A PROSPECTIVE ANALYSIS OF BODY FAT AND BONE MINERAL ACCRUAL DURING PUBERTAL GROWTH

by

KATHERINE LEIGH HARRIS

(Under the Direction of Richard D. Lewis)

ABSTRACT

This project sought to determine the relationship between accumulation of body fat and bone mineral content accrual in females followed over a period of up to 11 years from pre-pubertal (n = 203; aged 4 to 8 years) to pubertal maturation (n = 71; aged 13 to 19 years). Body fat percentage and BMC were measured using DXA. The slope of the regressions of each subject's repeated measurements over time was calculated for each subject for BMC, fat mass, and percent body fat. Pearson correlations and partial correlations, corrected for total body lean mass and height, were calculated. When adjusted for height and total body lean mass, percent body fat was positively related to BMC of the total body (R=0.52, P<0.0001), total proximal femur (R=0.35, P<0.0001), and non-dominant forearm (R=0.32, P=<0.0001). Similarly, total body fat mass was positively related to BMC of the total body (R=0.65, P<0.0001), total proximal femur (P=0.31, P<0.0001), and non-dominant forearm (R=0.34, P=<0.0001). These longitudinal data of females followed from childhood through adolescence indicate a significant positive impact of adiposity on BMC accrual.

INDEX WORDS: Fat mass, Percent body fat, Puberty, Bone mineral content accrual

A PROSPECTIVE ANALYSIS OF BODY FAT AND BONE MINERAL ACCRUAL DURING PUBERTAL GROWTH

by

KATHERINE LEIGH HARRIS

BS, The University of South Carolina, 2007

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

MASTER OF SCIENCE

ATHENS, GEORGIA

2010

A PROSPECTIVE ANALYSIS OF BODY FAT AND BONE MINERAL ACCRUAL DURING PUBERTAL GROWTH

by

KATHERINE LEIGH HARRIS

Major Professor: Richard D. Lewis

Committee: Clifton A. Baile
Jung Sun Lee

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May, 2010

DEDICATION

This thesis is dedicated to the friends and family who have never stopped supporting me in all my endeavors, academic and otherwise. To my mother, Beth Harris, who is still my biggest cheerleader; to my sister, Allison Kuenzli, and nephew, Grayson who have both helped keep my head up and my heart filled with love; and to Kris, my soldier, best friend, and source of inspiration and encouragement: thank you for understanding my dedication to this project and for supporting my passion for a professional career that will fulfill God's purpose for my life.

ACKNOWLEDGEMENTS

I am overwhelmed by the gracious support, guidance, and inspiration I've received while pursuing this project and collaborating with the entire Lewis Lab of UGA. Dr. Lewis, I'm awed by the balance you maintain between leadership in a demanding ever-changing, field and the relaxed, friendly, open-door mentorship you offer every student equally and unwaveringly. Thank you for pushing me to become better, to know more, and to strive for excellence instead of adequacy. I've realized that if I hadn't pushed myself to strive for excellence (and your approval), I would not have achieved nearly what I have over the past 2 years. I'll be indebted to you for a lifetime and words cannot express how grateful I am to have had your stewardship for my professional and academic careers. Emma, you are an unbelievable source of inspiration, wisdom, and compassion. There is no way I would have accomplished this project without your guidance and support. Thank you for always making time for me, both in regards to school and personal matters. The past two years have been more fun because of your infectious positive attitude, and I believe I'm a better person because I've learned from your helpfulness, your kindness, and your calmness in the midst of chaos. I will miss your cheerful smile that I can always bet on to help me get through a tough day. You have such a beautiful family, which has given me hope that I will be able to balance a professional and personal life successfully. You are a shining example of the person I want to be. I'm also grateful to have had the opportunity to share the lab with a great group of girls, Ashley, Claire, and Maria. Thanks for giving me comic relief (I'll never

forget your laugh Claire), including me in your social lives, and helping me get "Athenized". Also, Ruth and Jessica, you've made my time in the lab more fun and shown me how to get a job done right even when the odds are stacked against you. I envy your perseverance and organization. Lastly, I want to remind myself that, although the road was rough and the path less traveled, the journey here has made me a stronger, wiser, more resilient and well-rounded person with experiences that not many have the chance to gain in their lifetime. Thank you all for making that possible.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Osteoporosis is a worldwide public health concern associated with decreased quality of life and an increased morbidity and mortality for those afflicted with the disease.[1] This disease is characterized by increased bone porosity and fragility, decreased areal bone mineral density (aBMD) and strength, and elevated risk for fractures. Osteoporosis has been diagnosed in an estimated 10 million individuals in the U.S. and low aBMD is apparent in an additional 34 million Americans, who are at increased risk of developing the disease. Currently, approximately 19 billion healthcare dollars are spent for osteoporosis-related treatments, and these expenditures are expected to surpass 25 billion dollars by 2025.[2]

