF TABLES.....	v
CHAPTER.....	
1. INTRODUCTION.....	9
REFERENCES.....	12
2. REVIEW OF LITERATURE	16
Introduction.....	16
Bone biology.....	17
Bone strength.....	20
Determinants of bone strength in adolescents.....	21
Rationale for studying fat and bone.....	27
Imaging methods to assess bone.....	29
Adiposity and the material properties of bone.....	32
Adiposity and bone geometry.....	34
Adiposity and bone microarchitecture.....	37
Mechanism behind fat and bone.....	39
Summary.....	41
REFERENCES.....	42
3. OBESITY, TRABECULAR MICROARCHITECTURE AND.....	54
Abstract.....	56
Introduction.....	57

Materials and methods.....	59
Results.....	66
Discussion.....	69
REFERENCES.....	73
Tables.....	79
4. SUMMARY AND CONCLUSIONS.....	92
REFERENCES.....	97
APPENDICES.....	111

APPENDICES

	Page
A. Telephone screening questionnaire.....	112
B. Anthropometric data sheet (session 2)	115
C. Anthropometric data sheet (session 3)	117
D. Health history questionnaire and weight chart.....	119
E. 3-day diet record.....	123
F. 7-day physical activity recall.....	127
G. Bone Loading History Questionnaire.....	131

LIST OF TABLES

	Page
Table 3.1: Participant characteristics by adiposity group.....	79
Table 3.2: Magnetic resonance imaging bone characteristics by adiposity....	81
Table 3.3: Dual-energy X-ray absorptiometry bone characteristics by.....	84
Table 3.4: Bivariate correlations of magnetic resonance imaging-assessed...	86
Table 3.5: Partial correlations of magnetic resonance imaging-assessed.....	89

CHAPTER 1

INTRODUCTION

An estimated 44 million U.S. women and men aged 50 and older are diagnosed with osteoporosis and low bone mass, representing 55% of older adults in the United States. The goals of osteoporosis prevention are to reduce bone loss in later life and to promote optimal bone mass during childhood and adolescence (Looker et al 2012; NOF 2002). Peak bone growth occurs during childhood and adolescence between the ages of 12 and 20 years and is influenced by lifestyle choices. For this reason, some believe that osteoporosis may have its origins in childhood and that prevention should start earlier in life. Any condition or disorder that attenuates maximal bone strength during the growing years theoretically jeopardizes peak bone formation and may increase the risk of osteoporotic fractures (Dimitri et al 2010). Obesity is typically associated with chronic diseases like diabetes, hypertension and cardiovascular disease, but has recently been linked with fractures and lower bone strength (Dimitri et al 2012; Dimitri et al 2010; Taylor et al 2006). Importantly, the connection between adiposity and poorer bone health is of concern because of the high prevalence of obesity among adults and children and adolescents (aged 2-19 years) in the United States is 35.6% (Flegal et al 2012) and 16.9% (Ogden et al 2012) respectively.

For years, researchers have thought that excess body weight in the form of fat is advantageous to the skeleton due to the additional weight and mechanical loading effects it has on bones (Zaidi et al 2012). Recently, however, this hypothesis has been questioned. Adipocytes and osteoblasts are derived from common multipotential mesenchymal stem cells and it is

believed that with obesity there is a preference towards the production of adipocytes, not osteoblasts, leading to reduced bone formation. Moreover, adipocytes produce inflammatory adipokines, which have been linked with impaired skeletal development (Cao 2011).

During the growing years, children and adolescents who are obese have been shown to have more fractures compared to non-overweight children (Dimitri et al 2010; Taylor et al 2006). The causality of the higher fracture rates is not known, but Dimitri et al (Dimitri et al 2010) found that obese children with a history of fracture had less bone mass when compared to obese children with no prior fracture. These fracture data are likely responsible for an increasing number of investigations seeking to better understand the fat-bone relationships in children and adolescents.

To date, the findings regarding the relationships between fat and bone strength in children are equivocal and may be linked to the use of different imaging methods, statistical approaches and populations studied. When examining bone outcomes using dual-energy X-ray absorptiometry (DXA), adiposity is reported to be positively associated with bone mineral content (BMC) or areal bone mineral density (aBMD) when adjusted for height or fat-free soft tissue (FFST); (Clark et al 2006; Ellis et al 2003; Goulding et al 2002; Goulding et al 2000; Hong et al 2010; Krug et al 2008). However, when correcting for body weight (Goulding et al 2002; Goulding et al 2000; Hong et al 2010), leg length or truncal height (Janicka et al 2007), fat is not advantageous to bone.

One limitation of using DXA to assess bone, however, is that it is unable to distinguish between cortical and trabecular bone or account for differences in bone size. This is important because bone strength depends not only on the material properties of bone, but also, the geometrical and microarchitectural qualities. When assessing bone strength using peripheral

quantitative computed tomography (pQCT), fat mass (FM) was shown to be associated with greater trabecular and cortical bone strength at the radius and tibia in 7-10 year old children (Ducher et al 2009) and the tibia of 9-11 year olds (Wetzsteon et al 2008), when FFST is not considered. Fat mass was also shown to be a strong stimulus for accrual of cortical bone mass in girls 15 years of age (Sayers and Tobias 2010). Conversely, when controlling for muscle cross-sectional area (MCSA), a surrogate of FFST, distal radius (4%) cross-sectional area and cortical thickness were lower in obese than normal-weight children (Ducher et al 2009). When controlling for MCSA and limb length in late adolescent females, FM was negatively associated with radial and tibial cortical area, total area, cortical BMC, periosteal circumference and strength-strain index (SSI), a valid measure of cortical bone strength, at the 20% site (Pollock et al 2007). Also, the late adolescent females in the high-fat vs normal-fat group had significantly lower SSI, a valid measure of bone strength, at the 20% site of the tibia and radius than the late adolescent females in the normal fat (NF) group. No significant relationships were found between fat and bone measures at the 4% site, a site of primarily trabecular bone (Pollock et al 2007). Wetzsteon et al (Wetzsteon et al 2008) suggests that bone does not adapt to excess fat, but to the muscle and accounting for FFST is essential in studies of adiposity and bone. It appears that bone located at both metaphyseal and diaphyseal bone sites made up of predominantly trabecular and cortical bone, respectively are affected by excess body fat (Ducher et al 2009; Pollock et al 2007; Pollock et al 2011; Wetzsteon et al 2008).

Specific skeletal sites may be affected differently by fat depending on whether they are a weight bearing or non-weight bearing skeletal site (Shapses SA 2012). Although Pollock et al (Pollock et al 2007; Pollock et al 2011) showed no limb differences with respect to fat influences on tibial or radial strength, Ducher et al (Ducher et al 2009) reported differences in radial and

tibial trabecular and geometrical properties in obese children, most likely due to the greater fat to muscle ratio in the forearm than in the tibia.

To date, the trabecular bone outcomes reported with respect to the fat-bone link have been limited to area and density measures. To our knowledge, there are no published studies that have examined the impact of obesity on trabecular microarchitecture using magnetic resonance imaging (MRI). The purpose of the current study is to determine if adiposity influences radial and tibial trabecular architecture using MRI in obese and normal-weight 18-19 year old white females. A secondary aim was to assess bone geometry and bone mineral using MRI and DXA, respectively.

An important goal of this thesis project was to help the laboratory with the feasibility and development of expertise in the use of MRI for assessing bone strength. Furthermore, this project was intended to generate preliminary data for NIH grants and larger scale MRI studies exploring the links between body fat distribution, metabolic factors and bone strength. If obesity and excess fat accumulation does negatively impact bone strength and increase the risk of fractures, the sustained, long-term exposure to fat in children and adolescents, may exacerbate these harmful influences. The results of this study should contribute to the body of knowledge regarding the fat-bone link and provide a basis for further obesity prevention efforts.

REFERENCES

Looker AC, Borrud LG, Dawson-Hughes BA, Shepherd JA, Wright NC. Osteoporosis or low bone mass at the femur neck or lumbar spine in older adults: United States, 2005-2008. NCHS Data Brief 2012(93):1-8.

NOF. Internet: <http://www.nof.org/advocacy/resources/prevalencereport> (accessed June 18, 2012).

Dimitri P, Wales JK, Bishop N. Fat and bone in children: differential effects of obesity on bone size and mass according to fracture history. *J Bone Miner Res* 2010;25(3):527-36.

Dimitri P, Bishop N, Walsh JS, Eastell R. Obesity is a risk factor for fracture in children but is protective against fracture in adults: a paradox. *Bone* 2012;50(2):457-66.

Taylor ED, Theim KR, Mirch MC, et al. Orthopedic complications of overweight in children and adolescents. *Pediatrics* 2006;117(6):2167-74.

Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA: The Journal Of The American Medical Association* 2012;307(5):491-7.

Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity and trends in body mass index among US children and adolescents, 1999-2010. *JAMA: The Journal Of The American Medical Association* 2012;307(5):483-90.

Zaidi M, Buettner C, Sun L, Iqbal J. Minireview: The link between fat and bone: does mass beget mass? *Endocrinology* 2012;153(5):2070-5.

Cao JJ. Effects of obesity on bone metabolism. *Journal Of Orthopaedic Surgery And Research* 2011;6:30-.

Clark EM, Ness AR, Tobias JH. Adipose tissue stimulates bone growth in prepubertal children. *J Clin Endocrinol Metab* 2006;91(7):2534-41.

Ellis KJ, Shypailo RJ, Wong WW, Abrams SA. Bone mineral mass in overweight and obese children: diminished or enhanced? *Acta Diabetol* 2003;40 Suppl 1:S274-S7.

Goulding A, Taylor RW, Jones IE, Manning PJ, Williams SM. Spinal overload: a concern for obese children and adolescents? *Osteoporos Int* 2002;13(10):835-40.

Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, Williams SM. Overweight and obese children have low bone mass and area for their weight. *International Journal of Obesity* 2000;24(5):627-32.

Hong X, Arguelles LM, Liu X, et al. Percent fat mass is inversely associated with bone mass and hip geometry in rural Chinese adolescents. *J Bone Miner Res* 2010;25(7):1544-54.

Krug R, Carballido-Gamio J, Burghardt AJ, et al. Assessment of trabecular bone structure comparing magnetic resonance imaging at 3 Tesla with high-resolution peripheral quantitative computed tomography ex vivo and in vivo. *Osteoporos Int* 2008;19(5):653-61.

Janicka A, Wren TAL, Sanchez MM, et al. Fat mass is not beneficial to bone in adolescents and young adults. *J Clin Endocrinol Metab* 2007;92(1):143-7.

Ducher G, Bass SL, Naughton GA, Eser P, Telford RD, Daly RM. Overweight children have a greater proportion of fat mass relative to muscle mass in the upper limbs than in the lower limbs: implications for bone strength at the distal forearm. *The American Journal Of Clinical Nutrition* 2009;90(4):1104-11.

Wetzsteon RJ, Petit MA, Macdonald HM, Hughes JM, Beck TJ, McKay HA. Bone structure and volumetric BMD in overweight children: a longitudinal study. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2008;23(12):1946-53.

Sayers A, Tobias JH. Fat mass exerts a greater effect on cortical bone mass in girls than boys. *J Clin Endocrinol Metab* 2010;95(2):699-706.

Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, Lewis RD. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. *Am J Clin Nutr* 2007;86(5):1530-8.

Pollock NK, Laing EM, Hamrick MW, Baile CA, Hall DB, Lewis RD. Bone and fat relationships in postadolescent black females: a pQCT study. *Osteoporos Int* 2011;22(2):655-65.

Shapses SA LR, Pollack N. Relationships between fat and bone in children and adults. Edition ed. In: Anderson JB GS, Klemmer PJ, ed. *Calcium and Phosphorus in Health and Disease*. Boca Raton, FL: CRC Press, 2012.

Pollock NK, Bernard PJ, Gutin B, Davis CL, Zhu H, Dong Y. Adolescent obesity, bone mass, and cardiometabolic risk factors. *The Journal Of Pediatrics* 2011;158(5):727-34.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

Peak bone growth occurs in childhood and adolescence between the ages of 12 and 18 years, depending on the skeletal site. Any condition or disorder that disrupts normal bone growth potentially jeopardizes peak bone formation and increases the risk of adult osteoporotic fractures (Janicka et al 2007; Pollock et al 2007). For years, researchers have thought that excess weight in the form of fat is advantageous to the skeleton due to mechanical loading effects (Zaidi et al 2012). However, recently, this hypothesis has been questioned. Obesity may in fact be a disorder that negatively influences bone strength and development of peak bone mass (PBM). For example, fractures have been shown to be more prevalent in obese children and adolescents compared to non-overweight children (Taylor et al 2006). Even with the increasing focus on fat, bone and energy metabolism, there is still uncertainty regarding the impact of excess fat on bone. The following literature review will aim to address issues related to adiposity and bone. Prior to discussing the issues surrounding the fat and bone debate, I will address bone biology, including the two main bone types, bone cells and bone modeling and remodeling. Then, bone development during the growing years will be addressed as well as the meaning of bone strength and factors that influence bone strength. Finally, the studies that address adiposity and bone measured by dual-energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT) and magnetic resonance imaging (MRI) will be discussed. The hypothesized mechanisms behind the fat-bone link will also be highlighted.

Bone biology

Make-up of bone

Bones are vital to the body for functioning in movement, mechanical support, protection of vital organs and maintenance of mineral homeostasis (Favus 2006). Bone composition is 50-70% mineral, 20-40% organic matrix, and the rest is water and lipid. The organic matrix is mainly comprised of type I collagen fibrils that are organized to provide a combination of strength and elasticity. Non-collagenous proteins also make up the organic matrix and are required for proper mineralization of the matrix. The inorganic component is crystalline hydroxyapatite, which is formed from calcium and phosphorous. The skeleton accounts for 98% of calcium in the body (IOM 2011).

Anatomy

There are two main types of osseous tissue that form bones: cortical bone and trabecular bone. Cortical bone comprises roughly 80% of the skeleton, and is found in the shaft of long bones. Cortical bone is also known as compact bone, is dense in nature, and is characterized as being strong and rigid. Trabecular bone makes up the remaining 20% of the skeleton and is found in the ends of long bones. Trabecular bone is also called cancellous bone or spongy bone, is more metabolically active, and is characterized as being weak and soft. The combination of trabecular and cortical bone makes up the contradictory nature of bone; being stiff yet flexible, and light yet strong. Bones must be stiff so as not to fracture when loaded, yet they must be flexible in order to absorb energy from loading. Bone must also be light to allow rapid movement, yet strong in order to withstand everyday activities (Seeman 2008).

Bone cells

There are three types of bone cells: osteoclasts, osteoblasts and osteocytes. Osteoclasts are bone-resorbing cells, osteoblasts are bone-forming cells and osteocytes are cells derived from osteoblasts. These bone cells are responsible for modeling and remodeling and will be discussed below. Osteoclasts are generally located on or recruited to the surface of bone so as to remove damaged bone tissue (Downey and Siegel 2006). The plasma membrane of osteoclasts has an infolded appearance known as a ruffled border. These deep infolds of the osteoclasts attach themselves to bone, and once attached, secrete enzymes that break down bone, creating a resorption cavity known as a Howship lacuna (Favus 2006). Osteoblasts are derived from undifferentiated mesenchymal cells that are responsible for the production and deposition of bone matrix. The number of osteoblasts is directly related to the rate of bone formation. Eventually, osteoblasts either evolve into osteocytes or become inactive cells that line bone surfaces. Osteocytes resemble osteoblasts but make up 90 to 95% percent of bone cells. As osteocytes mature, more matrix is laid down, and these bone cells are incorporated deeper within the lacuna, or bone tissue space. The network of osteocytes communicates through canaliculi and responds to mechanical loads placed on the skeleton during physical activity. It is also believed that the cellular network senses the mechanical deformation within bone that leads to the coordinated formation and resorption of bone (Downey and Siegel 2006).

Modeling and remodeling

Bone structure and makeup define the loads it can support. Likewise, loads placed on the bone also influence its structure. Bone modifies its material framework and structure through bone modeling and remodeling. Bone modeling occurs during childhood until early adolescence, is a process that alters bone size and shape, and ultimately, leads to bone growth and elongation

(Seeman 2009). Bone remodeling occurs throughout the entire lifecycle and is important for mineral homeostasis and bone maintenance (Favus 2006). Modeling and remodeling both involve a complex process in which osteoblasts form bone while osteoclasts resorb bone (Lemaire et al 2004). During bone modeling, cellular activity occurs at different sites on the bone and osteoblast activities surpass osteoclast activities such that formation exceeds resorption resulting in a net gain of bone. Conversely, during bone remodeling, osteoclast and osteoblast activities are coupled throughout most of young adulthood and occur at the same site on bone resulting in bone mass conservation (Favus 2006; Seeman 2009). Osteoclasts resorb bone and then osteoblasts are recruited to the site to form bone. Approximately 20% of the skeleton is constantly undergoing remodeling activity (Hill 1998). Cortical bone has a relatively low turnover rate of 2-3% per year whereas the rate of trabecular bone turnover is higher (Clarke 2008). The process of bone modeling and remodeling are affected and influenced by multiple factors including dietary intake, physical activity, hormones and medications such as corticosteroids. For example, during growth, changes in modeling and remodeling occur in response to high impact loads often seen with artistic gymnastic maneuvers. These adaptations result in wider, stronger bones (Nickols-Richardson et al 1999).

Bone development

During growth, children experience bone enlargement and expansion due to the combination of modeling and remodeling processes, under the regulation of growth and sex hormones (Clarke 2008). The growth phase between childhood and adolescence is a crucial stage for developing optimal bone strength since the majority of bone mineral accrual occurs between 12 to 18 years of age (Pollock et al 2007). Between puberty and adulthood, young women accrue half of their skeletal bone mass. Approximately 90% of this gain occurs by the end of puberty.

Following the period of peak height velocity (PHV) and rapid bone elongation, there is a six-month lag period before the newly expanded bones undergo significant mineralization or peak bone mineralization (PBM). Consequently, this six-month lag time is a time that the under-mineralized bones are more susceptible to fracture.

The timing and tempo of PBM accrual are site specific (Baxter-Jones et al 2011; Recker and Heaney 1993). A longitudinal study of bone area (BA) development and accrual of bone mineral content (BMC) at multiple bone sites found that PBM occurs earlier at the hip and spine than at the whole body. Plateaus in total-body PBM was reached at age 19 for females (7 years after PHV), lumbar spine and total-hip PBM was reached at age 17 for females (5 years after PHV) and femoral neck peak BMC was reached at age 15 for females (3 years after PHV; (Baxter-Jones et al 2011). The majority of studies suggest that PBM, depending on the skeletal site, is likely attained by the second or third decade of life, yet the exact timing of PBM attainment is marginally variable between individuals (Heaney et al 2000; Matkovic et al 1994; Recker et al 1992).

Bone strength

Bone strength is defined as the amount of loading force required to cause the material to fail under a certain loading condition (Van der Meulen et al 2001). Or, more simply, bone strength is the relative ability of a skeletal structure to sustain the loads it is likely to experience in everyday life without fracturing (Heaney 2005). To effectively study bone strength, all aspects of bone must be taken into account including the material properties (bone mineral) of bone, bone geometry and the 3-dimensional architecture of bone. All three aspects contribute to bone strength and are measured by different instruments. However, 2-dimensional areal bone mineral density (aBMD) alone is often an inaccurate assessment of bone strength, especially in children.

These outcomes, measured by DXA, do not account for the changes that occur in bone size during growth. Bone material and geometric properties have been shown to more accurately estimate bone strength (Petit et al 2005).

Body composition, diet, physical activity and hormones all influence the variance in PBM and strength (Lloyd et al 2002; Pollock et al 2007). Therefore, any condition that reduces bone formation or increases bone resorption during growth may lead to suboptimal bone accrual, reduced bone strength, and presumably, a greater risk of osteoporotic fractures later in life (Pollock et al 2007).

Determinants of bone strength in adolescents

Fat-free soft tissue mass

As children's bones grow and elongate, there are multiple factors that influence this process. One such factor is fat-free soft tissue mass (FFST). Faulkner et al (Faulkner et al 1993) showed that total body FFST was highly related to BMC in boys and girls 8-16 years of age. A study by Janicka et al (Janicka et al 2007) found a strong positive effect of FFST on all computed tomography (CT)- and DXA-derived bone measurements in the appendicular and axial skeleton of 150 females and 150 males. In contrast, fat mass had a negative or no relationship to bone geometrical properties. A study in premenopausal women found that those with high muscle/low fat had greater aBMD at the femoral neck than those with low muscle/high fat. Thus, low muscle mass is a risk factor for low aBMD, suggesting that fat mass is protective only when associated with substantial muscle mass (Sowers et al 1992). More recent studies using pQCT have also supported the idea that FFST is an important influence of bone strength and that bone adapts to the muscle forces (Ducher et al 2009; Petit et al 2005; Pollock et al 2007; Wetzsteon et al 2008).