The impact of poor bone health is more commonly seen in older populations, usually culminating in a fracture that can heal sluggishly, causing unbearable pain, disability, deformities, and possibly fatal complications.[2] Disease-related fractures cause immobility, decreased efficiency in daily activities, loss of independence, and physical and emotional stress. Osteoporosis prevention and treatment will persistently be at the forefront of healthcare as the population of Americans older than 65 years, which is expected to double by 2050, continues to expand.[2]

The primary target age of osteoporosis prevention should be the pediatric years, when the rate of bone mineralization is greatest and bone has the capacity to alter its strength in response to environmental stimuli.[3] The growth period from childhood to

adolescence may be the most critical time to positively impact bone development through influential interventions, assuming positive gains in bone strength are sustained into adulthood.[4-6] Bone mineral acquisition reaches maximum velocity from 12 to 18 years of age[7] when approximately 90% of peak bone mass accrues.[8] Unfavorable alterations of bone modeling and remodeling could result in suboptimal skeletal development and, likely, an increased risk for osteoporosis.[3, 9] Peak bone mass is affected by the interaction of multiple factors encompassing both genetic[10] and environmental[11] determinants. Fully understanding the interactions between modifiable and non-modifiable factors on peak bone mass accretion is pertinent to osteoporosis prevention programs. For example, both genetic properties and environmental variables can significantly contribute to population variances in fat mass, fat-free mass and percent body fat, which are regarded as highly influential to optimal bone development.

Findings from cross-sectional studies that utilized dual energy X-ray absorptiometry (DXA)-derived aBMD or BMC in overweight youth have been mixed. Evidence of less than expected BMC at the total body[12,13] and lumbar spine[14] and a lower ratio of BMC to total body weight in obese vs. normal weight children[15] supports the theory that a higher level of body fat is unfavorable for the growing skeleton. Moreover, a negative association between BMC and fat mass has been shown in obese children with previous fractures.[16-18] Despite greater lean mass relative to height, obese children in a study by Dimitri et al.[16] had lower total body BMC compared to non-obese children without fractures. Furthermore, children with high body mass index (BMI), greater amounts of total body fat mass, or high percentage body fat have reported more first time[17-19] and repeated [20-22] fractures.

Conversely, several studies suggest that fat mass positively impacts both BMC and aBMD and that overweight and obese children have greater bone mass compared to normal weight peers when adjusting for height, maturation, or lean mass.[23-27] The statistical adjustments for either body weight, height, maturation, and/or fat-free mass and the use of aBMD as the primary outcome variable, may likely have contributed to these discrepant findings with respect to fat and bone. In addition, the synonymous reporting of aBMD and BMC may lead to misinterpretation of important differences among growing children. Bone mineral content and bone area are of greater reliability in childhood and adolescent bone studies because these measurements account for bone size and detect significant changes that can be overlooked by measures of aBMD alone.[28-29] Furthermore, the cross-sectional designs of these studies limit the observation of changes in body composition and bone mineral accrual before, during, and after the maturational period.

Few prospective studies exist in the literature that examine fat and bone gains during growth. Two prospective DXA investigations support a positive association between changes in total body fat mass and total body BMC accrual when data are unadjusted or are corrected for height, maturation, or lean mass.[30,31] Despite following subjects through the maturational period, the conclusions of Young et al.[30] are primarily supported by measures of aBMD, limiting the scope of implications for growing children. To date, the only BMC data reported have been at the total body, and did not show significant relationships with fat mass when height was accounted for during the growth phase.[30] Similarly, the prospective data from Clark et al.[31] report only total body (less head) BMC data, which showed positive relationships with fat mass in peripubertal

children, aged ~10 years. Additionally, this study grouped subjects by stages of breast development as outlined by Tanner[32] but limited the follow up period to two years. Therefore, the narrow age range captured in this study may have limited the potential to assess a wide range of pubertal maturation stages in relation to fat mass. Pubertal maturation may influence the effects of fat mass and bone if the positive relationship between fat mass and BMC becomes negative in more mature females.[31,33] The evidence of these studies is again limited by the absence of observation throughout the full maturational spectrum. Therefore, it is crucial to extend the period of observation from childhood throughout the pubertal growth phase and into post-menarche ages to collect evidence that explores the theory that pubertal maturation affects the degree and/or direction of influence of adiposity on BMC.