Calcium intake

During growth, children and adolescents are accruing 300-400 mg/day of calcium. During this period of time when there is high rate of calcium deposition, it is thought that supplementation of calcium may improve bone mineral accrual and PBM (IOM 2011). Numerous studies have shown that calcium intake, via food and nonfood supplements, is beneficial to bone (Bonjour et al 1997; Boonen et al 2006; Cameron et al 2004; Greene and Naughton 2011; Lambert et al 2008; Lloyd et al 1993; Matkovic et al 2005; Matkovic et al 2004; Nowson et al 1997). Matkovic et al (Matkovic et al 2004) studied the long-term effects of supplemental calcium and dairy intake on aBMD of the hip and spine and on the bone geometry and volumetric bone mineral density (vBMD) of the forearm in young females during late adolescence. This 3-year trial used DXA to assess the hip and spine and pQCT to assess the forearm. Supplemented participants had significantly higher aBMD at the femur trochanter (3%; $P = 0.0024$) and volumetric BMD at the proximal radius ($P = 0.018$). Those who had high dairy consumption had higher aBMD of the spine at age 15 years, and this was maintained up to the age of 18 years. Therefore, the results showed that calcium supplementation positively influences vBMD while dairy intake may additionally stimulate bone expansion. Both calcium supplementation and dairy products, however, showed a positive influence on aBMD (Matkovic et al 2004). Additionally, in three twin studies, calcium supplementation resulted in greater gains in aBMD when compared to a placebo group (Cameron et al 2004; Greene and Naughton 2011; Nowson et al 1997). All three studies had female twin pairs where one member in each twin pair was given a calcium supplement and the other twin was given a matched placebo. Cameron et al (Cameron et al 2004) found that 1,200 mg of calcium supplementation (as calcium carbonate) over a 2-year period in 8-13 year old girls increased aBMD at the total hip, lumbar spine and femoral neck. The increases were effective in enhancing bone accrual over the first 12–18

months, but these gains were not maintained to 24 months, possibly because the effect of calcium is waned over time. Nowson et al (Nowson et al 1997) also observed an increase in aBMD at the spine and total hip in females aged 10-17 years. The greatest gain was seen in the first 6 months of supplementation. From 6 to 18 months, the difference was maintained, but there was no accelerated increase in aBMD associated with calcium supplementation. Greene and Naughton (Greene and Naughton 2011) conducted a 6-month pQCT study on 9-13 year old girls. Supplementation of calcium with vitamin D was associated with an increase in trabecular area, trabecular density and strength strain index (SSI) at the ultra-distal tibia and radius and an increase in cortical area at the tibial mid-shaft. Data from NHANES 2003-2006 (IOM 2011) show that the median calcium intake from food sources only for adolescent girls aged 14-18 is 826 mg/day. When supplements were taken into account, there was a modest increase in intake to 867 mg/day. This is slightly below the recommended dietary allowance (RDA) of 1,300 mg/day (IOM 2011). While it is unlikely that calcium intakes will influence bone measures in the current study, calcium intake will potentially be used as a covariate in this study.

Vitamin D

Vitamin D is essential to the growth and regulation of bone. Poor vitamin D status in the elderly is related to low bone mass and risk of fracture, but with vitamin D supplementation, there is a decreased risk of falling, calcium absorption is increased and parathyroid hormone (PTH) is suppressed (Dawson-Hughes and Bischoff-Ferrari 2007; Tang et al 2007). While much is known regarding serum vitamin D levels and bone in adults (Bischoff-Ferrari et al 2005), major gaps still exist with regard to vitamin D status in growing children. Studies investigating the relationships between adolescent females, vitamin D status and bone indices report inconsistent findings, with some studies showing no relationships (Kremer et al 2009;

Kristinsson et al 1998), or modest improvements in bone mass with supplementation, but usually in those who are vitamin D deficient (Breen et al 2011; El-Hajj Fuleihan et al 2006; Kremer et al 2009; Kristinsson et al 1998; Outila et al 2001; Viljakainen et al 2006).

In a 12-month, randomized double blind, placebo-controlled trial, adolescent girls were given either 200 or 400 IU of vitamin D₃. Following the intervention, significant increases in femur BMC or spine BMC were observed, but only in the compliance based analysis (Viljakainen et al 2006). Another study found that vitamin D supplementation behaves in a dose-dependent manner. Females 10 to 17 years of age were supplemented with 1,400 IU/week (~200 IU/day), 14,000 IU/week (~2,000 IU/day), or placebo. After 1 year of supplementation, the participants showed substantial increments in lean mass, bone area, and bone mass, with a trend for the increments in bone mass to be larger at the high dose (El-Hajj Fuleihan et al 2006). The equivocal findings in the observational studies and the modest gains in the intervention trials may be attributed to varying maturational stages and initial vitamin D concentrations of participants. The biggest influence on circulating vitamin D concentrations is UVB exposure. Data from NHANES 2003-2006 (IOM 2011) show that the median vitamin D intake from food sources alone for adolescent girls aged 14-18 is 132 IU/day. When supplements were taken into account, there was a slight increase in intake to 144 IU/day. This is significantly below the RDA of 600 IU/day (IOM 2011). While it is unlikely that vitamin D intakes will influence bone measures in the current study, vitamin D intakes will potentially be used as a covariate.

Physical activity

It has been proposed that physical activity is a better determinant of bone mass and bone density throughout growth than diet (Anderson 2000). Besides nutrition, physical activity is the only other modifiable lifestyle factor that improves peak bone mineral accrual and bone strength.

Several cross sectional studies have shown the importance of physical activity on bone, in particular high impact load activities. For example, artistic gymnasts have been shown to have higher BMC and aBMD at various bone sites (Laing et al 2002; Nickols-Richardson et al 1999; Pollock et al 2006).

Prospective studies in children demonstrate the importance of physical activity on bone benefits during growth (Bailey et al 1999; Bakker et al 2003; Baxter-Jones et al 2008; Ianc et al 2006; Teegarden et al 1996). Two publications from the Saskatchewan Bone Mineral Accrual Study are particularly noteworthy (Bailey et al 1999; Baxter-Jones et al 2008). In the study by Bailey et al (Bailey et al 1999), when controlling for maturational and size difference, active boys and girls showed 9% and 17% greater total body BMC, respectively, compared to inactive peers. The study by Baxter-Jones et al (Baxter-Jones et al 2008) incorporates prospectively collected data showing there is a positive effect of physical activity during the time of peak bone mineral accrual that persists into young adulthood. At one year post PHV, active adolescent females had 9% and 10% more adjusted BMC at the total hip and femoral neck. The male and female adolescent active groups were still significantly more active than their peers, even in young adulthood. These results suggest that the skeletal benefits of physical activity in adolescents are maintained into young adulthood (Baxter-Jones et al 2008). In one jumping intervention trial in children, McKay et al (McKay et al 2005) found that children produced maximal ground reaction forces (GRF) ranging from 3.5 to 5 times body weight and rates of force of around 500 times body weight showed that even a small amount of jump repetitions may benefit bone health (McKay et al 2005). With respect to high impact loading activities, Dolan and collaborators (Dolan et al 2006) created to estimate a bone loading score, a score that reflects the historical impact loading activity. The combination of cross-sectional, prospective and

intervention trials demonstrate the strong influence of physical activity on bone. Therefore, it will be important to consider historical loading activity as a potential covariate in the analyses performed in Chapter 3.

Hormones

An array of hormones helps to regulate bone remodeling throughout the lifespan including growth factors and sex steroids. In addition, there are endocrine aspects of adipose tissue that most likely play an important role in bone strength, and this will be discussed in a later section. The ability of hormones to function properly is dependent on a variety of factors, including body weight, age, dietary intake, and health status. Bone growth is positively affected by growth hormone (GH) stimulation of insulin-like growth factor-I (IGF-I). IGF-I stimulates osteoblast activity and collagen synthesis. In a study spanning nine years that included pubertal growth, increasing levels of IGF-I were strongly associated with BMC accrual at multiple skeletal sites (Breen et al 2011). Estrogen is a sex steroid that plays an important role in skeletal maturation and mineralization because it regulates the rate of bone formation and bone resorption, prevents calcium loss and maintains circulating vitamin D concentrations. Estrogen suppresses bone remodeling and inhibits bone resorption, resulting in decreased bone turnover (Soyka et al 2000). Therefore, estrogen deficiency or GH deficiency during adolescence may lead to osteopenia in adulthood. Research on the complexity of how hormones influence bone is both lacking and inconsistent, and it is not yet known which hormones exert the greatest influences on BMC accrual during bone growth (Breen et al 2011; Soyka et al 2000).

Rationale for studying fat and bone

Fat mass and bone in adults

Almost one-third of US adults are obese, which is associated with multiple comorbidities including diabetes, cardiovascular disease, hypertension, and cancer. Only more recently has obesity been linked with osteoporosis and it is still debated whether obesity negatively affects bone strength leading to increased fractures. It was originally thought that obesity decreased the risk for developing osteoporosis due to reduced bone loss related to mechanical loading on the skeleton and the effects of hormones. Many studies have shown a positive correlation between fat mass and aBMD. For example, a meta-analysis of 12 prospective, population-based studies showed that fractures were inversely related to body mass index (BMI) in both men and women (De Laet et al 2005). Another large epidemiological study in Caucasian women over the age of 75 supports this finding, showing that the risk of a hip fracture increased by 40% for each standard deviation reduction in fat mass (Dimitri et al 2012). Conversely, many studies have shown a negative correlation between fat mass and aBMD. For example, a study by Bhupathiraju et al (Bhupathiraju et al 2011) in Puerto Rican men and women aged 47-49 years found that higher body weight-adjusted abdominal fat mass (AFM) was associated with poor bone health. The inverse association with AFM and DXA-derived aBMD measurements was seen at the femoral neck, trochanter, total femur, and lumbar spine (L2-L4) in women and at the femoral neck in men (Bhupathiraju et al 2011). Another recent study demonstrated that visceral adipose tissue (VAT) potentially has detrimental effects on trabecular BMD of the lumbar spine. Bredella et al (Bredella et al 2011) studied healthy obese premenopausal women and used quantitative computed tomography (QCT) to assess body composition and lumbar trabecular BMD. There was an inverse association between L4 trabecular BMD and VAT ($P = 0.003$) independent of age

and BMI. A second study by Bredella et al (Bredella et al 2011) tested the relationship between vertebral bone marrow fat (BMF) and trabecular BMD again in premenopausal obese women. Vertebral BMF was measured using proton magnetic resonance spectroscopy (1H-MRS) and trabecular BMD was measured using CT. The researchers found that those with high visceral fat subsequently had higher BMF than women with low visceral fat. Additionally, BMF was positively correlated to visceral fat, independent of BMD and vertebral BMF was inversely associated with trabecular BMD (Bredella et al 2011). Both studies support the hypothesis that VAT has adverse effects on bone health in premenopausal obese women (Bredella et al 2011; Bredella et al 2011).

Fat mass and bone in children

Ultimately, the risk of developing osteoporosis in late adulthood powers the need to better understand the relationship between fat and bone in childhood. Therefore, the hypothesis that fat may negatively influence bone strength is not limited to adults. A study by Taylor et al (Taylor et al 2006) found that the prevalence of documented skeletal fractures (odds ratio [OR]: 4.54; 95% confidence interval [CI]: (1.6, 13.2); P = 0.0053) and musculoskeletal discomfort (OR: 4.04; 95% CI: (1.5, 10.6); P = 0.0073) was significantly greater in overweight children compared to non-overweight children. Also, when compared to non-overweight children, the overweight children had greater impairment in mobility (mobility score: 17.0 ± 6.8 vs 11.6 ± 2.8). Secondary results related to skeletal fractures, musculoskeletal discomfort, and impaired mobility include a decreased likelihood that children would engage in physical activity, thus perpetuating the cycle of excess weight accumulation and musculoskeletal discomfort (Taylor et al 2006). Another study assessing the effect of obesity on bone mass in children with and without a history of fractures observed similar results. Dimitri et al (Dimitri et al 2010) found that obese

children with a history of fracture had less bone mass when compared to no prior fracture obese children and prior fracture non-obese children. Furthermore, this study reported that the most commonly fractured bone site for children was the distal radius, which is similar to other studies. This may be due to mechanical forces generated on a weaker bone area as the forearm is considered a non-weight bearing skeletal site. The findings from this study suggest that fat mass inhibits bone accrual in children with prior fracture (Dimitri et al 2010).

To date, the findings regarding fat and bone strength are equivocal and may be linked to the use of different imaging methods used, statistical approaches and the populations studied. Much of the existing work on fat and bone has been gained using DXA and pQCT and is summarized below. However, in order to better understand the work that will be presented in the area of fat and bone strength (in Chapter 3), it is important to better understand the imaging instruments that have been utilized. Each imaging technique generates different bone outcomes and has strengths and limitations, which partially contribute to the equivocal findings related to the influence of fat on bone.

Imaging methods to assess bone

Dual-energy X-ray absorptiometry (DXA)

DXA is a two-dimensional X-ray imaging technique that provides an estimate of the area and mineral content of bone including BA (cm^2), BMC (mg), and aBMD (g/cm^2 ; (Lewiecki et al 2008). Multiple sites can be assessed including the total body, lumbar spine, hip and radius.

DXA is used clinically to determine fracture risk in adults and is the standard clinical method for osteoporosis assessment. Typically, aBMD measures are compared to the age-matched population (Z-score) or to a young adult norm (T-score), the latter of which is used in the definition of osteoporosis in adults. When comparing DXA bone measures in young adults, like

the populations studies in the current project, the Z-score is typically used (McKiernan et al 2011). DXA is also capable of measuring soft tissue masses including FFST and total body fat mass. The soft-tissue mass outcomes are an important contribution to the study of fat and bone relationships. Instead of using BMI as a proxy for adiposity, DXA provides an accurate and reliable measure of fat mass. Moreover, since FFST is an important predictor of bone strength in children, FFST assessment is essential. However, DXA has several limitations. DXA provides only areal measures and cannot account for true volumetric changes that occur with childhood bone growth (Liu et al 2007). The areal measurements only present the length and width of bone, but not the depth, which does not fully explain structural strength or to what extent a child's bone is growing. (Heaney 2005). Unlike other imaging techniques, cortical and trabecular bone cannot be distinguished using DXA. Finally, it is thought that the material properties outcomes provided by DXA only contribute a fraction to overall estimate of bone strength (Krug et al 2008).

Peripheral quantitative computer tomography (pQCT)

Peripheral QCT, like DXA, uses a low dose X-ray. However, pQCT is a three-dimensional bone-imaging instrument that assesses the geometrical properties of bone at trabecular and cortical bone sites on the appendicular skeleton (tibia and radius) and provides a more complete assessment of bone strength than DXA (Liu et al 2007). Peripheral QCT outcome measures include: vBMD (mg/cm^3), BMC (mg/mm), cortical vBMD, (mg/cm^3), cortical thickness (mm), cross-sectional area (CSA; mm^2), bone strength index (BSI; mg^2/mm^4) and SSI (mm^3 ; (Lewiecki et al 2008; Pollock et al 2011). Peripheral QCT is preferred for pediatric studies because it can detect the changing size and shape of the growing skeleton. While pQCT does provide a good estimate of bone geometry and strength, it is used primarily for research purposes and is not used clinically. One limitation of pQCT, especially the Stratec 2000, is the gantry size.

When working with obese subjects, the gantry size may not be sufficient to accommodate the larger tibia of an obese individual. Also, when measuring the 4% site of the tibia or radius, an estimate of trabecular geometry and strength can be obtained, but not the three-dimensional microarchitecture of bone.

Magnetic resonance imaging (MRI)

MRI is a three-dimensional bone imaging technique that has traditionally been used clinically to examine soft tissue, but more recently, has been used in skeletal research. Unlike DXA and pQCT, MRI offers multi-planar bone image acquisition by using non-ionizing radiation via radio frequencies (Krug et al 2008). Like pQCT, MRI is capable of distinguishing between trabecular and cortical bone geometry, depending on the protocols employed. What makes MRI unique is that it is capable of assessing trabecular architecture through high-resolution imaging (Liu et al 2007). Modlesky et al (Modlesky et al 2008) have shown that MRI provides a valid assessment of the three-dimensional structure of bone, including apparent trabecular bone volume to total volume (appBV/TV), apparent trabecular number (appTbN), apparent trabecular thickness (appTbTh) and apparent trabecular separation (appTbSp; (Modlesky et al 2008). Cortical assessments by MRI are cortical CSA (mm^2), cortical volume (mm^3), medullary volume (mm^3), medullary CSA (mm^2), total bone volume (mm^3) and total bone CSA (mm^2). Other calculated measures of MRI include cross-sectional moment of inertia (CSMI; mm^4), section modulus (Z), and polar moment of inertia (J) used to estimate bone strength (Petit et al 2005). As discussed earlier, bone strength depends on the material, geometric and microarchitectural properties of bone. Using a combination of all three bone-imaging machines allow for a complete assessment of bone strength and body composition.

Adiposity and the material properties of bone

Studies that use DXA to interpret bone strength in overweight adolescents have shown equivocal findings. A DXA study by Goulding et al (Goulding et al 2000) found that age-adjusted total body BMC relative to body weight was lower than predicted values in both overweight and obese children when compared to normal children. These results showed a mismatch between body weight and bone development during growth in overweight and obese boys and girls (Goulding et al 2000). A subsequent study conducted by Goulding et al (Goulding et al 2002) found that overweight and obese children had smaller lumbar vertebral area when compared to children with normal adiposity, after adjusting for body size. This study showed that both sexes did not adequately increase their spine BMC in relation to their excessive body weight (Goulding et al 2002). Hong et al (Hong et al 2010) used DXA to study BA, BMC, and CSA (by DXA) using hip structure analysis (HSA) in relation to percent fat mass (PFM) in lean, same-sex, Chinese-twins. After controlling for body weight and other pertinent covariates, PFM was found to be inversely associated with BA, BMC, and hip geometry in both males and females. Compared with the lowest age- and gender-specific tertile of PFM, males in the highest tertile of PFM had lower measures of whole-body-less-head BA (WB-BA), lumbar spine BA (L2–L4-BA), total-hip BA, total-hip BMC, and CSA (adjusted $P < 0.05$ for all). Similar inverse relationships were seen in all DXA-derived bone parameters for females except WB-BA and L2–L4-BA (Hong et al 2010).

Conversely, other studies have found that BMC is higher in children with above normal adiposity. A study by Ellis et al (Ellis et al 2003) examined the relationship between BMC and body fatness (%fat) in healthy children and found that total body BMC was greater in obese versus normal adiposity females ($1,616 \pm 596$ vs $1,242 \pm 533$; $P < 0.0005$), even after adjusting

for height. The authors suggested that fat mass was advantageous for the growing skeleton (Ellis et al 2003). Similar results were found in a combined cross-sectional and prospective study, which examined the relationship between total-body fat mass in boys and girls approximately 10 years old, and total-body-less-head (TBLH) BA over a 2-year period by comparing DXA results obtained at age 10 with those at age 12 (Clark et al 2006). After adjusting for both lean mass and height, positive associations were found between fat mass and TBLH bone area (OR: 0.274; 95% CI: (0.258, 0.290); $P < 0.001$) and BMC (OR: 0.266; 95% CI: (0.247, 0.285); $P < 0.001$). Additionally, fat mass was positively related to the change in TBLH BA and the change in BMC in all boys and girls in Tanner stage 1. This study concluded that fat mass was a positive, independent determinant of bone mass and size and that adipose tissue acted to stimulate bone growth indicated by the increases in the bone parameters over a 2-year period. However, this relationship was weakened by puberty (Clark et al 2006).

Similarly, in a more recent study by Pollock et al (Pollock et al 2010), total body BMC (TBBMC) was found to be positively related to fat mass ($P = 0.01$) in prepubertal overweight children with and without diabetes after adjusting for sex, race, height, and FFST. The authors also found that TBBMC was lower in those overweight children with diabetes, suggesting that the health status of the overweight child or adolescent may be a mitigating factor with respect to the bone-fat relationships (Pollock et al 2010). Part of the explanation for the above findings may be related to the location of fat (visceral versus subcutaneous) because metabolic abnormalities are more strongly associated with visceral adipose tissue (VAT), rather than with subcutaneous adipose tissue (SAT). Unlike the preceding DXA studies, the authors further explored these relationships based on the location of the fat and found that TBBMC was inversely correlated with VAT and subcutaneous fat (both $P < 0.03$) determined by MRI (Pollock et al 2010). Hence,

the health of the child and location of the fat, both probably linked together, may be critical factors to consider when interpreting fat-bone relationships. Pollock et al (Pollock et al 2011) confirmed these ideas in a study of overweight adolescents in which overweight adolescents with one metabolic risk factor had 5% lower TBBMC than healthy overweight adolescents.