Questions still remain as to the effects of increased adiposity on BMC accrual throughout childhood growth and during puberty as previous studies have focused mainly on aBMD as measured by DXA with contradictory results. No previous studies to date have analyzed fat accumulation and BMC accrual at multiple anatomical sites prospectively throughout the maturational period from childhood to post-menarche. Furthermore, previous research has neglected to recognize important covariates, including height and/or lean mass, in the examination of the effects of fat mass on bone mineral accrual. Therefore, the purpose of this study is to investigate how increased adiposity affects BMC accrual during growth and to test the hypothesis that accumulation of higher levels of body fat during growth from childhood through puberty is associated with lower BMC accrual in females. With the exponential increase in childhood and adolescent obesity rates during the last three decades[34] it is crucial to establish the

effects of excess adiposity in youth. The evidence and conclusions of this research project will contribute to determining if high body fat is detrimental to bone mineral accrual during growth.

References

1. Vicente-Rodriguez G. How does Exercise Affect Bone Development during Growth? Sports Medicine 2006;7:561-9.

2. National Osteoporosis Foundation. Clinicians Guide to Prevention and Treatment of Osteoporosis. Washington, DC: 2008.

3. Carmona R, and Moritsugu, KP. The 2004 Surgeon General's Report on Bone Health and Osteoporosis: What is Means to You US Department of Health and Human Services, Office of the Surgeon General 2004.

4. Nurmi-Lawton JA, Baxter-Jones AD, Mirwald RL, et al. Evidence of sustained skeletal benefits from impact-loading exercise in young females: a 3-year longitudinal study. J Bone Miner Res 2004;19:314-22.

5. Kannus P, Haapasalo H, Sankelo M, et al. Effect of starting age of physical activity on bone mass in the dominant arm of tennis and squash players. Annals of Internal Medicine 1995;123:27-31.

6. 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. J Bone Miner Res 1999;14:1672-9.

7. Recker RR. Peak bone mineral density in young women. JAMA 1993;24:2926-7.

8. 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. J Clin Invest 1994;93:799-808.

9. Hui SL, Slemenda CW, Johnston CC. Age and bone mass as predictors of fracture in a prospective study. J Clin Invest 1988;81:1804-9.

10. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults. A twin study. J Clin Invest 1987;80:706-10.

11. Rizzoli R. Determinants of peak bone mass. Ann Endocrinol 2006;67:114-5.

12. 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. Int J Obes Relat Metab Disord 2000;24:627-32.

13. Weiler HA, Janzen L, Green K, Grabowski J, Seshia M, Yuen K. Percent body fat and bone mass in healthy Canadian females 10 to 19 years of age. Bone 2000;27:203-7.

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

15. Rocher E, Chappard C, Jaffre C, Benhamou CL, Courteix D. Bone mineral density in prepubertal obese and control children: relation to body weight, lean mass, and fat mass. J Bone Miner Metab 2008;26:73-8.

16. Dimitri P, Wales J, Bishop N. Fat and Bone in Children - Differential Effects of Obesity on Bone Size and Mass According to Fracture History. J Bone Miner Res 2009.

17. Goulding A, Cannan R, Williams SM, Gold EJ, Taylor RW, Lewis-Barned NJ. Bone mineral density in girls with forearm fractures. J Bone Miner Res 1998;13:143-8.

18. Goulding A, Jones IE, Taylor RW, Williams SM, Manning PJ. Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. J Pediatr 2001;139:509-15.

19. Skaggs DL, Loro ML, Pitukcheewanont P, Tolo V, Gilsanz V. Increased body weight and decreased radial cross-sectional dimensions in girls with forearm fractures. J Bone Miner Res 2001;16:1337-42.

20. Goulding A, Grant AM, Williams S. Bone and body composition of children and adolescents with repeated forearm fractures. J Bone Miner Res 2005;20:2090-6.

21. Goulding A, Jones IE, Taylor RW, Manning PJ, Williams SM. More broken bones: a 4-year double cohort study of young girls with and without distal forearm fractures. J Bone Miner Res 2000;15:2011-8.