With respect to the DXA studies, some of the discrepancies may be related to statistical approaches employed (Reid 2009) and whether adjustments were made for body weight, height and or FFST. The DXA studies concluding that fat was detrimental to bone adjusted for weight (Goulding et al 2002; Goulding et al 2000; Hong et al 2010), whereas the DXA studies adjusting for height found that adiposity was advantageous for bone (Clark et al 2006; Ellis et al 2003; Goulding et al 2002; Goulding et al 2000; Hong et al 2010; Krug et al 2008). While DXA is limited in that it only provides a two-dimensional assessment of bone, other factors to consider should be the health status of the population and the location of the fat. Three-dimensional imaging techniques such as pQCT and MRI are able to assess bone geometry and microarchitecture, respectively, and are therefore able to offer additional information on fat and bone strength relationships.

Adiposity and bone geometry

Bone studies using pQCT also show conflicting results. Sayers and Tobias (Sayers and Tobias 2010) studied whether fat mass and lean mass differ in the way they influence cortical bone development by examining relationships between fat mass, lean mass, and tibial pQCT parameters in boys and girls (mean age 15.5 years). Lean mass was strongly related to cortical BMC to a similar extent in boys (OR: 0.955; 95% CI: [0.900, 1.011]; $P < 0.0001$) and girls (OR: 0.947; 95% CI: [0.872, 1.021]; $P < 0.0001$). There was also a strong positive association between fat mass and cortical BMC in both girls and boys, but this relationship was considerably stronger

in girls (coefficients, 0.227 and 0.355 in boys and girls, respectively). The researchers found that fat mass was a strong stimulus for accrual of cortical bone mass in girls, showing a positive effect of fat on bone. However, they neglected to account for FFST, which substantially impacts bone (Sayers and Tobias 2010). Ducher et al (Ducher et al 2009) studied the influence of adiposity on bone strength measured at the 4% and 66% site of the radius and tibia in prepubertal normal-weight and overweight children. The overweight children had greater values for bone variables (0.3–1.3 SD; $P < 0.0001$) at both sites and limbs and greater fat-muscle ratio, particularly in the forearm ($92 \pm 28\%$ compared with $57 \pm 17\%$), when compared to normal-weight children. Conversely, the fat-muscle ratio correlated negatively with all bone variables, after adjusting for body weight ($r = -0.17$ to -0.54 ; $P < 0.0001$). Therefore, the findings showed that overweight prepubertal children had larger and stronger bones than did their normal-weight peers at both the forearm and tibia, that the researchers suspect is primarily due to greater muscle size and not to increased adiposity. These skeletal benefits seen in overweight children were more pronounced in the lower limbs than in the upper limbs. Again, this may be due to the lower limbs being a primarily weight bearing site (i.e., supporting the entire body weight). However, the researchers did report that the overweight children had a high proportion of fat relative to muscle in the forearm, which was associated with reduced bone strength (Ducher et al 2009). When taking muscle into account, the fat seemed likely to reduce bone strength at a non-weight bearing site.

Along the same lines, Pollock et al (Pollock et al 2007) used pQCT to determine both bone material and the 3-dimensional geometric properties of bone. This study was one of the first to use 3-D imaging, showing that adiposity may not be beneficial for bone strength. In a cross-sectional study, Pollock et al (Pollock et al 2007) used both pQCT and DXA to assess bone

strength in late adolescent females. Bone measurements with pQCT were taken on the non-dominant tibia and radius at the 4%, 20% and 66% sites from the distal metaphyses to represent areas of high trabecular bone, cortical bone, and muscle cross-sectional area (MCSA), respectively. The MSCA measurement provides an estimate of muscle size and density, and is used as a surrogate of muscle force to which the tibial and radial bones are exposed. When controlling for MCSA and limb length, Pollock et al (Pollock et al 2007) found that %fat was negatively associated with radial and tibial cortical area, total area, cortical BMC, periosteal circumference, and SSI at the 20% site (all $P < 0.05$). When controlling for MCSA, the high fat (HF) group had significantly lower SSI at the 20% site of the tibia and radius than the normal fat (NF) group. No significant relationships were found between fat and bone measures at the 4% site, a site of primarily trabecular bone (Pollock et al 2007). Another study by Pollock et al (Pollock et al 2011) used pQCT to assess the effect of adiposity on bone structure and strength in late adolescent, black females. Black females were studied since that population experiences the highest rates of obesity. After controlling for either body weight or FFST, the HF vs the NF group had lower total CSA (9–17%), cortical CSA (6–15%), and SSI (13–24%) at the cortical site of the tibia (all $P < 0.05$). After controlling for body weight, the HF vs NF group had lower total CSA (14%, $P = 0.03$), cortical CSA (9%, $P = 0.04$), and SSI (15%, $P = 0.07$) at the cortical site of the radius. In the HF group, the lower cortical bone strength was due to smaller bone dimensions [total CSA (9%, $P = 0.01$) and cortical CSA (6%, $P = 0.05$)] compared to the NF group. Interestingly, there were no significant differences observed between adiposity groups at the tibial and radial trabecular bone sites (4%) after controlling for either body weight or FFST mass. These results are consistent with previous findings conducted by the same researchers,

showing that obesity may adversely influence cortical bone strength in late-adolescent, black females entering adulthood (Pollock et al 2011).

With little focus on trabecular bone, additional information on the fat and bone strength relationship may be gained utilizing MRI. To date, there are no published studies using MRI to assess the effects of fat on trabecular bone architecture. In order to better understand the influence of adiposity on bone strength, future studies should consider the use of MRI.

Adiposity and bone microarchitecture

When assessing the fat-bone relationship using DXA, cortical or trabecular bone cannot be assessed. When considering pQCT studies, it seems that fat has a negative effect on bone, and is more prominent at cortical bone sites than at trabecular bone sites. However the pQCT doesn't provide information about the microarchitecture of trabecular bone. To our knowledge there are no published studies examining the link between fat and bone microarchitecture determined by MRI. High resolution MRI has been shown to be a good methodology for assessing bone microarchitecture. Two studies conducted by Modlesky et al (Modlesky et al 2008; Modlesky et al 2008) demonstrate that MRI is a good methodology for comparing different population groups. Modlesky et al (Modlesky et al 2008) studied a small sample of eight NCAA Division I female college gymnasts and eight female controls matched for age, height, body mass, and race. In order to determine if the trabecular bone microarchitecture in the proximal tibia of female college artistic gymnasts was enhanced compared to controls, MRI was used to measure trabecular appBV/TV, appTbN, appTbTh and appTbSp in the non-dominant leg. Before this study, the status of trabecular microarchitecture in the weight-bearing bone of gymnasts was unknown. Modlesky et al (Modlesky et al 2008) found that gymnasts had higher appBV/TV (13.6%, $d = 1.22$) and appTbN (8.4%, $d = 1.45$) and lower appTbSp (13.7%, $d = 1.33$) than

controls ($P < 0.05$). Although not statistically significant ($P = 0.121$), there was a trend that gymnasts had higher appTbTh (6.3%, $d = 0.83$) than controls. Additionally, gymnasts had higher tibial aBMD and BMC, although the differences were smaller in magnitude ($d = 0.75$ and 0.74 , respectively) and not statistically significant ($P > 0.05$). Modlesky et al (Modlesky et al 2008) found that high-load physical activity, seen in gymnastics, may enhance the trabecular microarchitecture of weight-bearing bones (Modlesky et al 2008). Although the current project did not compare athletes to non-athletes, a similar population group in age and gender was studied.

A second MRI study by Modlesky and colleagues (Modlesky et al 2008) compared trabecular microarchitecture values of non-ambulatory children with cerebral palsy (CP) and typically developing children to determine the short-term reliability of trabecular bone microarchitecture assessment in children using high-resolution MRI. In children with CP, the coefficients of variation for repeat measures of appBV/TV, appTbN, appTbTh and appTbSp were 2.18, 1.98, 3.00 and 2.26%, respectively, in the distal femur. For typically developing children, similar coefficients of variation were reported (2.03, 2.73, 1.80 and 3.49%, respectively). The high degree of reproducibility was confirmed by very strong intraclass correlation coefficients for the combined sample of children with CP and typically developing children (0.971, 0.964, 0.942 and 0.982 for appBV/TV, appTbN, appTbTh and appTbSp, respectively, $P < 0.001$). According to Modlesky et al (Modlesky et al 2008), the reproducibility found was similar to, or even better than the reproducibility reported for adults. The findings suggest that MRI is reliable to assess trabecular bone microarchitecture in children. This study showcases that MRI is feasible in assessing trabecular bone, can show differences in trabecular architecture, and can distinguish between bones of varying strengths.

Another bone imaging methodology that is more recently being used is high-resolution pQCT (HR-pQCT). Krug et al (Krug et al 2008) sought to measure trabecular bone micro-architecture and provide structural information by comparing the performance and capability of HR-pQCT to a 3.0 Tesla MRI. Correlations between the two machines for appTbN and appTbSp were found to be high ($r > 0.7$). High correlations ($r > 0.8$) were also found for 2-dimensional and 3-dimension analysis of all structural bone parameter measurements. From this study, the researchers concluded that both modalities were capable of offering meaningful information on trabecular structure and performed well regarding trabecular bone measurements (Krug et al 2008). It is expected that more studies will be published looking at trabecular microarchitecture using HR-pQCT.

Mechanism behind fat and bone

The mechanisms by which fat may influence bone development are actively being explored. For years, the primary hypothesis was that excess fat had a beneficial effect on the skeleton due to mechanical loading effects (Zaidi et al 2012). Many studies have reported a positive relationship between body weight and BMC (Clark et al 2006; Ducher et al 2009; Ellis et al 2003; Sayers and Tobias 2010). More recently, there has been interest in non-weight bearing effects of fat tissue on bone. In fact, there is a new hypothesis that suggests that fat may influence bone through the secretion of adipokines and that in fact, bone and fat, may communicate with each other. Bone has recently been shown to influence energy metabolism, which is mediated by osteocalcin (Pittas et al 2009). Now the interplay between the two tissues, thought for a long time to be related only by a weight-bearing mechanism, has been called into question.

Adipocytes and osteoblasts are derived from a common multipotential mesenchymal stem cell. It is thought that with greater adiposity there is a preference towards the production of adipocytes and not osteoblasts, leading to reduced bone formation (Cao 2011). For example, higher levels of bone marrow fat were associated with lower trabecular BMD at the lumbar spine (Bredella et al 2011). Adipose tissue produces the adipokines, leptin and adiponectin, and cytokines resistin, adipsin, tumor necrosis factor and interleukins (Sheu and Cauley 2011). Any change in the expression or secretion of the adipokines may cause altered bone mass (Rosen and Klibanski 2009). Currently, the most understood adipokines are leptin and adiponectin, which are also the most abundant in circulation (Hill et al 2009). Leptin affects food regulation, energy expenditure, and bone mass and is highly correlated with fat mass. Leptin also has a direct anabolic effect on osteoblast activity and may indirectly decrease osteoclast activity, resulting in decreased bone resorption (Cirmanova et al 2008). Paradoxically, adiponectin levels are inversely related to visceral fat mass and body mass index, as well as aBMD (Shetty et al 2009). Recent studies indicate that adiponectin acts directly on bone cells. However, the results are conflicting; in-vitro data show a positive correlation of adiponectin with bone-mass formation whereas in vivo data show a negative correlation (Shetty et al 2009). In obesity there is increased secretion of leptin and/or decreased production of adiponectin that may either directly affect bone formation or indirectly affect bone resorption through up-regulated pro-inflammatory cytokine production (Cao 2011).

Resistin, a pro-inflammatory cytokine and linked to central adiposity, is up-regulated in obesity and insulin resistance. Modest increases in resistin promote proliferation of osteoblasts and increase the formation of osteoclasts in cell culture studies. Still, the influence resistin has on bone mass is not currently known (Russell et al 2010).

The characteristics of adipocytes may be dependent upon the fat depot site and the proportion of VAT versus subcutaneous adipose tissue (SAT; (Russell et al 2010). Recent studies indicate that central obesity may have more of an effect on bone health than total body fat or SAT. Elevated VAT is associated with increased levels of pro-inflammatory cytokines and these cytokines promote increased bone resorption and bone loss (Cao 2011). Adiponectin and leptin, which stimulate the proliferation and differentiation of osteoblasts, and estrogen, which reduce osteoclast activity, are less abundant in the VAT.

Assessment of VAT using CT and MRI found that VAT was negatively associated with aBMD, BMC, structure, and strength. Pollock et al (Pollock et al 2011) found that total body BMC was negatively associated with VAT ($P < 0.04$) However, when studies use surrogate measures of central obesity such as waist circumference, waist-to-hip ratio and abdominal fat measured by DXA, the relationship between BMD and VAT was inconclusive. The inconsistent results may be due to the inability to separate VAT from other soft tissues (Sheu and Cauley 2011). Although there is an inverse relationship between VAT and BMD using CT or MRI measurement, there is not an association for SAT. Using multiple linear regression, adjusting for age, sex, race, height, and FFST mass, Pollock et al (Pollock et al 2011) found no relation between bone mass and SAT. It is undecided whether the effects of SAT and bone are similar or opposite to the effects seen with VAT on bone (Sheu and Cauley 2011).

Summary

Though there have been numerous studies assessing fat and bone, there is conflicting evidence due to differing methodologies and statistical adjustments employed. Therefore, the extent to which excess body fat influences bone strength is still unclear. Additionally, there is no known published data using MRI to assess the influence of fat on trabecular microarchitecture.

Therefore, the collective findings warrant a study assessing trabecular architecture to better understand fat and bone strength.

REFERENCES

Janicka A, Wren TAL, Sanchez MM, et al. Fat mass is not beneficial to bone in adolescents and young adults. *J Clin Endocrinol Metab* 2007;92(1):143-7.

Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, Lewis RD. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. *Am J Clin Nutr* 2007;86(5):1530-8.

Zaidi M, Buettner C, Sun L, Iqbal J. Minireview: The link between fat and bone: does mass beget mass? *Endocrinology* 2012;153(5):2070-5.

Taylor ED, Theim KR, Mirch MC, et al. Orthopedic complications of overweight in children and adolescents. *Pediatrics* 2006;117(6):2167-74.

Favus M. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 6th ed. Washington, D.C.: American Society for Bone and Mineral Research, 2006.

IOM IoM. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, D.C.: The National Academic Press, 2011.

Seeman E. Bone quality: the material and structural basis of bone strength. *J Bone Miner Metab* 2008;26(1):1-8.

Downey PA, Siegel MI. Bone biology and the clinical implications for osteoporosis. *Physical Therapy* 2006;86(1):77-91.

Seeman E. Bone modeling and remodeling. *Critical Reviews In Eukaryotic Gene Expression* 2009;19(3):219-33.

Lemaire V, Tobin FL, Greller LD, Cho CR, Suva LJ. Modeling the interactions between osteoblast and osteoclast activities in bone remodeling. *Journal Of Theoretical Biology* 2004;229(3):293-309.

Hill PA. Bone remodelling. *British Journal Of Orthodontics* 1998;25(2):101-7.

Clarke B. Normal bone anatomy and physiology. *Clinical Journal Of The American Society Of Nephrology: CJASN* 2008;3 Suppl 3:S131-S9.

Nickols-Richardson SM, O'Connor PJ, Shapses SA, Lewis RD. Longitudinal bone mineral density changes in female child artistic gymnasts. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 1999;14(6):994-1002.

Baxter-Jones ADG, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2011;26(8):1729-39. doi: 10.1002/jbmr.412.

Recker RR, Heaney RP. Peak bone mineral density in young women. *JAMA: The Journal Of The American Medical Association* 1993;270(24):2926-7.

Heaney RP, Abrams S, Dawson-Hughes B, et al. Peak bone mass. *Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA* 2000;11(12):985-1009.

Matkovic V, Jelic T, Wardlaw GM, et al. Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. *The Journal Of Clinical Investigation* 1994;93(2):799-808.

Recker RR, Davies KM, Hinders SM, Heaney RP, Stegman MR, Kimmel DB. Bone gain in young adult women. *JAMA: The Journal Of The American Medical Association* 1992;268(17):2403-8.

Van der Meulen MC, Jepsen KJ, Mikiřá B. Understanding bone strength: size isn't everything. *Bone* 2001;29(2):101-4.

Heaney RP. BMD: the problem. *Osteoporos Int* 2005;16(9):1013-5.

Petit MA, Beck TJ, Kontulainen SA. Examining the developing bone: What do we measure and how do we do it? *Journal Of Musculoskeletal & Neuronal Interactions* 2005;5(3):213-24.

Lloyd T, Beck TJ, Lin HM, et al. Modifiable determinants of bone status in young women. *Bone* 2002;30(2):416-21.

Faulkner RA, Bailey DA, Drinkwater DT, Wilkinson AA, Houston CS, McKay HA. Regional and total body bone mineral content, bone mineral density, and total body tissue composition in children 8-16 years of age. *Calcified Tissue International* 1993;53(1):7-12.

Sowers MF, Kshirsagar A, Crutchfield MM, Updike S. Joint influence of fat and lean body composition compartments on femoral bone mineral density in premenopausal women. *American Journal Of Epidemiology* 1992;136(3):257-65.

Ducher G, Bass SL, Naughton GA, Eser P, Telford RD, Daly RM. Overweight children have a greater proportion of fat mass relative to muscle mass in the upper limbs than in the lower limbs: implications for bone strength at the distal forearm. *Am J Clin Nutr* 2009;90(4):1104-11.

Petit MA, Beck TJ, Shults J, Zemel BS, Foster BJ, Leonard MB. Proximal femur bone geometry is appropriately adapted to lean mass in overweight children and adolescents. *Bone* 2005;36(3):568-76.

Wetzsteon RJ, Petit MA, Macdonald HM, Hughes JM, Beck TJ, McKay HA. Bone structure and volumetric BMD in overweight children: a longitudinal study. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2008;23(12):1946-53.

Bonjour JP, Carrie AL, Ferrari S, et al. Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. *The Journal Of Clinical Investigation* 1997;99(6):1287-94.

Boonen S, Vanderschueren D, Haentjens P, Lips P. Calcium and vitamin D in the prevention and treatment of osteoporosis - a clinical update. *Journal Of Internal Medicine* 2006;259(6):539-52.

Cameron MA, Paton LM, Nowson CA, Margerison C, Frame M, Wark JD. The effect of calcium supplementation on bone density in premenarcheal females: a co-twin approach. *The Journal Of Clinical Endocrinology And Metabolism* 2004;89(10):4916-22.

Greene DA, Naughton GA. Calcium and vitamin-D supplementation on bone structural properties in peripubertal female identical twins: a randomised controlled trial. *Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA* 2011;22(2):489-98.

Lambert HL, Eastell R, Karnik K, Russell JM, Barker ME. Calcium supplementation and bone mineral accretion in adolescent girls: an 18-mo randomized controlled trial with 2-y follow-up. *The American Journal Of Clinical Nutrition* 2008;87(2):455-62.

Lloyd T, Andon MB, Rollings N, et al. Calcium supplementation and bone mineral density in adolescent girls. *JAMA: The Journal Of The American Medical Association* 1993;270(7):841-4.

Matkovic V, Goel PK, Badenhop-Stevens NE, et al. Calcium supplementation and bone mineral density in females from childhood to young adulthood: a randomized controlled trial. *The American Journal Of Clinical Nutrition* 2005;81(1):175-88.

Matkovic V, Landoll JD, Badenhop-Stevens NE, et al. Nutrition influences skeletal development from childhood to adulthood: a study of hip, spine, and forearm in adolescent females. *The Journal Of Nutrition* 2004;134(3):701S-5S.

Nowson CA, Green RM, Hopper JL, et al. A co-twin study of the effect of calcium supplementation on bone density during adolescence. *Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA* 1997;7(3):219-25.

Dawson-Hughes B, Bischoff-Ferrari HA. Therapy of osteoporosis with calcium and vitamin D. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2007;22 Suppl 2:V59-V63.

Tang BMP, Eslick GD, Nowson C, Smith C, Bensoussan A. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet* 2007;370(9588):657-66.

- Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *JAMA: The Journal Of The American Medical Association* 2005;293(18):2257-64.
- Kremer R, Campbell PP, Reinhardt T, Gilsanz V. Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. *The Journal Of Clinical Endocrinology And Metabolism* 2009;94(1):67-73.
- Kristinsson JO, Valdimarsson O, Sigurdsson G, Franzson L, Olafsson I, Steingrimsdottir L. Serum 25-hydroxyvitamin D levels and bone mineral density in 16-20 years-old girls: lack of association. *Journal Of Internal Medicine* 1998;243(5):381-8.
- Breen ME, Laing EM, Hall DB, et al. 25-hydroxyvitamin D, insulin-like growth factor-I, and bone mineral accrual during growth. *The Journal Of Clinical Endocrinology And Metabolism* 2011;96(1):E89-E98.
- El-Hajj Fuleihan G, Nabulsi M, Tamim H, et al. Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. *The Journal Of Clinical Endocrinology And Metabolism* 2006;91(2):405-12.
- Outila TA, K√ § rkk√ § inen MU, Lamberg-Allardt CJ. Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: associations with forearm bone mineral density. *The American Journal Of Clinical Nutrition* 2001;74(2):206-10.
- Viljakainen HT, Natri A-M, K√ § rkk√ § inen M, et al. A positive dose-response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. *Journal Of*

Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research 2006;21(6):836-44.

Anderson JJ. The important role of physical activity in skeletal development: how exercise may counter low calcium intake. The American Journal Of Clinical Nutrition 2000;71(6):1384-6.

Laing EM, Massoni JA, Nickols-Richardson SM, Modlesky CM, O'Connor PJ, Lewis RD. A prospective study of bone mass and body composition in female adolescent gymnasts. The Journal Of Pediatrics 2002;141(2):211-6.

Pollock NK, Laing EM, Modlesky CM, O'Connor PJ, Lewis RD. Former college artistic gymnasts maintain higher BMD: a nine-year follow-up. Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA 2006;17(11):1691-7.

Bailey DA, McKay HA, Mirwald RL, Crocker PR, Faulkner RA. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research 1999;14(10):1672-9.

Bakker I, Twisk JWR, Van Mechelen W, Roos JC, Kemper HCG. Ten-year longitudinal relationship between physical activity and lumbar bone mass in (young) adults. Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research 2003;18(2):325-32.

- Baxter-Jones ADG, Kontulainen SA, Faulkner RA, Bailey DA. A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. *Bone* 2008;43(6):1101-7.
- Ianc D, Serbescu C, Bembea M, Benhamou L, Lespessailles E, Courteix D. Effects of an exercise program and a calcium supplementation on bone in children: a randomized control trial. *International Journal Of Sport Nutrition And Exercise Metabolism* 2006;16(6):580-96.
- Teegarden D, Proulx WR, Kern M, et al. Previous physical activity relates to bone mineral measures in young women. *Medicine And Science In Sports And Exercise* 1996;28(1):105-13.
- McKay H, Tsang G, Heinonen A, MacKelvie K, Sanderson D, Khan KM. Ground reaction forces associated with an effective elementary school based jumping intervention. *British Journal Of Sports Medicine* 2005;39(1):10-4.
- Dolan SH, Williams DP, Ainsworth BE, Shaw JM. Development and reproducibility of the bone loading history questionnaire. *Medicine And Science In Sports And Exercise* 2006;38(6):1121-31.
- Soyka LA, Fairfield WP, Klibanski A. Clinical review 117: Hormonal determinants and disorders of peak bone mass in children. *The Journal Of Clinical Endocrinology And Metabolism* 2000;85(11):3951-63.
- De Laet C, Kanis JA, Odén A, et al. Body mass index as a predictor of fracture risk: a meta-analysis. *Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA* 2005;16(11):1330-8.

Dimitri P, Bishop N, Walsh JS, Eastell R. Obesity is a risk factor for fracture in children but is protective against fracture in adults: a paradox. *Bone* 2012;50(2):457-66.

Bhupathiraju SN, Dawson-Hughes B, Hannan MT, Lichtenstein AH, Tucker KL. Centrally located body fat is associated with lower bone mineral density in older Puerto Rican adults. *The American Journal Of Clinical Nutrition* 2011;94(4):1063-70.

Bredella MA, Torriani M, Ghomi RH, et al. Determinants of bone mineral density in obese premenopausal women. *Bone* 2011;48(4):748-54.

Bredella MA, Torriani M, Ghomi RH, et al. Vertebral bone marrow fat is positively associated with visceral fat and inversely associated with IGF-1 in obese women. *Obesity (Silver Spring, Md)* 2011;19(1):49-53.

Dimitri P, Wales JK, Bishop N. Fat and bone in children: differential effects of obesity on bone size and mass according to fracture history. *J Bone Miner Res* 2010;25(3):527-36.

Lewiecki EM, Gordon CM, Baim S, et al. International Society for Clinical Densitometry 2007 Adult and Pediatric Official Positions. *Bone* 2008;43(6):1115-21.

McKiernan FE, Berg RL, Linneman JG. The utility of BMD Z-score diagnostic thresholds for secondary causes of osteoporosis. *Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA* 2011;22(4):1069-77.

Krug R, Carballido-Gamio J, Burghardt AJ, et al. Assessment of trabecular bone structure comparing magnetic resonance imaging at 3 Tesla with high-resolution peripheral quantitative computed tomography ex vivo and in vivo. *Osteoporos Int* 2008;19(5):653-61.

Liu D, Manske SL, Kontulainen SA, et al. Tibial geometry is associated with failure load ex vivo: a MRI, pQCT and DXA study. *Osteoporos Int* 2007;18(7):991-7.

Pollock NK, Laing EM, Hamrick MW, Baile CA, Hall DB, Lewis RD. Bone and fat relationships in postadolescent black females: a pQCT study. *Osteoporos Int* 2011;22(2):655-65.

Modlesky CM, Subramanian P, Miller F. Underdeveloped trabecular bone microarchitecture is detected in children with cerebral palsy using high-resolution magnetic resonance imaging. *Osteoporos Int* 2008;19(2):169-76.

Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, Williams SM. Overweight and obese children have low bone mass and area for their weight. *International Journal of Obesity* 2000;24(5):627-32.

Goulding A, Taylor RW, Jones IE, Manning PJ, Williams SM. Spinal overload: a concern for obese children and adolescents? *Osteoporos Int* 2002;13(10):835-40.

Hong X, Arguelles LM, Liu X, et al. Percent fat mass is inversely associated with bone mass and hip geometry in rural Chinese adolescents. *J Bone Miner Res* 2010;25(7):1544-54.

Ellis KJ, Shypailo RJ, Wong WW, Abrams SA. Bone mineral mass in overweight and obese children: diminished or enhanced? *Acta Diabetol* 2003;40 Suppl 1:S274-S7.

Clark EM, Ness AR, Tobias JH. Adipose tissue stimulates bone growth in prepubertal children. *J Clin Endocrinol Metab* 2006;91(7):2534-41.

Pollock NK, Bernard PJ, Wenger K, et al. Lower bone mass in prepubertal overweight children with prediabetes. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2010;25(12):2760-9. doi: 10.1002/jbmr.184.

Pollock NK, Bernard PJ, Gutin B, Davis CL, Zhu H, Dong Y. Adolescent obesity, bone mass, and cardiometabolic risk factors. *The Journal Of Pediatrics* 2011;158(5):727-34.

Reid IR. Adipose Tissue and Bone. *Clinic Rev Bone Miner Metab* 2009;7:207-9.

Sayers A, Tobias JH. Fat mass exerts a greater effect on cortical bone mass in girls than boys. *J Clin Endocrinol Metab* 2010;95(2):699-706.

Modlesky CM, Majumdar S, Dudley GA. Trabecular bone microarchitecture in female collegiate gymnasts. *Osteoporos Int* 2008;19(7):1011-8.

Ducher G, Bass SL, Naughton GA, Eser P, Telford RD, Daly RM. Overweight children have a greater proportion of fat mass relative to muscle mass in the upper limbs than in the lower limbs: implications for bone strength at the distal forearm. *The American Journal Of Clinical Nutrition* 2009;90(4):1104-11.

Pittas AG, Harris SS, Eliades M, Stark P, Dawson-Hughes B. Association between serum osteocalcin and markers of metabolic phenotype. *The Journal Of Clinical Endocrinology And Metabolism* 2009;94(3):827-32.

Cao JJ. Effects of obesity on bone metabolism. *Journal Of Orthopaedic Surgery And Research* 2011;6:30-.

Sheu Y, Cauley JA. The role of bone marrow and visceral fat on bone metabolism. *Current Osteoporosis Reports* 2011;9(2):67-75.

Rosen CJ, Klibanski A. Bone, fat, and body composition: evolving concepts in the pathogenesis of osteoporosis. *The American Journal Of Medicine* 2009;122(5):409-14.

Hill MJ, Kumar S, McTernan PG. Adipokines and the clinical laboratory: what to measure, when and how? *Journal Of Clinical Pathology* 2009;62(3):206-11.

Cirmanova V, Bayer M, Starka L, Zajickova K. The effect of leptin on bone: an evolving concept of action. *Physiological Research / Academia Scientiarum Bohemoslovaca* 2008;57 Suppl 1:S143-S51.

Shetty S, Kusminski CM, Scherer PE. Adiponectin in health and disease: evaluation of adiponectin-targeted drug development strategies. *Trends In Pharmacological Sciences* 2009;30(5):234-9.

Russell M, Mendes N, Miller KK, et al. Visceral fat is a negative predictor of bone density measures in obese adolescent girls. *The Journal Of Clinical Endocrinology And Metabolism* 2010;95(3):1247-55.

CHAPTER 3
OBESITY, TRABECULAR MICROARCHITECTURE AND CORTICAL BONE STRENGTH
IN LATE ADOLESCENT FEMALES

To be submitted to the Journal of Bone and Mineral Research.

Goff, H.L.¹, Modlesky, C.M.², Laing, E.M.¹, Pollock, N.K.³, Baile, C.A.¹, Lewis R.D.¹

¹Department of Foods and Nutrition, The University of Georgia, Athens, GA, USA ²Department of Kinesiology, The University of Delaware, Newark, DE, USA

³Department of Pediatrics, Prevention Institute, Georgia Health Sciences University, Augusta, GA, USA

OBESITY, TRABECULAR MICROARCHITECTURE AND CORTICAL BONE STRENGTH
IN LATE ADOLESCENT FEMALES

Goff, H.L.¹, Modlesky, C.M.², Laing, E.M.¹, Pollock, N.K.³, Baile, C.A.¹, Lewis R.D.¹

¹Department of Foods and Nutrition, The University of Georgia, Athens, GA, USA ²Department of Kinesiology, The University of Delaware, Newark, DE, USA

³Department of Pediatrics, Prevention Institute, Georgia Health Sciences University, Augusta, GA, USA

Running Title: OBESITY, TRABECULAR AND CORTICAL BONE STRENGTH

Emails:

hlgoft@uga.edu

modlesky@UDel.Edu

emonk@uga.edu

NPOLLOCK@georgiahealth.edu

cbaile@uga.edu

rlewis@fcs.uga.edu

Correspondence and reprint requests should be addressed to: Richard D. Lewis, Ph.D., Room 279 Dawson Hall, Department of Foods and Nutrition, The University of Georgia, Athens, GA 30602. Phone: 706-542-4901. Fax: 706.542.5059. Email: rlewis@fcs.uga.edu

Abstract

The influence of excess body fat on bone strength is unclear. This cross-sectional study determined if adiposity influences bone strength in late adolescent white females. Participants, 18-19 years of age, were grouped into obese ($n = 12$) and normal-weight ($n = 12$) based on %fat, BMI/age% and waist circumference (WC). Participants from each group were matched for age and height. Apparent trabecular bone volume to total volume (appBV/TV), apparent trabecular number (appTbN), apparent trabecular thickness (appTbTh) and apparent trabecular separation (appTbSp) as well as cortical volume, polar moment of inertia (J), section modulus (Z), cross-sectional moment of inertia (CSMI), medullary volume, and total bone volume were determined by MRI (GE 3.0 Tesla). DXA (Discovery A) was used to assess bone area, bone mineral content and areal bone mineral density for total body, lumbar spine, total hip, femoral neck, trochanter and radius. Groups were compared using t-test and ANCOVA. Obesity was associated with lower appTbTh at the proximal tibia ($P < 0.03$), appBV/TV ($P < 0.002$) and appTbTh ($P < 0.03$) at the distal radius and higher measures of appTbSp at the distal radius ($P < 0.02$). No differences were seen at the mid-tibia or mid-radius for cortical bone or DXA outcomes. After adjustment for fat-free soft-tissue (FFST), appBV/TV and appTbTh at the distal radius, but not the proximal tibia, remained lower ($P < 0.009$) in obese participants, whereas, appTbN at the proximal tibia was higher ($P < 0.02$) with obesity. Adjusted cortical volume, J, Z, CSMI and total bone volume at the mid-tibia and cortical volume, J, Z, and CSMI at the mid-radius, were lower in obese participants. DXA bone outcomes were also lower in obese subjects following FFST adjustment. Significant inverse correlations existed between several obesity indicators and MRI and DXA bone outcome measures. These data suggest that obesity is associated with lower bone strength in late adolescent females. Lower trabecular bone outcomes in the distal radius, but not proximal

tibia, suggests a protective effect of weight bearing in the lower limbs in the presence of excess fat. Future studies should examine potential mechanisms and the clinical consequences.

Key words: Obesity, bone, trabecular, cortical, MRI

Introduction

For years, researchers have thought that excess body weight in the form of fat is advantageous to the skeleton due to the additional weight and mechanical loading effects it has on bones (Zaidi et al 2012). Recently, however, this hypothesis has been questioned. Adipocytes and osteoblasts are derived from common multipotential mesenchymal stem cells and it is believed that with obesity there is a preference towards the production of adipocytes, not osteoblasts, leading to reduced bone formation. Moreover, adipocytes produce inflammatory adipokines, which have been linked with impaired skeletal development (Cao 2011).

During the growing years, children and adolescents who are obese have been shown to have more fractures compared to non-overweight children (Dimitri et al 2010; Taylor et al 2006). The causality of the higher fracture rates is not known, but Dimitri et al (Dimitri et al 2010) found that obese children with a history of fracture had less bone mass when compared to obese children with no prior fracture. These fracture data are likely responsible for an increasing number of investigations seeking to better understand the fat-bone relationships in children and adolescents.

To date, the findings regarding the relationships between fat and bone strength in children are equivocal and may be linked to the use of different imaging methods, statistical approaches and populations studied. When examining bone mineral content (BMC) using dual-energy X-ray absorptiometry (DXA), adiposity is reported to be positively associated with bone outcomes when adjusted for height or fat-free soft tissue (FFST); (Clark et al 2006; Ellis et al 2003;

Goulding et al 2002; Goulding et al 2000; Hong et al 2010; Krug et al 2008). However, when correcting for body weight (Goulding et al 2002; Goulding et al 2000; Hong et al 2010), leg length or truncal height (Janicka et al 2007), fat is not advantageous to bone.

One limitation of using DXA to assess bone, however, is that it is unable to distinguish between cortical and trabecular bone or account for differences in bone size. This is important because bone strength depends not only on the material properties of bone, but also, the geometrical and microarchitectural qualities. When assessing bone strength using peripheral quantitative computed tomography (pQCT), fat mass (FM) was shown to be associated with greater trabecular and cortical bone strength at the radius and tibia in 7-10 year old children (Ducher et al 2009) and the tibia of 9-11 year olds (Wetzsteon et al 2008), when FFST is not considered. Fat mass was also shown to be a strong stimulus for accrual of cortical bone mass in girls 15 years of age (Sayers and Tobias 2010). Conversely, when controlling for muscle cross-sectional area (MCSA), a surrogate of FFST, distal radius (4%) cross-sectional area and cortical thickness were lower in obese than normal-weight children (Ducher et al 2009). When controlling for MCSA and limb length in late adolescent females, FM was negatively associated with radial and tibial cortical area, total area, cortical BMC, periosteal circumference and strength-strain index (SSI), a valid measure of cortical bone strength, at the 20% site (Pollock et al 2007). Also, the late adolescent females in the high-fat vs normal-fat group had significantly lower SSI, a valid measure of bone strength, at the 20% site of the tibia and radius than the late adolescent females in the normal fat (NF) group. No significant relationships were found between fat and bone measures at the 4% site, a site of primarily trabecular bone (Pollock et al 2007). Wetzsteon et al (Wetzsteon et al 2008) suggests that bone does not adapt to excess fat, but to the muscle and accounting for FFST is essential in studies of adiposity and bone. It appears

that bone located at both metaphyseal and diaphyseal bone sites made up of predominantly trabecular and cortical bone, respectively are affected by excess body fat (Ducher et al 2009; Pollock et al 2007; Pollock et al 2011; Wetzsteon et al 2008).

Specific skeletal sites may be affected differently by fat depending on whether they are a weight bearing or non-weight bearing skeletal site (Shapses SA 2012). Although Pollock et al (Pollock et al 2007; Pollock et al 2011) showed no limb differences with respect to fat influences on tibial or radial strength, Ducher et al (Ducher et al 2009) reported differences in radial and tibial trabecular and geometrical properties in obese children, most likely due to the greater fat to muscle ratio in the forearm than in the tibia.

To date, the trabecular bone outcomes reported with respect to the fat-bone link have been limited to area and density measures assessed by pQCT. To our knowledge, there are no published studies that have examined the impact of obesity on trabecular microarchitecture using magnetic resonance imaging (MRI). The purpose of the current study was to determine if adiposity influences bone strength in adolescents by examining radial and tibial trabecular architecture using MRI in obese and normal-weight 18-19 year old white females.

Materials and methods

Study design and participants

The study design was cross-sectional, comparing two groups of Caucasian females with normal (n=12) and high (n=12) body fat. Participants were 18 to 19 years of age and enrolled at The University of Georgia. Subjects were recruited through presentations in classes, email listservs, announcements on websites, and campus fliers. A telephone pre-screening questionnaire was used to determine initial eligibility based on self-reported chronological age, onset of menarche, duration of oral contraceptive use, height and weight (and calculated BMI-

for-age-percentile from reported anthropometry), weight history, eating disorder diagnosis, chronic diseases, and use of medications or herbal supplements known to affect bone metabolism. Females were excluded if they reported significant weight loss or gain in the past 6 months ($\pm 10\%$ initial body weight), participated in Division-I college athletics, had been diagnosed with an eating disorder, had irregular menstrual cycle or had not reached menarche, had a chronic disease, or reported use of medications or herbal supplements known to affect bone metabolism.

If self-reported inclusion criteria were met, participants attended a second screening session, in which anthropometric measurements (height, weight, and waist circumference [WC]) and DXA-assessed % body fat (%BF) were obtained. BMI-for-age percentile was calculated using Epi Info software (v. 3.5.3). Females were excluded if their BMI was $< 20^{\text{th}}$ percentile or between the 80^{th} - 90^{th} percentile. A third inclusion criterion was waist circumference (WC), where subjects had to fall either between the 25^{th} - 75^{th} percentile or $\geq 90^{\text{th}}$ percentile. Obese females were defined as having $\text{BF} \geq 32\%$, BMI-for-age $> 90^{\text{th}}$ percentile and $\text{WC} \geq 90^{\text{th}}$ percentile and normal-weight females were defined as having $\text{BF} < 30\%$, BMI-for-age between the 20^{th} - 79^{th} percentile and WC between the 25^{th} - 75^{th} percentile. Height and muscle mass are strong predictors of bone measurements in youth combined with the fact that obese individuals, in general, tend to be taller with greater levels of muscle mass than their normal-weight counterparts (Bachrach et al 1999). Therefore, we minimized these potential confounding effects on bone measurements in the group comparisons by initially matching those in the normal and high-fat groups for age, height and oral contraceptive (OC) use. Normal-weight participants were matched to obese participants within 6 months of their birthday, within 1 inch of their height, and for positive or negative OC use.

Sample size was calculated using SPSS (Sample Power, software version 2.0, Chicago, IL) and was estimated from adiposity and bone data previously collected in our laboratory (Modlesky et al 2008; Pollock et al 2007; Pollock et al 2011). In non-athletic females ranging in age from 18 to 22 years (Pollock et al 2007; Pollock et al 2011), pQCT-derived cortical bone structural measurements at the tibia were 4.1% to 13.2% lower in females with high vs normal levels of body fat. From MRI-derived trabecular bone data collected in non-athletic females, aged 19-25 years (Modlesky et al 2008), the mean \pm SD tibial microarchitectural bone values for apparent trabecular bone volume to total volume (appBV/TV), apparent trabecular number (appTbN; mm^{-1}), apparent trabecular thickness (appTbTh; mm) and apparent trabecular separation (appTbSp; mm) were $0.274 \pm 0.032\%$, $1.309 \pm 0.084 \text{ mm}^{-1}$, $0.209 \pm 0.013 \text{ mm}$, and $0.561 \pm 0.065 \text{ mm}$, respectively (Modlesky et al 2008). Based on these trabecular bone microarchitecture data and the adiposity and pQCT-derived bone findings, we estimated that 7-12 subjects in each adiposity group would provide 80-85% power ($\alpha = 0.05$) to detect at least a 10% difference in trabecular appBV/ TV, appTbN, appTbTh and appTbSp at the tibia. Using these estimated sample sizes from our bone variables of interest, 12 subjects per adiposity group were recruited. Prior to the beginning of the study all protocols and procedures were approved by the Institutional Review Board for Human Subjects at The University of Georgia, and all participants provided written consent.