22. Manias K, McCabe D, Bishop N. Fractures and recurrent fractures in children; varying effects of environmental factors as well as bone size and mass. Bone 2006.

23. Ellis KJ, Shypailo RJ, Wong WW, Abrams S. Bone mineral mass in overweight and obese children: diminished or enhanced? Acta Diabetol 2003;40 Suppl 1:S274-7.

24. Leonard MB, Shults J, Wilson BA, Tershakovec AM, Zemel BS. Obesity during childhood and adolescence augments bone mass and bone dimensions. Am J Clin Nutr 2004;80:514-23.

25. 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;36:568-76.

26. El Hage RC, CB. Jacob, C. Jaffre, C. . Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys. European Journal of Applied Physiology 2009:759-64.

27. El Hage RJ, C. Moussa, E. Benhamou, CL. Jaffre, C. Total body, lumbr spine and hip bone mineral density in overweight adolescent girls: decreased or increased? J Bone Miner Metab 2009;27:629-33.

28. Rockell JE, Williams SM, Taylor RW, Grant AM, Jones IE, Goulding A. Two-year changes in bone and body composition in young children with a history of prolonged milk avoidance. Osteoporos Int 2005;16:1016-23.

29. Petit MA, Beck TJ, Kontulainen S. Examining the developing bone: What do we measure and how do we do it? J Musculoskelet Neuronal Interact 2005;5:213-24.

30. Young D, Hopper JL, Macinnis RJ, Nowson CA, Hoang NH, Wark JD. Changes in body composition as determinants of longitudinal changes in bone mineral measures in 8 to 26-year-old female twins. Osteoporos Int 2001;12:506-15.

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

32. Tanner J. Growth and Adolescence. 2nd ed. Oxford: Blackwell Scientific Publications; 1962.

33. Petit MA, Beck TJ, Hughes JM, Lin HM, Bentley C, Lloyd T. Proximal femur mechanical adaptation to weight gain in late adolescence: a six-year longitudinal study. J Bone Miner Res 2008;23:180-8.

34. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. Jama 2006;295:1549-55.

CHAPTER 2

REVIEW OF LITERATURE

In order to better understand the basis for this project on pubertal growth, fat mass, and BMC accrual, background information on bone biology, bone growth, factors that determine optimal bone growth, adolescent bone development, and body composition changes will be presented. This chapter will focus on the effects of body composition change on bone mineral accrual during growth with particular attention to body fat accrual as a determinant of bone mineral acquisition.

Bone Biology

Anatomy

Structurally, bones are essential to the body, allowing it to move, providing mechanical support, and protecting organs. Physiologically, bone is imperative for blood cell production and to serve as a reservoir of minerals that are required to maintain homeostasis.[1] The anatomy of bone is complex whereby it serves as both an organ and a tissue and is comprised of three matrices: organic, inorganic, and a water component. The organic matrix contains Type 1 collagen fibers, noncollagenous proteins, and bone cells. This component establishes the majority of the structure, mechanics, and biochemical properties of bone. The inorganic phase of bone consists of the compound hydroxyapatite, which is a platelike crystal of calcium and phosphate surrounding collagen fibers. Crystalline hydroxyapatite harbors 98% of the body's calcium with the remaining 2% circulating in the blood and tissues.[2]

Bone Cells

The structure of bones and the skeleton endure lifelong adjustments through the synchronized effects of osteoblasts, i.e. cells that produce bone matrix, and osteoclasts, i.e. cells responsible for bone resorption.[3] Osteoblasts mature to become either bone lining cells or osteocytes deposited within small bone cavities.[4] Osteocytes interact with other osteocytes, bone lining cells, and osteoblasts at the bone surface, creating a complex network of bone matrix that provides communication between bone cells during growth.[3]

Types of Bone

The bones of the skeleton are divided into two groups, those of the axial skeleton and those of the appendicular skeleton. The axial skeleton includes the head and trunk while the appendicular skeleton is comprised of the limbs and the pelvic girdle. The skeleton can also be separated into long bones and flat bones. Long bones, which include the femur, tibia, and humerus, have three parts, the diaphysis, metaphysis, and epiphysis. Found at either end of long bones, the epiphysis is demarcated by a layer of growth cartilage. The metaphysis connects the epiphysis and diaphysis, found in the center, conjoining the wider head of the long bone to the slender bone shaft.[5]