Anthropometrics

Participants' height, weight, radial length, and waist circumference were measured in light indoor clothing and without shoes. All measures were obtained twice and then averaged. If the two measures differed by 1 cm or 1 kg, a third measure was taken. Height was measured with the use of a wall-mounted stadiometer (Novel Products Inc, Rockton, IL) and rounded to the

nearest 0.1 cm. Body weight was measured with an electronic scale (Seca Bella 840, Columbia, MD) and rounded to the nearest 0.1 kg. Radial length and waist circumference were measured with anthropometric tape (Rosscraft, Inc, Surrey, Canada) and rounded to the nearest 0.10 mm. The radius was defined as the distance between the ulnar styloid process and olecranon. Waist circumference was determined by placing the tape at the top of the hipbone, encircling the waist so the tape was level with the navel and parallel to the floor. All height, weight, radius length and waist circumference measurements were performed by the same researcher.

Adipose tissue measures

DXA (Discovery A; Hologic Inc., Waltham, MA) was used to assess total body fat mass (FM; kg), fat-free soft-tissue mass (FFST; kg), and %BF. The same technician analyzed the scans using APEX software, version 3.3. Quality assurance for FM, FFST, and %BF were carried out by calibration against a three-step soft tissue wedge provided by Hologic, Inc., composed of different thickness levels of aluminum and Lucite, calibrated against stearic acid (100% fat) and water (8.6% fat). Reliability was determined using a one-way random effects model, single measure ICC in five females, aged 18 to 30 years, scanned twice in our lab during a seven day period for FM, FFST, and %BF (all $R \geq 0.87$; (Pollock et al 2007; Pollock et al 2011).

Diet and physical activity

Daily average intakes of energy, macronutrients, calcium and vitamin D were estimated using a three-day diet diary. Two weekdays and one weekend day were included and one trained operator analyzed the three-day diet records using FOOD PROCESSOR for WINDOWS software (version 10.8; 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 three-day diet records twice during a 2-week period. One-factor random effects model ICCs were computed for three-day energy intake ($R = 0.47$) and three-day calcium intake ($R = 0.71$); (Pollock et al 2007). Physical activity was assessed by an interviewer-administered 7-day physical activity recall questionnaire, valid in females within this age group (Washburn et al 2003). The questionnaire evaluated time spent sleeping and time spent performing moderate, hard, and very hard activities. Participants' average daily energy expenditure was estimated (in kcal/d; Appendix E; (Pollock et al 2007). A second physical activity questionnaire, the Bone Loading History Questionnaire, was used to assess lifetime participation in sports and activities that generate specific loads on the hip and spine. Taking into account a bone loading unit for each sport or exercise (based on ground reaction forces), the age of onset, seasons participated and years of participation and frequency, bone loading exposure scores for the hip and spine were generated. The bone loading exposure scores are significantly related to hip (0.317 ; $P < 0.004$) and spine (0.338 ; $P < 0.002$) aBMD values. ICCs for test-retest reliability were $R = 0.92$ ($P < 0.001$) for the hip and $R = 0.89$ ($P < 0.001$) for the spine (Dolan et al 2006).

Trabecular microarchitecture, cortical bone geometry and bone mass

Participants completed MRI scans performed on the nondominant tibia and radius at the Bio-imaging Research Center (BIRC) on The University of Georgia's campus. Trabecular and cortical bone images were acquired using a General Electric 16-channel fixed-site Signa HDx 3.0 Tesla MRI magnet. For the high-resolution (HR) trabecular bone scans, the forearm was placed in an 8-channel wrist coil (Invivo, Inc.). The distal radius was identified as being 7mm below from the radial plateau. Contiguous images of the distal radius, 0.5mm thickness, were acquired using a 3-D Fast Gradient Echo pulse sequence and 1 NEX. The average scan time for the distal radius was 8 min 20 sec. For the tibia, the lower-leg was placed in a single-channel phase array

knee coil (GE). Contiguous images of the proximal tibia, 1mm thickness, were acquired using a 3-D Fast Gradient Echo pulse sequence and 2 NEX. The average scan time for the proximal tibia was 12 min 40 sec. Trabecular bone microarchitecture, including trabecular appBV/TV, appTbN, appTbTh and appTbSp were determined using the procedure described by Majumdar et al (Majumdar et al 1997). Fifteen images representing the distal radius and proximal tibia images were analyzed using custom semi-automated software created with Interactive Data Language (IDL; Research Systems, Inc, Boulder, CO), as previously described (Modlesky et al 2008; Modlesky et al 2008). A low-pass filter-based correction was applied to images to eliminate inhomogeneity. Image signal intensity was inverted to facilitate visualization. Regions of interest containing trabecular bone and marrow were manually identified in each image. The coefficient of variation for test-retest reliability of appBV/TV, appTbN, appTbTh and appTbSp in the proximal tibia is 4.0, 3.3, 1.4 and 4.6%, respectively (Modlesky et al 2008).

The single-channel phase array knee coil was also used on cortical bone measurements at both the radius and tibia. The same protocol was used for both sites. Subjects placed their forearm or lower-leg into the coil at the 50% site where 25 slices with 0.6mm thickness and 0.6cm spacing were generated. A spin echo, 2 NEX, pulse sequence was used. The average scan time for the mid radius was 6 min 30 sec and the mid tibia was 5 min 30 sec. The 13th slice approximated the 50% site of the limb, as an equal number of slices were acquired below and above the 50% site. Cortical bone samples were taken from the cortical rim of each image and used as a calibration during the separation of pixels into bone and marrow phases. Images of the radial shaft and tibial shaft at the 50% level of each bone were analyzed using custom automated software created with IDL and a procedure similar to that previously described (Johnson et al 2009; Modlesky et al 2009). For each image, a gradient image was created using Sobel operators.

The optimal segmentation threshold was determined by maximizing the correlation between the original image and the gradient image. Images were then median filtered and segmented with a fuzzy C-means clustering algorithm (Suckling et al 1999). Regions representing cortical bone, the medullary cavity and adipose tissue were identified and automatically summed to determine their cross-sectional areas. The volume of each region was determined by accounting for image thickness and spacing between images. Mid-radius and mid-tibia total volumes were determined by summing cortical and medullary volumes. The mass of the adipose tissue in the mid-forearm and the mid-leg was determined by multiplying adipose tissue volume by 0.923 g/cm³, the estimated density of adipose tissue (Wang and Pierson 1976). Cross-sectional moment of inertia (CSMI) of the mid-radius and mid-tibia was determined in the anterior-posterior and medial-lateral directions using the parallel-axis theorem (Turner 2001) and the average value is reported. Polar moment of inertia (J) was calculated by summing CSMI in the two directions. Section modulus (Z) was calculated by dividing CSMI by the furthest distance from the neutral axis.

Bone area (BA), BMC, areal bone mineral density (aBMD) and corresponding Z-scores of the total body (TB), lumbar spine (LS), nondominant proximal femur (PF), femoral neck (FN), trochanter (TR) and nondominant radius (R) were assessed using the Discovery A DXA, software APEX version 3.3.

Statistical analyses

Normal distribution and homogeneity of variances were confirmed by Shapiro-Wilks W and Levene's tests, respectively. Group differences for anthropometric, body composition and unadjusted bone variables were determined using independent samples two-tailed t-tests. Analysis of covariance was used to compare the differences in bone response variables between the obese and normal-weight groups after adjusting for total body FFST. All data were analyzed

using SPSS software package (version 18.02; PASW Statistics, Chicago, IL) and statistical significance was set at $P < 0.05$. Descriptive statistics for raw variables and the estimated means of bone variables in the adjusted analyses are reported as means \pm SD.

Results

Participant characteristics

Participant characteristics are presented in **Table 3.1**. There were no differences in age, height or limb lengths. Mean BW, BMI, BMI/age%, WC, FFST, leg and forearm muscle masses, fat mass, %fat, and leg and forearm adipose tissue masses, were all significantly higher (all $P < 0.0001$) in the obese vs the normal-weight group. Vitamin D intakes were lower and energy expenditure (kcal/d) higher in obese vs normal-weight participants ($P < 0.05$). However, when expressed by kcal/kg bw/d, energy expenditure was not different between groups.

Bone measurement comparisons between obese and normal-weight groups before and after adjustment for total body FFST mass

MRI

The trabecular and cortical MRI-derived bone parameters are listed in **Table 3.2**. Proximal tibia appTbTh and distal radius appBV/TV and appTbTh were significantly lower and proximal radius appTbS was significantly higher in obese vs normal-weight participants. After controlling for total body FFST (TB FFST), the obese group vs normal-weight group had greater appTbN at the proximal tibia and lower appBV/TV and appTbTh at the distal radius. At the diaphysis (50% site), there were no significant differences between obese and normal-weight participants for mid-tibia or mid-radius bone measures. However, once adjusted for TB FFST, the cortical volume, J, Z and CSMI were all significantly lower in obese vs normal-weight

participants. Additionally, total bone volume was lower in the obese vs normal-weight participants at the mid-tibia.

DXA

Total body, PF, TR, FN, LS and R BA, BMC and aBMD are found in **Table 3.3**. There were no significant differences between groups in TB, PF, TR, FN, LS and R BA, BMC and aBMD. However, when corrected for TB FFST, TB and LS BA, BMC, aBMD were all significantly lower in the obese participants compared to the normal-weight participants. The PF, TR and R BA and BMC were also lower in obese vs normal-weight participants. Mean FN BMC and aBMD were lower in the obese vs the normal-weight group.

Bivariate correlations of BW, BMI, BMI/age%, waist circumference, total body FM, %fat, tibia or radius FM and FFST mass with bone measurements

Bivariate relations between MRI- and DXA-derived bone parameters and body weight, BMI, BMI/age%, WC, TB FM, %fat, leg or forearm adipose tissue mass and FFST mass are shown in **Table 3.4**. BMI, FM and %fat were significantly inversely related to appTbTh at the proximal tibia and distal radius, while BW was also inversely related to appTbTh at the proximal tibia. BW, BMI, BMI/age%, WC, FM, %fat, and leg and forearm adipose tissue mass were all inversely correlated with appBV/TV and positively related to appTbSp (except %fat) at the distal radius. FFST was positively related to appTbSp at the distal radius. No significant bivariate correlations were observed between any cortical bone outcomes and body weight, BMI, BMI/age%, WC, TB fat mass, leg or forearm adipose tissue mass and FFST mass. With respect to the DXA-derived bone, BW was positively correlated with TB BA and FFST was significantly correlated with TB BA, LS BMC, LS aBMD and radius BMC.

Partial correlations of BW, BMI, BMI/age%, waist circumference, total body FM, %fat and tibia or radius FM with bone measurements after adjustment for total body FFST

Partial correlations between MRI- and DXA-derived bone parameters and BW, BMI, BMI/age%, WC, TB FM, %fat and tibia or radius FM, adjusted for TB FFST, are found in **Table 3.5**. After adjustment for FFST, significant positive correlations were found for BW, WC and TB FM with appTbN at both the proximal tibia and the distal radius. At the distal radius, significant inverse relationships were found between BW, BMI/age%, WC, TB FM, and leg and forearm adipose tissue mass and appBV/TV and appTbTh. At the mid-tibia, BMI and BMI/age% were negatively correlated with cortical volume. Additionally, BMI and %fat were inversely related to J, Z and CSMI at the mid-tibia. Body weight, BMI, FM, and %fat were negatively associated with medullary volume and total bone volume. At the mid-radius similar associations were found. Waist circumference, FM and %fat were inversely correlated with cortical volume, while BW, BMI, WC, FM, %fat and leg or forearm adipose tissue mass were negatively associated with J, Z and CSMI. FM and %fat were inversely related to total bone volume. When corrected for TB FFST, BW was negatively associated with PF and TR BA. BMI, BMI/age% and WC were negatively associated with TB, LS, PF, and R BA and BMC. BMI was also negatively correlated with FN BA and BMC and TR BA. Waist circumference was negatively associated with TB aBMD, FN BMC and TR BA and BMC. Total body FM was negatively correlated with TB BA, PF BA and BMC, TR BA, and R BA and BMC, while %fat was negatively associated with TB BA, BMC and aBMD, PF BA and BMC, TR BMC and R BA and BMC.

Discussion

This is the first study to examine the microarchitectural, geometrical and material properties of bone with respect to adiposity in late adolescent females. Taking into account both

MRI- and DXA-derived bone measures, our results suggest that obese females have weaker bones than their normal-weight peers. Without statistical adjustment, obesity was associated with lower MRI measures of appTbTh at the proximal tibia and appBV/TV and appTbTh at the distal radius, and higher measures of appTbSp at the distal radius. Moreover, unadjusted cortical bone measures at the mid-tibia and mid-radius and BA, BMC and aBMD values assessed by DXA at all bone sites were similar between obese and normal-weight participants indicating that extra body weight is not advantageous to the skeleton. Once total body FFST was taken into account, numerous MRI and DXA bone indices were lower in obese vs normal-weight participants. For example, trabecular indices, appBV/TV and appTbTh at the distal radius, but not the proximal tibia, remained lower in obese participants, whereas, appTbN at the proximal tibia was significantly higher in obese vs normal-weight participants. Cortical indices, such as cortical volume, J, Z and CSMI at the mid-tibia and mid-radius, were lower in obese participants. The majority of the DXA bone outcomes were also lower in obese subjects following FFST adjustment, most notably BMC and aBMD of the lumbar spine and femoral neck. The statistically significant inverse correlations between several obesity indicators and MRI and DXA bone measures further support the belief that obesity is associated with lower bone strength.

Trabecular connectivity is a key factor associated with trabecular bone strength (Davison et al 2006) and of the trabecular variables reported in the current study, appTbN is best associated with connectivity. In the unadjusted analyses we found no differences in appTbN between the two groups, but when adjusted for FFST, contrary to what we hypothesized, appTbN was higher in the obese vs the normal-weight group at the proximal tibia, but not distal radius. Those findings combined with the fact that the distal radius appBV/TV and appTbTh

were lower and appTbSp greater in obese vs normal-weight subjects, may indicate compromised bone strength in unloaded limbs such as the forearm. For decades it has been thought that a protective influence of fat on bone is through additional body weight with obesity and greater sustained loading on bones in the lower extremities. It may be that trabecular microarchitecture is compromised in obese compared to normal-weight but that loading associated with a greater body weight attenuates this negative influence in the weight bearing limbs. Ducher et al (Ducher et al 2009; Pollock et al 2007) also reported weaker and smaller bones at the distal radius, but not at the distal tibia, after correction for MCSA in overweight compared to normal-weight males and females. Because the distal radius is a bone site associated with a high incidence of fractures in children and adolescents (Cooper et al 2004) negative effects of fat on trabecular bone at the distal radius could have significant health implications.

Prior studies that have examined relationships between adiposity and 3-dimensional bone strength and geometric indices in children and adolescents have used pQCT or CT. Because the metaphyseal bone variables acquired using these instruments, such as trabecular or cancellous density and total bone density differ from the three-dimensional characteristics acquired with MRI, interpreting and comparing data between studies using the two instruments should be done so cautiously. Pollock et al (Pollock et al 2007) studied a similar age group to the current study and found that after correcting for MCSA (a surrogate for muscle strength; (Petit et al 2005), there were no group differences between obese and normal-weight participants at the 4% site, suggesting that extra fat is not advantageous to areas of the skeleton high in trabecular bone. In contrast, other investigators have reported that overweight prepubertal children, 7-11 years of age, have larger and stronger bones than normal-weight peers at the 4% and 66% of the tibia and radius (Ducher et al 2009) or for most bone outcomes at the 8%, 50% or 66% site site of the tibia

(Wetzsteon et al 2008). In the study conducted by Ducher et al (Ducher et al 2009), the total area and cortical thickness at the distal radius were smaller in overweight vs normal-weight subjects after correcting for muscle cross-sectional area, but, trabecular density remained higher in overweight subjects. Why trabecular density remained higher in obese compared to normal-weight subjects is unclear. We found that distal radius appTbTh and appBV/TV were significantly lower in obese subjects compared to normal-weight subjects, whether or not we corrected for FFST. The differing trabecular bone results in prior studies (Ducher et al 2009; Wetzsteon et al 2008) compared to the current study may be related to the different methodologies used, the definition of obesity, and the potential fat exposure or duration of obesity. Peripheral QCT may not be sensitive enough to detect the differences in the 3-dimensional structure of trabecular bone vs the high resolution imaging with MRI. One strength of the current study was that subjects were matched for height and age and ensured that all obese subjects met %fat, waist circumference and BMI-for-age percentile criteria defining obesity. Both Ducher et al (Ducher et al 2009) and Wetzsteon et al (Wetzsteon et al 2008) used BMI to define their overweight populations. Because of the young age of prepubertal subjects in these studies, it could be hypothesized that the potential cumulative exposure to body fat would be far less than the older participants in the current study. Unlike results that showed higher tibia (66% site) cortical thickness, cortical area and BMC in overweight vs normal-weight subjects after adjustment for MCSA (Ducher et al 2009), our data demonstrate that once corrected for FFST most bone outcomes at the diaphysis or 50% site of the tibia and radius were lower in obese vs normal-weight participants. It is clear that cortical bone strength was attenuated in obese participants, once the 9kg of extra FFST in the obese group was accounted for. Similarly, Pollock et al (Pollock et al 2007) found that once cortical bone measures at the radius and tibia

were corrected for MCSA, the high vs normal fat group had lower cortical bone area and bone strength. One noteworthy aspect of our study is that few changes occurred at the metaphyses of the tibia and radius after the data were corrected for FFST. As FFST is one of the most important determinants of bone strength and bone mineral accrual during growth (Rauch et al 2004), it may be that muscle has differential effects on cortical and trabecular bone based on our findings.

The mechanisms by which fat may influence bone are unclear. The observed differences in trabecular bone at the radius and tibia support the idea that greater loading with a higher body weight attenuates the negative influences of fat on bone. However, other mechanisms may be involved. There has been recent interest in the location of fat and the hypothesis that central adiposity, specifically visceral adipose tissue (VAT), may be more detrimental to the skeleton than total body fat. Moreover, it has been suggested that bone marrow fat and muscle fat are also linked to lower bone strength (Bredella et al 2011; Farr et al 2011; Pollock et al 2011). A limitation of the current study is that we did not assess specific fat depots. Because we observed differences in bone strength between obese and normal-weight subjects, it would have been informative from a mechanistic standpoint to discern if specific fat depots were associated with the observed differences. Collection of serum samples for assessment of specific adipokines may have also been helpful in understanding mechanisms because VAT is associated with secretion of inflammatory adipokines. Future studies should take into account fat location and serum adipokines to better understand the metabolic consequences of excess fat on the skeleton.

One of the strengths of this study is that subjects were carefully matched for height and age, and that we ensured that the obese subjects met strict criteria for defining obesity. It is possible that factors other than adiposity may explain the differences in bone strength between the two groups, but it is unlikely. Hormonal differences may have contributed to bone

differences between groups; however, all subjects had reached menarche, were regularly menstruating and were matched based on oral contraceptive use and duration. Dietary intakes were similar between the two groups except for vitamin D, which was higher in the obese group. While physical activity is an important determinant of bone strength during growth, it is doubtful that differences in physical activity explain the bone differences between the groups, especially since there were no differences in energy expenditure (kcal/kg bw/d) and mean bone loading scores between groups.

The current study is the first to investigate the microarchitectural, geometrical and material properties simultaneously in a carefully controlled group of obese and normal-weight late adolescent white females. We hypothesized that excess adiposity would be associated with lower indices of trabecular microarchitecture and our data confirm this for the distal radius. Overall, the data suggest that obese late adolescent females have weaker bones than normal-weight late adolescent females. Future studies should explore the possibility that skeletal exposure to excess fat is cumulative and becomes more apparent in late adolescent and young adults as opposed to prepubertal children. Moreover, loading activities that target the forearm may be clinically relevant with respect to fracture prevention.

REFERENCES

- Zaidi M, Buettner C, Sun L, Iqbal J. Minireview: The link between fat and bone: does mass beget mass? *Endocrinology* 2012;153(5):2070-5.
- Cao JJ. Effects of obesity on bone metabolism. *Journal Of Orthopaedic Surgery And Research* 2011;6:30-.
- Dimitri P, Wales JK, Bishop N. Fat and bone in children: differential effects of obesity on bone size and mass according to fracture history. *J Bone Miner Res* 2010;25(3):527-36.

Taylor ED, Theim KR, Mirch MC, et al. Orthopedic complications of overweight in children and adolescents. *Pediatrics* 2006;117(6):2167-74.

Clark EM, Ness AR, Tobias JH. Adipose tissue stimulates bone growth in prepubertal children. *J Clin Endocrinol Metab* 2006;91(7):2534-41.

Ellis KJ, Shypailo RJ, Wong WW, Abrams SA. Bone mineral mass in overweight and obese children: diminished or enhanced? *Acta Diabetol* 2003;40 Suppl 1:S274-S7.

Goulding A, Taylor RW, Jones IE, Manning PJ, Williams SM. Spinal overload: a concern for obese children and adolescents? *Osteoporos Int* 2002;13(10):835-40.

Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, Williams SM. Overweight and obese children have low bone mass and area for their weight. *International Journal of Obesity* 2000;24(5):627-32.

Hong X, Arguelles LM, Liu X, et al. Percent fat mass is inversely associated with bone mass and hip geometry in rural Chinese adolescents. *J Bone Miner Res* 2010;25(7):1544-54.

Krug R, Carballido-Gamio J, Burghardt AJ, et al. Assessment of trabecular bone structure comparing magnetic resonance imaging at 3 Tesla with high-resolution peripheral quantitative computed tomography ex vivo and in vivo. *Osteoporos Int* 2008;19(5):653-61.

Janicka A, Wren TAL, Sanchez MM, et al. Fat mass is not beneficial to bone in adolescents and young adults. *J Clin Endocrinol Metab* 2007;92(1):143-7.

Ducher G, Bass SL, Naughton GA, Eser P, Telford RD, Daly RM. Overweight children have a greater proportion of fat mass relative to muscle mass in the upper limbs than in the lower limbs: implications for bone strength at the distal forearm. *The American Journal Of Clinical Nutrition* 2009;90(4):1104-11.

- Wetzsteon RJ, Petit MA, Macdonald HM, Hughes JM, Beck TJ, McKay HA. Bone structure and volumetric BMD in overweight children: a longitudinal study. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2008;23(12):1946-53.
- Sayers A, Tobias JH. Fat mass exerts a greater effect on cortical bone mass in girls than boys. *J Clin Endocrinol Metab* 2010;95(2):699-706.
- Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, Lewis RD. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. *Am J Clin Nutr* 2007;86(5):1530-8.
- Pollock NK, Laing EM, Hamrick MW, Baile CA, Hall DB, Lewis RD. Bone and fat relationships in postadolescent black females: a pQCT study. *Osteoporos Int* 2011;22(2):655-65.
- Shapses SA LR, Pollack N. Relationships between fat and bone in children and adults. Edition ed. In: Anderson JB GS, Klemmer PJ, ed. *Calcium and Phosphorus in Health and Disease*. Boca Raton, FL: CRC Press, 2012.
- Bachrach LK, Hastie T, Wang MC, Narasimhan B, Marcus R. Bone mineral acquisition in healthy Asian, Hispanic, black, and Caucasian youth: a longitudinal study. *The Journal Of Clinical Endocrinology And Metabolism* 1999;84(12):4702-12.
- Zin S. *Clinical methods: The history, physical, and laboratory examinations*. 3rd edition ed. Boston: Butterworths, 1990.
- Modlesky CM, Majumdar S, Dudley GA. Trabecular bone microarchitecture in female collegiate gymnasts. *Osteoporos Int* 2008;19(7):1011-8.

- Washburn RA, Jacobsen DJ, Sonko BJ, Hill JO, Donnelly JE. The validity of the Stanford Seven-Day Physical Activity Recall in young adults. *Medicine And Science In Sports And Exercise* 2003;35(8):1374-80.
- Dolan SH, Williams DP, Ainsworth BE, Shaw JM. Development and reproducibility of the bone loading history questionnaire. *Medicine And Science In Sports And Exercise* 2006;38(6):1121-31.
- Majumdar S, Genant HK, Grampp S, et al. Correlation of trabecular bone structure with age, bone mineral density, and osteoporotic status: in vivo studies in the distal radius using high resolution magnetic resonance imaging. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 1997;12(1):111-8.
- Modlesky CM, Subramanian P, Miller F. Underdeveloped trabecular bone microarchitecture is detected in children with cerebral palsy using high-resolution magnetic resonance imaging. *Osteoporos Int* 2008;19(2):169-76.
- Johnson DL, Miller F, Subramanian P, Modlesky CM. Adipose tissue infiltration of skeletal muscle in children with cerebral palsy. *The Journal Of Pediatrics* 2009;154(5):715-20.
- Modlesky CM, Kanoff SA, Johnson DL, Subramanian P, Miller F. Evaluation of the femoral midshaft in children with cerebral palsy using magnetic resonance imaging. *Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA* 2009;20(4):609-15.

- Suckling J, Sigmundsson T, Greenwood K, Bullmore ET. A modified fuzzy clustering algorithm for operator independent brain tissue classification of dual echo MR images. *Magnetic Resonance Imaging* 1999;17(7):1065-76.
- Wang J, Pierson RN, Jr. Disparate hydration of adipose and lean tissue require a new model for body water distribution in man. *The Journal Of Nutrition* 1976;106(12):1687-93.
- Turner CH, and D. B. Burr. *Experimental techniques for bone mechanics*. Boca Raton: CRC Press, 2001.
- Petit MA, Beck TJ, Kontulainen SA. Examining the developing bone: What do we measure and how do we do it? *Journal Of Musculoskeletal & Neuronal Interactions* 2005;5(3):213-24.
- Cooper C, Dennison EM, Leufkens HGM, Bishop N, van Staa TP. Epidemiology of childhood fractures in Britain: a study using the general practice research database. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2004;19(12):1976-81.
- Rauch F, Bailey DA, Baxter-Jones A, Mirwald R, Faulkner R. The 'muscle-bone unit' during the pubertal growth spurt. *Bone* 2004;34(5):771-5.
- Bredella MA, Torriani M, Ghomi RH, et al. Vertebral bone marrow fat is positively associated with visceral fat and inversely associated with IGF-1 in obese women. *Obesity (Silver Spring, Md)* 2011;19(1):49-53.
- Farr JN, Funk JL, Chen Z, et al. Skeletal muscle fat content is inversely associated with bone strength in young girls. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2011;26(9):2217-25. doi: 10.1002/jbmr.414.

Pollock NK, Bernard PJ, Gutin B, Davis CL, Zhu H, Dong Y. Adolescent obesity, bone mass, and cardiometabolic risk factors. *The Journal Of Pediatrics* 2011;158(5):727-34.

Table 3.1 Participant characteristics by adiposity group

Variable	Total Sample ^a N = 24	Obese ^a n = 12	Normal-Weight ^a n = 12	P-Value ^b	η^2 ^c
Age (years)	19.0 ± .43	19.0 ± .49	19.1 ± .39	.945	.000
Height (cm)	166 ± 8.3	166 ± 8.2	166 ± 8.7	.821	.002
Weight (kg)	74.6 ± 17.0	88.9 ± 11.0	60.2 ± 6.0	.000	.742
BMI (kg/m ²)	27.0 ± 5.6	32.0 ± 2.5	22.0 ± 2.2	.000	.835
BMI-For-Age (%)	72.8 ± 25.7	94.9 ± 1.6	50.6 ± 17.5	.000	.777
Waist Circumference (cm)	88.5 ± 14.5	102 ± 6.0	75.2 ± 4.4	.000	.874
Tibia Length (cm)	37.7 ± 2.4	37.7 ± 2.5	37.7 ± 2.5	.974	.000
Radius Length (cm)	26.1 ± 1.4	26.4 ± 1.4	25.9 ± 1.5	.481	.023
Fat-Free Soft Tissue Mass (g)	48472 ± 7517	53869 ± 5270	43075 ± 5177	.000	.538
Leg Muscle Mass (g)	690 ± 96.0	571 ± 84.9	631 ± 107	.004	.320
Forearm Muscle Mass (g)	186 ± 21.4	164 ± 22.2	175 ± 24.1	.020	.222
Fat Mass (g)	24915 ± 10434	34026 ± 6584	15803 ± 1777	.000	.796
Body Fat (%)	31.7 ± 6.7	37.5 ± 3.5	25.9 ± 2.9	.000	.779
Leg Adipose Tissue Mass (g)	342 ± 121	438 ± 90.7	246 ± 48.1	.000	.656
Forearm Adipose Tissue Mass (g)	104 ± 48.6	143 ± 37.6	66.3 ± 18.4	.000	.644
Dietary Energy (Kcal)	1626 ± 488	1647 ± 536	1605 ± 462	.844	.002
Dietary Calcium (mg)	693 ± 277	667 ± 253	716 ± 307	.679	.008
Dietary Vitamin D (IU)	113 ± 81.8	78.8 ± 55.5	145 ± 91.0	.050	.171
Energy Expenditure (kcal)	2093 ± 526	2558 ± 315	1667 ± 221	.000	.749
Energy Expenditure (kcal/kg bw/d)	28.4 ± 2.2	29.1 ± 1.4	27.7 ± 2.7	.128	.102
Bone Loading Score	19.3 ± 11.3	18.5 ± 14.1	20.2 ± 8.5	.727	.006

^a Values are means ± SD

^b Tests of significance between adiposity groups are based on one-way ANOVA; Significantly different if $P \leq 0.05$ and are in bold font

^c Partial eta squared (η^2) values indicate effect sizes (small ≥ 0.10 , medium ≥ 0.25 , and large ≥ 0.40)

Table 3.2 Magnetic resonance imaging bone characteristics by adiposity group, controlling for total body fat-free soft tissue mass (FFST)

Bone Variable ^a	Unadjusted				Adjusted for Total Body FFST			
	Obese ^b n = 12	Normal-Weight ^b n = 12	P-Value ^{c,d}	η^2 ^e	Obese ^f n = 12	Normal-Weight ^f n = 12	P-Value ^{c,d}	η^2 ^e
<i>Trabecular Bone</i>								
<u>Proximal Tibia</u>								
appBV/TV	.358 ± .018	.368 ± .022	.212	.070	.362 ± .007	.364 ± .007	.870	.001
appTbN	1.63 ± .030	1.60 ± .040	.089	.126	1.64 ± .012	1.59 ± .012	.019	.236
appTbTh	.220 ± .010	.230 ± .011	.027	.204	.220 ± .004	.229 ± .004	.193	.079
appTbSp	.395 ± .016	.296 ± .021	.984	.000	.390 ± .007	.401 ± .007	.295	.052
<u>Distal Radius</u>								
appBV/TV	.388 ± .015	.409 ± .014	.002	.359	.386 ± .005	.411 ± .005	.009	.282
appTbN	1.55 ± .033	1.55 ± .064	.983	.000	1.58 ± .017	1.53 ± .017	.104	.121
appTbTh	.250 ± .012	.264 ± .017	.030	.196	.245 ± .005	.270 ± .005	.008	.288
appTbSp	.395 ± .012	.382 ± .013	.016	.238	.391 ± .005	.386 ± .005	.458	.026
<i>Cortical Bone</i>								
<u>Mid-Tibia</u>								
Cortical Volume	31.1 ± 5.56	33.4 ± 5.95	.332	.043	28.0 ± 1.84	36.4 ± 1.84	.011	.268
J	2.57 ± .561	2.72 ± .866	.617	.012	2.16 ± .228	3.13 ± .228	.019	.236
Z	1.02 ± .178	1.07 ± .253	.633	.011	.898 ± .067	1.19 ± .067	.015	.250

CSMI	1.28 ± .281	1.36 ± .433	.617	.012	1.08 ± .114	1.56 ± .114	.019	.236
Medullary Volume	18.9 ± 4.92	19.9 ± 5.23	.606	.012	17.6 ± 1.84	21.1 ± 1.84	.264	.059
Total Bone Volume	49.9 ± 8.01	53.3 ± 10.0	.367	.037	45.7 ± 3.01	57.6 ± 3.01	.026	.214
<u>Mid-Radius</u>								
Cortical Volume	6.65 ± .722	6.86 ± 1.13	.580	.014	6.06 ± .281	7.45 ± .281	.007	.299
J	.154 ± .026	.175 ± .049	.199	.074	.134 ± .013	.194 ± .013	.009	.281
Z	.123 ± .015	.139 ± .027	.082	.131	.111 ± .007	.150 ± .007	.003	.345
CSMI	.077 ± .013	.087 ± .024	.199	.074	.067 ± .006	.097 ± .006	.009	.281
Medullary Volume	2.50 ± .848	2.46 ± .708	.916	.001	2.51 ± .290	2.45 ± .290	.896	.001
Total Bone Volume	9.14 ± .976	9.33 ± 1.61	.739	.005	8.57 ± .449	9.90 ± .449	.088	.132

^a Bone variables: appBV/TV = apparent trabecular bone volume to total volume, appTbN = apparent trabecular number (mm⁻¹), appTbTh = trabecular thickness (mm), appTbSp = apparent trabecular separation (mm), cortical volume = cortical cross-sectional area (mm³), J = polar moment of inertia (cm³), Z = section modulus (cm⁴), CSMI = cross-sectional moment of inertia (cm⁴), Medullary Volume = medullary cross-sectional area (mm³), total bone volume = total bone cross-sectional area (mm³)

^b Values are means ± SD

^c Tests of significance between adiposity groups are based on one-way ANOVA

^d Significant differences are indicated if $P \leq 0.05$ and are in bold font

^e Partial eta squared (η^2) values indicate effect sizes (small ≥ 0.10 , medium ≥ 0.25 , and large ≥ 0.40)

^f Values are estimated marginal means \pm SE

Table 3.3 Dual-energy X-ray absorptiometry bone characteristics by adiposity group, controlling for total body fat-free soft tissue mass (FFST)

Bone Variable	Unadjusted				Adjusted for Total Body FFST			
	Obese ^a n = 12	Normal-Weight ^a n = 12	P-Value ^b	η^2 ^c	Obese ^d n = 12	Normal-Weight ^d n = 12	P-Value ^{b,e}	η^2 ^c
<u>Total Body</u>								
Bone Area (cm ²)	1978 ± 153	1975 ± 170	.961	.000	1837 ± 32.1	2115 ± 32.1	.000	.567
Bone Mineral Content (g)	2214 ± 262	2321 ± 348	.400	.032	1975 ± 75.2	2560 ± 75.6	.000	.510
Areal Bone Mineral Density (g/cm ²)	1.12 ± .07	1.17 ± .09	.114	.110	1.07 ± .03	1.21 ± .03	.003	.348
<u>Lumbar Spine</u>								
Bone Area (cm ²)	58.3 ± 6.6	59.8 ± 7.0	.578	.014	54.3 ± 2.1	63.8 ± 2.1	.012	.264
Bone Mineral Content (g)	62.0 ± 11.5	61.7 ± 11.4	.994	.000	53.9 ± 3.1	69.8 ± 3.1	.005	.319
Areal Bone Mineral Density (g/cm ²)	1.06 ± .12	1.03 ± .11	.486	.022	.985 ± .033	1.10 ± .03	.047	.175
<u>Proximal Femur</u>								
Bone Area (cm ²)	32.7 ± 4.55	33.7 ± 4.24	.593	.013	28.9 ± .91	37.5 ± .91	.000	.606
Bone Mineral Content (g)	33.5 ± 6.06	34.6 ± 6.67	.669	.008	28.0 ± 1.33	40.0 ± 1.33	.000	.589
Areal Bone Mineral Density (g/cm ²)	1.02 ± .117	1.01 ± .095	.950	.000	.970 ± .035	1.07 ± .04	.094	.128
<u>Femoral Neck</u>								
Bone Area (cm ²)	4.65 ± .90	4.70 ± .49	.860	.001	4.30 ± .24	5.05 ± .24	.070	.148
Bone Mineral Content (g)	4.15 ± .86	4.39 ± .74	.481	.023	3.58 ± .22	4.96 ± .22	.001	.415

Areal Bone Mineral Density (g/cm ²)	.900 ± .135	.933 ± .120	.533	.018	.844 ± .043	.989 ± .043	.054	.165
<u>Trochanter</u>								
Bone Area (cm ²)	9.90 ± 1.40	10.5 ± 1.49	.343	.041	8.84 ± .378	11.5 ± .38	.000	.471
Bone Mineral Content (g)	7.41 ± 1.25	8.00 ± 1.68	.345	.041	6.44 ± .423	8.97 ± .423	.002	.385
Areal Bone Mineral Density (g/cm ²)	.752 ± .106	.758 ± .075	.867	.001	.734 ± .033	.777 ± .033	.447	.028
<u>Radius</u>								
Bone Area (cm ²)	12.8 ± 1.29	13.2 ± 1.46	.471	.024	11.8 ± .37	14.2 ± .37	.001	.408
Bone Mineral Content (g)	7.36 ± .93	7.62 ± 1.15	.548	.017	6.52 ± .25	8.46 ± .25	.000	.523
Areal Bone Mineral Density (g/cm ²)	.576 ± .048	.575 ± .046	.990	.000	.553 ± .015	.599 ± .015	.086	.134

^a Values are means ± SD

^b Tests of significance between adiposity groups are based on one-way ANOVA

^c Partial eta squared (η^2) values indicate effect sizes (small ≥ 0.10 , medium ≥ 0.25 , and large ≥ 0.40)

^d Values are estimated marginal means ± SE

^e Significant differences are indicated if $P \leq 0.05$ and are in bold font

Table 3.4 Bivariate correlations of magnetic resonance imaging-assessed bone outcomes with measures of adiposity and fat-free soft tissue mass (FFST)^a

Bone Variable ^b	Weight		BMI		BMI Percentile		Waist Circumference		Total Body Fat Mass		Total Body Percent Fat		Leg or Forearm Adipose Tissue Mass ^c		FFST	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
<i>Trabecular Bone</i>																
<u>Proximal Tibia</u>																
appBV/TV	-.318	.130	-.251	.236	-.168	.432	-.226	.288	-.290	.169	-.228	.285	-.078	.716	-.329	.117
appTbN	.205	.335	.352	.092	.277	.190	.279	.187	.283	.180	.388	.061	.363	.082	.034	.876
appTbTh	-.444	.030	-.436	.033	-.312	.138	-.377	.070	-.451	.027	-.432	.035	-.256	.227	-.374	.072
appTbSp	.109	.612	-.014	.947	-.036	.869	.009	.968	.052	.809	-.041	.849	-.131	.542	.201	.346
<u>Distal Radius</u>																
appBV/TV	-.492	.015	-.551	.005	-.531	.008	-.539	.007	-.560	.004	-.582	.003	-.546	.006	-.357	.087
appTbN	-.115	.593	.019	.930	-.003	.988	-.008	.969	.035	.870	.204	.339	.002	.992	-.313	.137
appTbTh	-.308	.114	-.419	.042	-.389	.060	-.396	.056	-.433	.035	-.531	.008	-.403	.051	-.109	.612
appTbSp	.486	.016	.428	.037	.436	.033	.440	.032	.420	.041	.303	.150	.441	.031	.538	.007
<i>Cortical Bone</i>																
<u>Mid-Tibia</u>																
Cortical Volume	.062	.774	-.205	.337	-.181	.398	-.020	.924	-.037	.862	-.197	.355	-.054	.801	.189	.298
J	.128	.550	-.102	.636	-.037	.862	.052	.809	-.004	.983	-.183	.391	-.124	.563	.286	.176

Z	.153	.476	-.081	.705	-.028	.898	.066	.758	.023	.915	-.158	.461	-.117	.586	.302	.151
CSMI	.128	.550	-.102	.636	-.037	.862	.052	.809	-.004	.983	-.183	.391	-.124	.563	.286	.176
Medullary Volume	-.104	.628	-.247	.244	-.074	.730	-.086	.691	-.204	.339	-.301	.153	-.226	.288	.074	.732
Total Bone Volume	-.018	.933	-.267	.208	-.156	.467	-.060	.780	-.136	.526	-.292	.167	-.159	.457	.161	.452
<u>Mid-Radius</u>																
Cortical Volume	.163	.446	.025	.907	.044	.840	-.012	.954	.002	.993	-.184	.390	-.062	.775	.318	.130
J	-.058	.786	-.181	.397	-.030	.890	-.158	.460	-.201	.347	-.337	.108	-.250	.239	.117	.585
Z	-.121	.573	-.245	.249	-.098	.649	-.229	.282	-.254	.230	-.386	.062	-.284	.178	.050	.818
CSMI	-.058	.786	-.181	.397	-.030	.890	-.158	.460	-.201	.347	-.337	.108	-.250	.239	.117	.585
Medullary Volume	-.070	.744	-.029	.894	.102	.636	.002	.994	-.113	.599	-.106	.623	-.135	.530	.004	.985
Total Bone Volume	.076	.726	.001	.996	.091	.673	-.008	.971	-.065	.763	-.193	.365	-.123	.567	.230	.280

^a Pearson's bivariate correlations were used to examine associations of bone outcomes at the tibia and radius with measures of adiposity and fat-free soft tissue mass (FFST) in this sample ($N=24$). Correlations are significantly different if $P \leq 0.05$ and are in bold font.

^b Bone variables: appBV/TV = apparent trabecular bone volume to total volume, appTbN = apparent trabecular number (mm⁻¹), appTbTh = apparent trabecular thickness (mm), appTbSp = apparent trabecular separation (mm), cortical volume= cortical cross-sectional area (mm³), J = polar moment of inertia (cm³),

Z = section modulus (cm^4), CSMI = cross-sectional moment of inertia (cm^4), medullary volume = medullary cross-sectional area (mm^3), total bone volume = total bone cross-sectional area (mm^3)

^c Leg adipose tissue mass measures are correlated with bone variables at the tibia and forearm adipose tissue mass measures are correlated with bone variables at the radius.

Table 3.5 Partial correlations of magnetic resonance imaging-assessed bone outcomes with measures of adiposity, adjusting for total body fat-free soft tissue mass^a

Bone Variable ^b	Weight		BMI		BMI Percentile		Waist Circumference		Total Body Fat Mass		Total Body Percent Fat		Leg or Forearm Adipose Tissue Mass ^c	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
<i>Trabecular Bone</i>														
<u>Proximal Tibia</u>														
appBV/TV	-.039	.859	.006	.977	.107	.627	.103	.640	-.048	.828	-.043	.845	.119	.587
appTbN	.459	.027	.517	.011	.366	.085	.470	.024	.428	.041	.457	.028	.407	.054
appTbTh	-.278	.199	-.248	.253	-.064	.772	-.122	.580	-.272	.210	-.282	.192	-.073	.739
appTbSp	-.206	.345	-.276	.203	-.268	.216	-.309	.152	-.185	.398	-.203	.353	-.285	.187
<u>Distal Radius</u>														
appBV/TV	-.455	.029	-.464	.026	-.423	.045	-.476	.022	-.490	.018	-.492	.017	-.443	.034
appTbN	.483	.020	.437	.037	.341	.112	.505	.014	.501	.015	.508	.013	.277	.201
appTbTh	-.548	.007	-.533	.009	-.453	.030	-.572	.004	-.580	.004	-.582	.004	-.437	.037
appTbSp	-.037	.866	.020	.928	.079	.720	-.034	.876	-.022	.920	-.023	.917	.150	.494
<i>Cortical Bone</i>														
<u>Mid-Tibia</u>														
Cortical Volume	-.303	.159	-.568	.005	-.469	.024	-.345	.107	-.321	.136	-.391	.065	-.186	.397
J	-.373	.080	-.535	.008	-.369	.083	-.370	.082	-.405	.055	-.456	.029	-.339	.114

Z	-.349	.102	-.525	.010	-.375	.078	-.372	.081	-.383	.071	-.439	.036	-.343	.110
CSMI	-.373	.080	-.535	.008	-.369	.083	-.370	.082	-.405	.055	-.456	.029	-.339	.114
Medullary Volume	-.455	.029	-.484	.019	-.186	.396	-.278	.199	-.440	.036	-.429	.041	-.313	.145
Total Bone Volume	-.446	.033	-.629	.001	-.400	.058	-.373	.080	-.448	.032	-.487	.019	-.292	.176
<u>Mid-Radius</u>														
Cortical Volume	-.364	.088	-.371	.081	-.286	.186	-.557	.006	-.445	.034	-.487	.018	-.364	.088
J	-.443	.034	-.435	.038	-.168	.444	-.486	.019	-.495	.016	-.508	.013	-.425	.043
Z	-.440	.036	-.450	.031	-.194	.375	-.509	.013	-.491	.017	-.517	.012	-.412	.051
CSMI	-.443	.034	-.435	.038	-.168	.444	-.486	.019	-.491	.016	-.508	.013	-.425	.043
Medullary Volume	-.195	.372	-.050	.819	.143	.515	-.003	.988	-.194	.375	-.134	.542	-.179	.414
Total Bone Volume	-.371	.081	-.289	.181	-.113	.608	-.390	.066	-.427	.042	-.420	.046	-.361	.090

^a Partial Pearson's correlations were used to examine associations of bone outcomes at the tibia and radius with measures of adiposity, adjusting for total body fat-free soft tissue mass in this sample ($N=24$). Correlations are significantly different if $P \leq 0.05$ and are in bold font.

^b Bone variables: appBV/TV = apparent trabecular bone volume to total volume, appTbN = apparent trabecular number (mm⁻¹), appTbTh = apparent trabecular thickness (mm), appTbSp = apparent trabecular separation (mm), cortical volume = cortical cross-sectional area (mm³), J = polar moment of inertia (cm³),

Z = section modulus (cm^4), CSMI = cross-sectional moment of inertia (cm^4), medullary volume = medullary cross-sectional area (mm^3), total bone volume = total bone cross-sectional area (mm^3)

^c Leg adipose tissue mass measures are correlated with bone variables at the tibia and forearm adipose tissue mass measures are correlated with bone variables at the radius.

CHAPTER 4

SUMMARY AND CONCLUSIONS

Obesity is a serious disorder in children and adults that leads to metabolic abnormalities and common chronic diseases like type 2 diabetes, hypertension and cardiovascular disease. One condition that is now being linked with obesity is osteoporosis and osteoporotic fractures. In fact obesity does contribute to poor bone health, with an increasing number of children and adolescents classified as obese, there will be serious concerns about long-term health, quality of life and health care costs issues. While there are still many unanswered questions regarding obesity and bone, this thesis project was intended to contribute to that knowledge base by utilizing high resolution imaging technology at the Bioimaging Research Center on the UGA campus to address the question of whether fat has negative effects on bone strength.

This is the first study to examine the microarchitectural, geometrical and material properties of bone with respect to adiposity in late adolescent females. Taking into account both MRI- and DXA-derived bone measures, our results suggest that obese females have weaker bones than their normal-weight peers. Without statistical adjustment, obesity was associated with lower MRI measures of appTbTh at the proximal tibia and appBV/TV and appTbTh at the distal radius, and higher measures of appTbSp at the distal radius. Moreover, unadjusted BA, BMC and aBMD values assessed by DXA were similar between obese and normal-weight participants at all bone sites indicating that extra body weight is not advantageous to the skeleton. Once total body FFST was taken into account, numerous MRI and DXA bone indices were lower in obese vs normal-weight participants. For example, trabecular indices, appBV/TV and appTbTh at the

distal radius, but not the proximal tibia, remained lower in obese participants, whereas, appTbN at the proximal tibia was significantly higher in obese vs normal-weight participants. Cortical indices, such as cortical volume, J, Z and CSMI at the mid-tibia and mid-radius, were lower in obese participants. The majority of the DXA bone outcomes were also lower in obese subjects following FFST adjustment, most notably BMC and aBMD of the lumbar spine and femoral neck. The statistically significant inverse correlations between several obesity indicators and MRI and DXA bone measures further support the idea that obesity is associated with lower bone strength.

Prior studies that have examined relationships between adiposity and 3-dimensional bone strength and geometric indices in children and adolescents have used pQCT or CT. Because the metaphyseal bone variables acquired using these instruments, such as trabecular or cancellous density and total bone density, differ from the three-dimensional characteristics acquired with MRI, interpreting and comparing data between studies using the two instruments should be done so cautiously. Pollock et al (Pollock et al 2007) studied a similar age group to the current study and found that after correcting for MCSA (a surrogate for muscle strength; (Petit et al 2005), there were no group differences between obese and normal-weight participants at the 4% site, suggesting that extra fat is not advantageous to areas of the skeleton high in trabecular bone. In contrast, other investigators have reported that overweight prepubertal children, 7-11 years of age, have larger and stronger bones than normal-weight peers at the 4% and 66% of the tibia and radius (Ducher et al 2009) or for most bone outcomes at the 8%, 50% or 66% site of the tibia (Wetzsteon et al 2008). In the study conducted by Ducher et al (Ducher et al 2009), the total area and cortical thickness at the distal radius were smaller in overweight vs normal-weight subjects after correcting for muscle cross-sectional area, but, trabecular density remained higher in

overweight subjects. Why trabecular density remained higher in obese compared to normal-weight subjects is unclear. We found that distal radius appTbTh and appBV/TV were significantly lower in obese subjects compared to normal-weight subjects, whether or not we corrected for FFST. The differing trabecular bone results in prior studies (Ducher et al 2009; Wetzsteon et al 2008) compared to the current study may be related to the different methodologies used, the definition of obesity, and the potential fat exposure or duration of obesity. Peripheral QCT may not be sensitive enough to detect the differences in the 3-dimensional structure of trabecular bone vs the high resolution imaging with MRI. One strength of the current study was that subjects were matched for height and age and ensured that all obese subjects met %fat, waist circumference and BMI-for-age percentile criteria defining obesity. Both Ducher et al (Ducher et al 2009) and Wetzsteon et al (Wetzsteon et al 2008) used BMI to define their overweight populations. Because of the young age of prepubertal subjects in these studies, it could be hypothesized that the potential cumulative exposure to body fat would be far less than the older participants in the current study.

For decades it has been thought that a protective influence of fat on bone is through additional body weight with obesity and greater loading on bones in the lower extremities. In the current study we observed limb differences in trabecular, but not cortical bone. Whether unadjusted or adjusted for FFST, the appBV/TV and appTbTh at the distal radius, but not proximal tibia, were lower in obese than normal-weight subjects. After adjustment for FFST, appTbN at the proximal tibia was higher in obese than normal-weight subjects and this may be reflective of sustained higher loads associated with higher fat and FFST masses with obesity. Ducher et al (Ducher et al 2009; Pollock et al 2007) also reported weaker and smaller bones at the distal radius, but not at the distal tibia, after correction for MCSA in overweight compared to

normal-weight males and females. Because the distal radius is a bone site associated with a high incidence of fractures in children and adolescents (Cooper et al 2004), negative effects of fat on trabecular bone at the distal radius could have significant health implications.

Unlike results that showed higher tibia (66% site) cortical thickness, cortical area and BMC in overweight vs normal-weight subjects after adjustment for MCSA (Ducher et al 2009), our data demonstrate that once corrected for FFST most bone outcomes at the diaphysis or 50% site of the tibia and radius were lower in obese vs normal-weight participants. It is clear that cortical bone strength was attenuated in obese participants, once the 9kg of extra FFST in the obese group was accounted for. Similarly, Pollock et al (Pollock et al 2007) found that once cortical bone measures at the radius and tibia were corrected for MCSA, the high vs normal fat group had lower cortical bone area and bone strength. One noteworthy aspect of our study is that few changes occurred at the metaphyses of the tibia and radius after the data were corrected for FFST. As FFST is one of the most important determinants of bone strength and bone mineral accrual during growth (Rauch et al 2004), it may be that muscle has differential effects on cortical and trabecular bone based on our findings.

The mechanisms by which fat may influence bone are unclear. The observed differences in trabecular bone at the radius and tibia support the idea that greater loading with a higher body weight attenuates the negative influences of fat on bone. However, other mechanisms may be involved. There has been recent interest in the location of fat and the hypothesis that central adiposity, specifically visceral adipose tissue (VAT), may be more detrimental to the skeleton than total body fat. Moreover, it has been suggested that bone marrow fat and muscle fat are also linked to lower bone strength (Bredella et al 2011; Farr et al 2011; Pollock et al 2011). A limitation of the current study is that we did not assess specific fat depots. Because we saw such

clear differences in bone strength between obese and normal-weight subjects, it would have been informative from a mechanistic standpoint to discern if specific fat depots, particularly VAT and bone marrow fat, were associated with the observed differences. Collection of serum samples for assessment of specific adipokines may have also been helpful in understanding mechanisms because VAT is associated with secretion of inflammatory adipokines. Future studies should take into account fat location and serum adipokines to better understand the metabolic consequences of excess fat on the skeleton.

One of the strengths of this study is that subjects were carefully matched for height and age, and that we ensured that the obese subjects met strict criteria for defining obesity. It is possible that factors other than adiposity may explain the differences in bone strength between the two groups, but it is unlikely. Hormonal differences may have contributed to bone differences between groups; however, all subjects had reached menarche, were regularly menstruating and were matched based on oral contraceptive use and duration. Dietary intakes were similar between the two groups except for vitamin D, which was higher in the obese group. While physical activity is an important determinant of bone strength during growth, it is doubtful that differences in physical activity explain the bone differences between the groups, especially since there were no differences in energy expenditure (kcal/kg bw/d) and mean bone loading scores between groups.

The current study is the first to investigate the microarchitectural, geometrical and material properties simultaneously in a carefully controlled group of obese and normal-weight late adolescent white females. We hypothesized that excess adiposity would be associated with lower indices of trabecular microarchitecture and our data confirm this at the radius. Overall, the data suggest that obese late adolescent females have weaker bones than normal-weight late

adolescent females. Future studies should explore the possibility that skeletal exposure to excess fat is cumulative and becomes more apparent in late adolescent and young adults as opposed to prepubertal children. Moreover, loading activities that target the forearm may be clinically relevant with respect to fracture prevention.

There were a couple of observations with respect to completing this thesis. One of the important aspects of conducting clinical work in this area is to carefully match participants by factors for factors that significantly impact bone. For example, we matched subjects by height, age and OC use. This additional work in the screening and recruitment phase of the study paid dividends during data collection and with respect to the novel findings. Hopefully, this study will lead to future work that will help us better understand the mechanisms related to adiposity and bone and the increasingly apparent cross communication between the two.

REFERENCES

Looker AC, Borrud LG, Dawson-Hughes BA, Shepherd JA, Wright NC. Osteoporosis or low bone mass at the femur neck or lumbar spine in older adults: United States, 2005-2008.

NCHS Data Brief 2012(93):1-8.

NOF. Internet: <http://www.nof.org/advocacy/resources/prevalencereport> (accessed June 18, 2012).

Dimitri P, Wales JK, Bishop N. Fat and bone in children: differential effects of obesity on bone size and mass according to fracture history. *J Bone Miner Res* 2010;25(3):527-36.

Dimitri P, Bishop N, Walsh JS, Eastell R. Obesity is a risk factor for fracture in children but is protective against fracture in adults: a paradox. *Bone* 2012;50(2):457-66.

Taylor ED, Theim KR, Mirch MC, et al. Orthopedic complications of overweight in children and adolescents. *Pediatrics* 2006;117(6):2167-74.

Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA: The Journal Of The American Medical Association* 2012;307(5):491-7.

Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity and trends in body mass index among US children and adolescents, 1999-2010. *JAMA: The Journal Of The American Medical Association* 2012;307(5):483-90.

Zaidi M, Buettner C, Sun L, Iqbal J. Minireview: The link between fat and bone: does mass beget mass? *Endocrinology* 2012;153(5):2070-5.

Cao JJ. Effects of obesity on bone metabolism. *Journal Of Orthopaedic Surgery And Research* 2011;6:30-.

Clark EM, Ness AR, Tobias JH. Adipose tissue stimulates bone growth in prepubertal children. *J Clin Endocrinol Metab* 2006;91(7):2534-41.

Ellis KJ, Shypailo RJ, Wong WW, Abrams SA. Bone mineral mass in overweight and obese children: diminished or enhanced? *Acta Diabetol* 2003;40 Suppl 1:S274-S7.

Goulding A, Taylor RW, Jones IE, Manning PJ, Williams SM. Spinal overload: a concern for obese children and adolescents? *Osteoporos Int* 2002;13(10):835-40.

Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, Williams SM. Overweight and obese children have low bone mass and area for their weight. *International Journal of Obesity* 2000;24(5):627-32.

Hong X, Arguelles LM, Liu X, et al. Percent fat mass is inversely associated with bone mass and hip geometry in rural Chinese adolescents. *J Bone Miner Res* 2010;25(7):1544-54.

Krug R, Carballido-Gamio J, Burghardt AJ, et al. Assessment of trabecular bone structure comparing magnetic resonance imaging at 3 Tesla with high-resolution peripheral

- quantitative computed tomography ex vivo and in vivo. *Osteoporos Int* 2008;19(5):653-61.
- Janicka A, Wren TAL, Sanchez MM, et al. Fat mass is not beneficial to bone in adolescents and young adults. *J Clin Endocrinol Metab* 2007;92(1):143-7.
- Ducher G, Bass SL, Naughton GA, Eser P, Telford RD, Daly RM. Overweight children have a greater proportion of fat mass relative to muscle mass in the upper limbs than in the lower limbs: implications for bone strength at the distal forearm. *The American Journal Of Clinical Nutrition* 2009;90(4):1104-11.
- Wetzsteon RJ, Petit MA, Macdonald HM, Hughes JM, Beck TJ, McKay HA. Bone structure and volumetric BMD in overweight children: a longitudinal study. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2008;23(12):1946-53.
- Sayers A, Tobias JH. Fat mass exerts a greater effect on cortical bone mass in girls than boys. *J Clin Endocrinol Metab* 2010;95(2):699-706.
- Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, Lewis RD. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. *Am J Clin Nutr* 2007;86(5):1530-8.
- Pollock NK, Laing EM, Hamrick MW, Baile CA, Hall DB, Lewis RD. Bone and fat relationships in postadolescent black females: a pQCT study. *Osteoporos Int* 2011;22(2):655-65.
- Shapses SA LR, Pollack N. Relationships between fat and bone in children and adults. Edition ed. In: Anderson JB GS, Klemmer PJ, ed. *Calcium and Phosphorus in Health and Disease*. Boca Raton, FL: CRC Press, 2012.

- Favus M. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 6th ed. ed. Washington, D.C.: American Society for Bone and Mineral Research, 2006.
- IOM IoM. Dietary Reference Intakes for Calcium and Vitamin D. Washington, D.C.: The National Academic Press, 2011.
- Seeman E. Bone quality: the material and structural basis of bone strength. *J Bone Miner Metab* 2008;26(1):1-8.
- Downey PA, Siegel MI. Bone biology and the clinical implications for osteoporosis. *Physical Therapy* 2006;86(1):77-91.
- Seeman E. Bone modeling and remodeling. *Critical Reviews In Eukaryotic Gene Expression* 2009;19(3):219-33.
- Lemaire V, Tobin FL, Greller LD, Cho CR, Suva LJ. Modeling the interactions between osteoblast and osteoclast activities in bone remodeling. *Journal Of Theoretical Biology* 2004;229(3):293-309.
- Hill PA. Bone remodelling. *British Journal Of Orthodontics* 1998;25(2):101-7.
- Clarke B. Normal bone anatomy and physiology. *Clinical Journal Of The American Society Of Nephrology: CJASN* 2008;3 Suppl 3:S131-S9.
- Nickols-Richardson SM, O'Connor PJ, Shapses SA, Lewis RD. Longitudinal bone mineral density changes in female child artistic gymnasts. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 1999;14(6):994-1002.
- Baxter-Jones ADG, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. *Journal Of Bone And*

- Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research 2011;26(8):1729-39. doi: 10.1002/jbmr.412.
- Recker RR, Heaney RP. Peak bone mineral density in young women. JAMA: The Journal Of The American Medical Association 1993;270(24):2926-7.
- Heaney RP, Abrams S, Dawson-Hughes B, et al. Peak bone mass. Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA 2000;11(12):985-1009.
- Matkovic V, Jelic T, Wardlaw GM, et al. Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. The Journal Of Clinical Investigation 1994;93(2):799-808.
- Recker RR, Davies KM, Hinders SM, Heaney RP, Stegman MR, Kimmel DB. Bone gain in young adult women. JAMA: The Journal Of The American Medical Association 1992;268(17):2403-8.
- Van der Meulen MC, Jepsen KJ, Mikić B. Understanding bone strength: size isn't everything. Bone 2001;29(2):101-4.
- Heaney RP. BMD: the problem. Osteoporos Int 2005;16(9):1013-5.
- Petit MA, Beck TJ, Kontulainen SA. Examining the developing bone: What do we measure and how do we do it? Journal Of Musculoskeletal & Neuronal Interactions 2005;5(3):213-24.
- Lloyd T, Beck TJ, Lin HM, et al. Modifiable determinants of bone status in young women. Bone 2002;30(2):416-21.
- Faulkner RA, Bailey DA, Drinkwater DT, Wilkinson AA, Houston CS, McKay HA. Regional and total body bone mineral content, bone mineral density, and total body tissue

- composition in children 8-16 years of age. *Calcified Tissue International* 1993;53(1):7-12.
- Sowers MF, Kshirsagar A, Crutchfield MM, Updike S. Joint influence of fat and lean body composition compartments on femoral bone mineral density in premenopausal women. *American Journal Of Epidemiology* 1992;136(3):257-65.
- Ducher G, Bass SL, Naughton GA, Eser P, Telford RD, Daly RM. Overweight children have a greater proportion of fat mass relative to muscle mass in the upper limbs than in the lower limbs: implications for bone strength at the distal forearm. *Am J Clin Nutr* 2009;90(4):1104-11.
- Petit MA, Beck TJ, Shults J, Zemel BS, Foster BJ, Leonard MB. Proximal femur bone geometry is appropriately adapted to lean mass in overweight children and adolescents. *Bone* 2005;36(3):568-76.
- Bonjour JP, Carrie AL, Ferrari S, et al. Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. *The Journal Of Clinical Investigation* 1997;99(6):1287-94.
- Boonen S, Vanderschueren D, Haentjens P, Lips P. Calcium and vitamin D in the prevention and treatment of osteoporosis - a clinical update. *Journal Of Internal Medicine* 2006;259(6):539-52.
- Cameron MA, Paton LM, Nowson CA, Margerison C, Frame M, Wark JD. The effect of calcium supplementation on bone density in premenarcheal females: a co-twin approach. *The Journal Of Clinical Endocrinology And Metabolism* 2004;89(10):4916-22.
- Greene DA, Naughton GA. Calcium and vitamin-D supplementation on bone structural properties in peripubertal female identical twins: a randomised controlled trial.

- Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA 2011;22(2):489-98.
- Lambert HL, Eastell R, Karnik K, Russell JM, Barker ME. Calcium supplementation and bone mineral accretion in adolescent girls: an 18-mo randomized controlled trial with 2-y follow-up. *The American Journal Of Clinical Nutrition* 2008;87(2):455-62.
- Lloyd T, Andon MB, Rollings N, et al. Calcium supplementation and bone mineral density in adolescent girls. *JAMA: The Journal Of The American Medical Association* 1993;270(7):841-4.
- Matkovic V, Goel PK, Badenhop-Stevens NE, et al. Calcium supplementation and bone mineral density in females from childhood to young adulthood: a randomized controlled trial. *The American Journal Of Clinical Nutrition* 2005;81(1):175-88.
- Matkovic V, Landoll JD, Badenhop-Stevens NE, et al. Nutrition influences skeletal development from childhood to adulthood: a study of hip, spine, and forearm in adolescent females. *The Journal Of Nutrition* 2004;134(3):701S-5S.
- Nowson CA, Green RM, Hopper JL, et al. A co-twin study of the effect of calcium supplementation on bone density during adolescence. *Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA* 1997;7(3):219-25.
- Dawson-Hughes B, Bischoff-Ferrari HA. Therapy of osteoporosis with calcium and vitamin D. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2007;22 Suppl 2:V59-V63.

Tang BMP, Eslick GD, Nowson C, Smith C, Bensoussan A. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet* 2007;370(9588):657-66.

Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *JAMA: The Journal Of The American Medical Association* 2005;293(18):2257-64.

Kremer R, Campbell PP, Reinhardt T, Gilsanz V. Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. *The Journal Of Clinical Endocrinology And Metabolism* 2009;94(1):67-73.

Kristinsson JO, Valdimarsson O, Sigurdsson G, Franzson L, Olafsson I, Steingrimsdottir L. Serum 25-hydroxyvitamin D levels and bone mineral density in 16-20 years-old girls: lack of association. *Journal Of Internal Medicine* 1998;243(5):381-8.

Breen ME, Laing EM, Hall DB, et al. 25-hydroxyvitamin D, insulin-like growth factor-I, and bone mineral accrual during growth. *The Journal Of Clinical Endocrinology And Metabolism* 2011;96(1):E89-E98.

El-Hajj Fuleihan G, Nabulsi M, Tamim H, et al. Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. *The Journal Of Clinical Endocrinology And Metabolism* 2006;91(2):405-12.

Outila TA, K√ § rkk√ § inen MU, Lamberg-Allardt CJ. Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: associations with forearm bone mineral density. *The American Journal Of Clinical Nutrition* 2001;74(2):206-10.

Viljakainen HT, Natri A-M, Kärkkäinen M, et al. A positive dose-response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2006;21(6):836-44.

Anderson JJ. The important role of physical activity in skeletal development: how exercise may counter low calcium intake. *The American Journal Of Clinical Nutrition* 2000;71(6):1384-6.

Laing EM, Massoni JA, Nickols-Richardson SM, Modlesky CM, O'Connor PJ, Lewis RD. A prospective study of bone mass and body composition in female adolescent gymnasts. *The Journal Of Pediatrics* 2002;141(2):211-6.

Pollock NK, Laing EM, Modlesky CM, O'Connor PJ, Lewis RD. Former college artistic gymnasts maintain higher BMD: a nine-year follow-up. *Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA* 2006;17(11):1691-7.

Bailey DA, McKay HA, Mirwald RL, Crocker PR, Faulkner RA. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 1999;14(10):1672-9.

Bakker I, Twisk JWR, Van Mechelen W, Roos JC, Kemper HCG. Ten-year longitudinal relationship between physical activity and lumbar bone mass in (young) adults. *Journal*

- Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research 2003;18(2):325-32.
- Baxter-Jones ADG, Kontulainen SA, Faulkner RA, Bailey DA. A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. *Bone* 2008;43(6):1101-7.
- Ianc D, Serbescu C, Bembea M, Benhamou L, Lespessailles E, Courteix D. Effects of an exercise program and a calcium supplementation on bone in children: a randomized control trial. *International Journal Of Sport Nutrition And Exercise Metabolism* 2006;16(6):580-96.
- Teegarden D, Proulx WR, Kern M, et al. Previous physical activity relates to bone mineral measures in young women. *Medicine And Science In Sports And Exercise* 1996;28(1):105-13.
- McKay H, Tsang G, Heinonen A, MacKelvie K, Sanderson D, Khan KM. Ground reaction forces associated with an effective elementary school based jumping intervention. *British Journal Of Sports Medicine* 2005;39(1):10-4.
- Dolan SH, Williams DP, Ainsworth BE, Shaw JM. Development and reproducibility of the bone loading history questionnaire. *Medicine And Science In Sports And Exercise* 2006;38(6):1121-31.
- Soyka LA, Fairfield WP, Klibanski A. Clinical review 117: Hormonal determinants and disorders of peak bone mass in children. *The Journal Of Clinical Endocrinology And Metabolism* 2000;85(11):3951-63.
- De Laet C, Kanis JA, Odén A, et al. Body mass index as a predictor of fracture risk: a meta-analysis. *Osteoporosis International: A Journal Established As Result Of Cooperation*

- Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA 2005;16(11):1330-8.
- Bhupathiraju SN, Dawson-Hughes B, Hannan MT, Lichtenstein AH, Tucker KL. Centrally located body fat is associated with lower bone mineral density in older Puerto Rican adults. *The American Journal Of Clinical Nutrition* 2011;94(4):1063-70.
- Bredella MA, Torriani M, Ghomi RH, et al. Determinants of bone mineral density in obese premenopausal women. *Bone* 2011;48(4):748-54.
- Bredella MA, Torriani M, Ghomi RH, et al. Vertebral bone marrow fat is positively associated with visceral fat and inversely associated with IGF-1 in obese women. *Obesity (Silver Spring, Md)* 2011;19(1):49-53.
- Lewiecki EM, Gordon CM, Baim S, et al. International Society for Clinical Densitometry 2007 Adult and Pediatric Official Positions. *Bone* 2008;43(6):1115-21.
- McKiernan FE, Berg RL, Linneman JG. The utility of BMD Z-score diagnostic thresholds for secondary causes of osteoporosis. *Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA* 2011;22(4):1069-77.
- Liu D, Manske SL, Kontulainen SA, et al. Tibial geometry is associated with failure load ex vivo: a MRI, pQCT and DXA study. *Osteoporos Int* 2007;18(7):991-7.
- Modlesky CM, Subramanian P, Miller F. Underdeveloped trabecular bone microarchitecture is detected in children with cerebral palsy using high-resolution magnetic resonance imaging. *Osteoporos Int* 2008;19(2):169-76.
- Pollock NK, Bernard PJ, Wenger K, et al. Lower bone mass in prepubertal overweight children with prediabetes. *Journal Of Bone And Mineral Research: The Official Journal Of The*

- American Society For Bone And Mineral Research 2010;25(12):2760-9. doi:
10.1002/jbmr.184.
- Pollock NK, Bernard PJ, Gutin B, Davis CL, Zhu H, Dong Y. Adolescent obesity, bone mass, and cardiometabolic risk factors. *The Journal Of Pediatrics* 2011;158(5):727-34.
- Reid IR. Adipose Tissue and Bone. *Clinic Rev Bone Miner Metab* 2009;7:207-9.
- Modlesky CM, Majumdar S, Dudley GA. Trabecular bone microarchitecture in female collegiate gymnasts. *Osteoporos Int* 2008;19(7):1011-8.
- Pittas AG, Harris SS, Eliades M, Stark P, Dawson-Hughes B. Association between serum osteocalcin and markers of metabolic phenotype. *The Journal Of Clinical Endocrinology And Metabolism* 2009;94(3):827-32.
- Sheu Y, Cauley JA. The role of bone marrow and visceral fat on bone metabolism. *Current Osteoporosis Reports* 2011;9(2):67-75.
- Rosen CJ, Klibanski A. Bone, fat, and body composition: evolving concepts in the pathogenesis of osteoporosis. *The American Journal Of Medicine* 2009;122(5):409-14.
- Hill MJ, Kumar S, McTernan PG. Adipokines and the clinical laboratory: what to measure, when and how? *Journal Of Clinical Pathology* 2009;62(3):206-11.
- Cirmanova V, Bayer M, Starka L, Zajickova K. The effect of leptin on bone: an evolving concept of action. *Physiological Research / Academia Scientiarum Bohemoslovaca* 2008;57 Suppl 1:S143-S51.
- Shetty S, Kusminski CM, Scherer PE. Adiponectin in health and disease: evaluation of adiponectin-targeted drug development strategies. *Trends In Pharmacological Sciences* 2009;30(5):234-9.

- Russell M, Mendes N, Miller KK, et al. Visceral fat is a negative predictor of bone density measures in obese adolescent girls. *The Journal Of Clinical Endocrinology And Metabolism* 2010;95(3):1247-55.
- Bachrach LK, Hastie T, Wang MC, Narasimhan B, Marcus R. Bone mineral acquisition in healthy Asian, Hispanic, black, and Caucasian youth: a longitudinal study. *The Journal Of Clinical Endocrinology And Metabolism* 1999;84(12):4702-12.
- Washburn RA, Jacobsen DJ, Sonko BJ, Hill JO, Donnelly JE. The validity of the Stanford Seven-Day Physical Activity Recall in young adults. *Medicine And Science In Sports And Exercise* 2003;35(8):1374-80.
- Majumdar S, Genant HK, Grampp S, et al. Correlation of trabecular bone structure with age, bone mineral density, and osteoporotic status: in vivo studies in the distal radius using high resolution magnetic resonance imaging. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 1997;12(1):111-8.
- Johnson DL, Miller F, Subramanian P, Modlesky CM. Adipose tissue infiltration of skeletal muscle in children with cerebral palsy. *The Journal Of Pediatrics* 2009;154(5):715-20.
- Modlesky CM, Kanoff SA, Johnson DL, Subramanian P, Miller F. Evaluation of the femoral midshaft in children with cerebral palsy using magnetic resonance imaging. *Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA* 2009;20(4):609-15.

- Suckling J, Sigmundsson T, Greenwood K, Bullmore ET. A modified fuzzy clustering algorithm for operator independent brain tissue classification of dual echo MR images. *Magnetic Resonance Imaging* 1999;17(7):1065-76.
- Wang J, Pierson RN, Jr. Disparate hydration of adipose and lean tissue require a new model for body water distribution in man. *The Journal Of Nutrition* 1976;106(12):1687-93.
- Turner CH, and D. B. Burr. *Experimental techniques for bone mechanics*. Boca Raton: CRC Press, 2001.
- Davison KS, Siminoski K, Adachi JD, et al. Bone strength: the whole is greater than the sum of its parts. *Seminars In Arthritis And Rheumatism* 2006;36(1):22-31.
- Cooper C, Dennison EM, Leufkens HGM, Bishop N, van Staa TP. Epidemiology of childhood fractures in Britain: a study using the general practice research database. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2004;19(12):1976-81.
- Rauch F, Bailey DA, Baxter-Jones A, Mirwald R, Faulkner R. The 'muscle-bone unit' during the pubertal growth spurt. *Bone* 2004;34(5):771-5.
- Farr JN, Funk JL, Chen Z, et al. Skeletal muscle fat content is inversely associated with bone strength in young girls. *Journal Of Bone And Mineral Research: The Official Journal Of The American Society For Bone And Mineral Research* 2011;26(9):2217-25. doi: 10.1002/jbmr.414.

APPENDICIES

APPENDIX A

Telephone Screening Questionnaire

Screening Phase 1: Telephone Questionnaire

Date: _____ Time: _____ Screen completed by: _____

- Participants' name: _____
- Participants' DOB: _____
- Participants' age: _____
- Participants' race: _____
- Approximately, how tall are you? _____ ft _____ in
- Approximately, how much do you weigh? _____ lbs
 - Calculate BMI: _____ kg/m^2 – plot on CDC growth chart – must either be between the 5th-85th or $\geq 95^{\text{th}}$ percentile BMI for age to qualify.
- Approximately, what size pants do you wear? _____ (for waist circumference)
- Have you lost or gained weight in the past 3 months? _____ YES _____ NO
 - If yes, how much? _____ lbsSignificant weight loss or gain in the past 6 mo is $\pm 10\%$ initial body weight
- Do you have any other significant weight changes, either weight loss or weight gain, in the past?

- Have you ever been diagnosed with an eating disorder? _____ YES _____ NO
 - If yes, what was it? _____
- Are you currently taking any medication? _____ YES _____ NO
 - If yes, what medication(s)? _____

(check approved and non-approved medication list)
- Please describe your menstruation history (irregular menstrual cycle or have not reached menarche): _____
- How many cycles do you currently have?
_____ ≥ 9 cycles/year _____ between 4-8 cycles/year _____ ≤ 3 cycles/year
- Are you currently taking any oral contraceptives (birth control)? _____ YES _____ NO
If yes, what is the name and duration of oral contraceptive use? _____

- Have you ever been diagnosed with any of the following disease/conditions?

- | | | | | |
|--|-------|-----|-------|----|
| <input type="radio"/> Bone Disease | _____ | YES | _____ | NO |
| <input type="radio"/> Diabetes | _____ | YES | _____ | NO |
| <input type="radio"/> High Blood Pressure | _____ | YES | _____ | NO |
| <input type="radio"/> High Cholesterol | _____ | YES | _____ | NO |
| <input type="radio"/> Renal Disease or Kidney Stones | _____ | YES | _____ | NO |
| <input type="radio"/> Cerebral Palsy | _____ | YES | _____ | NO |
| <input type="radio"/> Intestinal Malabsorption | _____ | YES | _____ | NO |
| <input type="radio"/> Juvenile Rheumatoid Arthritis | _____ | YES | _____ | NO |
| <input type="radio"/> Growth Disorders | _____ | YES | _____ | NO |
| <input type="radio"/> Thyroid Disease | _____ | YES | _____ | NO |
| <input type="radio"/> Zinc Malabsorption (e.g. acrodermatitis enteropathica) | _____ | YES | _____ | NO |
| <input type="radio"/> Psychological Illness | _____ | YES | _____ | NO |

- Have you ever, or are you currently participating in Division I college athletics?

_____YES _____NO

- In this study, all participants must provide 1 blood sample. Are you willing to do this?

_____YES _____NO

Collect the following information:

Address: _____

City: _____ Zip: _____

Phone Number: _____ (cell)

Email Address: _____

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_____

“This is 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 one 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 B

Anthropometric Data Sheet (session 2)

Bone Structure and Strength in Young Adult Females Study
Anthropometric Measurements

I. D. _____

Date: _____

Date of Birth: _____

Height:

1. _____ cm
2. _____ cm
3. _____ cm

Average: _____ cm _____ inches

Weight:

1. _____ pounds
2. _____ pounds
3. _____ pounds

Average: _____ pounds _____ kilograms

BMI Percentile:

Answer: _____

✓ if qualifies

Waist Circumference:

1. _____ cm
2. _____ cm
3. _____ cm

Average: _____ cm

✓ if qualifies

Forearm Length:

_____ cm ÷ 2.54 _____ inches

APPENDIX C

Anthropometric Data Sheet (session 3)

Bone Structure and Strength in Young Adult Females Study

I. D. _____

Date: _____

Blood Pressure

Left arm: _____ / _____ mmHg

Right arm: _____ / _____ mmHg

Forearm Length:

_____ cm

_____ cm

4%: _____ cm

66%: _____ cm

Leg Length:

_____ cm

_____ cm

4%: _____ cm

66%: _____ cm

APPENDIX D

Health History Questionnaire and Weight Chart

Bone Structure and Strength in Young Adult Females Study Health History Questionnaire

ID: _____ Age: _____ Date of Birth: _____

1. Do you smoke cigarettes now? ____ YES ____ NO
 - a. If yes, on the average, about how many cigarettes a day do you smoke now?
 ____ 1-5, ____ 6-14, ____ 15-24, ____ 25-35, ____ 35 or more
2. Have you ever smoked cigarettes? ____ YES ____ NO
 - a. If you used to smoke but do not smoke now, how long ago did you smoke?
 _____ years
 - b. On the average, about how many cigarettes a day did you smoke?
 ____ 1-5, ____ 6-14, ____ 15-24, ____ 25-35, ____ 35-more

3. Are you currently taking birth control? ____ YES ____ NO
 - a. If yes, what kind and what dose?

Name of Birth Control	Dose amount

- b. How old were you when you began using birth control pills? _____ years old
 - c. How long have you been using birth control pills? _____ years _____ months

4. Have you ever stopped using birth control pills? ____ YES ____ NO
 - a. If yes, please give name of birth control and start and end dates of birth control use.

Name of Birth Control	Start date of Birth Control	End Date of Birth Control

5. At what age did you start your menstrual cycle? _____ years old
6. Are your menstrual cycles regular? ____ YES ____ NO
 - a. If not, how many cycles do you have per year?
 ____ ≥ 9 cycles/year ____ between 4-8 cycles/year ____ ≤ 3 cycles/year

- b. How long have your cycles been irregular? _____year(s)
7. Are you taking any nutritional supplements? _____YES _____NO
- a. If yes, what kind and what dose?

Name of supplement	Dose amount

8. Are you currently dieting or on a special type of weight loss program (Weight Watchers, Atkins, self-regulating diet, etc.) ___YES ___NO
- a. If yes, what is the name of the program? _____
- b. If yes, how long have you been dieting/on the program? _____

9. Have you lost or gained weight in the past 6 months? _____YES _____NO
- a. If yes, how much? _____ lbs
(Significant weight loss or gain in the past 6 mo is $\pm 10\%$ initial body weight)

10. Do you have any other significant weight changes, either weight loss or weight gain, in the past? (see attached weight chart) _____

11. At what age did you reach your current weight? _____

12. Has any member of your family been diagnosed with osteoporosis? _____YES_____NO
- a. If yes, who? _____

13. List any fractures in your lifetime including the cause and your age at the time of fracture:

Fracture Site	Cause	Age at the time of Fracture

255																				
250																				
245																				
240																				
235																				
230																				
225																				
220																				
215																				
210																				
205																				
200																				
195																				
190																				
185																				
180																				
175																				
170																				
165																				
160																				
155																				
150																				
145																				
140																				
135																				
130																				
125																				
120																				
115																				
110																				
105																				
100																				
95																				
90																				
85																				
80																				
	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su
	Freshman HS				Sophomore HS				Junior HS				Senior HS				Freshman College			

Body Composition and Bone Strength Study - Weight History

Subject ID#: _____

APPENDIX E

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.

5. **Write down the date and day at the top of the form.**
6. **Write down the first foods you ate for that day. Write down:**
 8. The time of day you ate the food(s).
 9. Each food that you ate.
 10. How the food was prepared (baked, boiled, fried, microwaved).
 11. How much you ate (cup, 1/2 cup, pieces, tablespoons, teaspoons).
7. **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 there were in it and 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?**
4. **What is the complete name and brand name of bread that you eat most often?**

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

APPENDIX F

7-Day Physical Activity Recall

Subject Code No. _____

Date _____

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:

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

Household activities:

1. Raking the lawn
2. Sweeping and mopping
3. Mowing the lawn with a power mower
4. Cleaning windows

Sports Activities (Actual playing time)

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

Hard Activities

Occupational Tasks:

1. Heavy carpentry
2. Construction work – doing physical labor

Household Tasks:

1. Scrubbing floors

Sports Activities (Actual playing time):

1. Doubles tennis
2. Disco, Square, or Folk dancing

Very Hard Activity

Occupational Tasks:

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

Sports Activities (Actual playing time):

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

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:

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

APPENDIX G

Bone Loading History Questionnaire

Bone Structure and Strength in Young Adult Females Study Bone Loading History Questionnaire

ID: _____ Age: _____ Date of Birth: _____

1. Have you *ever* participated in any organized sports? (i.e. swimming, softball, soccer, basketball, gymnastics, ballet, cheerleading, track etc.)

Sport	Age of Onset	Season (W, Sp, Su, F)	Years of Participation	Frequency (day/mo/yr)
1.				
2.				
3.				
4.				
5.				
6.				

2. Have you *ever* participated in any recreational exercise? (i.e. hiking, spin classes, running, etc.)

Recreational Exercise	Age of Onset	Season (W, Sp, Su, F)	Years of Participation	Frequency (day/mo/yr)
1.				
2.				
3.				
4.				
5.				
6.				

3. Do you have any health problems that limit your physical activity? ___ YES ___ NO

a. If yes, what are they? _____

4. On average, how many hours of screen time (i.e. watching TV, on the computer, iPad, video games, cell phone, etc.) do you get per day? _____