The skeleton is comprised primarily of two bone types, cortical bone and cancellous bone. Cortical bone makes up approximately 80% of the skeletal mass while the other 20% is comprised of cancellous bone.[5] The densely calcified cortical bone tissue is found in the outer layers of most bones and along the shaft of long bones.[4] This type of bone consists of functional units named osteons that are comprised of collagen enclosed Haversian canals. The collagen is organized into concentric rings within the

osteons. Cavities within the collagen ring structures are termed lacunae, which house osteocytes. The Haversian canal, central within the osteon, is surrounded by blood vessels, nerve fibers, and lymphatic vessels. Osteocytes within the lacunae are capable of communicating with the Haversian canal through channels known as canaliculi (see Figure 1).[5]

Cancellous bone is also referred to as trabecular bone and is found in the axial skeleton, flat bones, and in both the metaphyses and epiphyses of long bones. In contrast to the osteon found in cortical bone, trabecular bone is composed of trabeculae, which are thin, calcified, honeycomb-like structures of bone tissue. The strength of trabecular bone is determined largely by its porosity and structure, which vary at different anatomic sites and with chronological age. The network of trabecular bone is metabolically active as it interacts with blood vessels, connective tissue, and bone marrow that are accessible in the pores created by the arrangement of its honeycomb-like structure.[5]

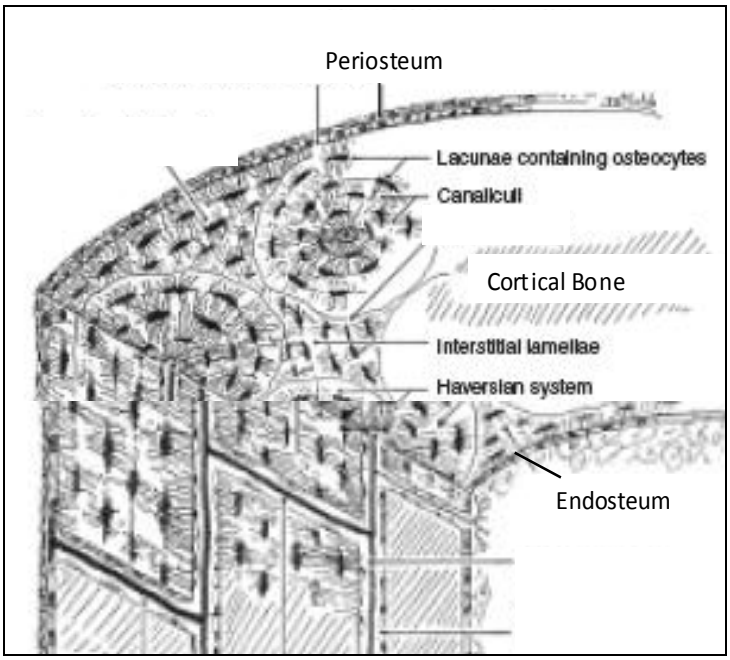


Figure 2.1 Structure of compact bone. Adapted from Ham.[6]

Bone Modeling and Remodeling

The structure of bones and the skeleton endure lifelong adjustments through the synchronized actions of bone modeling and remodeling. Bone modeling occurs during growth and consists of bone resorption and formation at separate anatomical sites resulting in changes in bone shape. Bone remodeling is a balance of removing bone and initiating new bone formation of the same site. Thus, the result should be no net gain of bone tissue if the coupling of resorption and formation are in balance (see Figure 2). Remodeling takes place to repair the microstructure of bone as fatigued bone is replaced with newly formed tissue in a process that is mediated by osteoclasts. Bone that is being remodeled is referred to as the basic structural unit while the cells that participate collectively are the basic multicellular unit. At any given time, approximately 20% of trabecular bone is being remodeled, and over an approximate two-year period, every bone surface is remodeled. With the conclusion of remodeling, osteoblasts are converted into osteocytes. Bone remodeling can be affected by environmental factors such as dietary intake and physical activity. The activity of osteoblasts and osteoclasts is hormonally influenced as well. If the homeostasis of remodeling becomes unbalanced and resorption and formation become uncoupled, bone is removed at a faster rate than it is replaced. Unbalanced bone resorption leads to bone loss, deterioration of bone structure, fragility, and fractures, as seen in those with osteoporosis.[7,8]

Bone modeling is predominant during growth or periods of bone healing during which new bone is deposited within existing bone. With bone modeling, resorption and formation occur at independent locations, which result in structural modifications that are

independent of the coupled activity of osteoblasts and osteoclasts. Bone modeling is controlled by genetic factors and can also be initiated in response to loading of bones.[3]

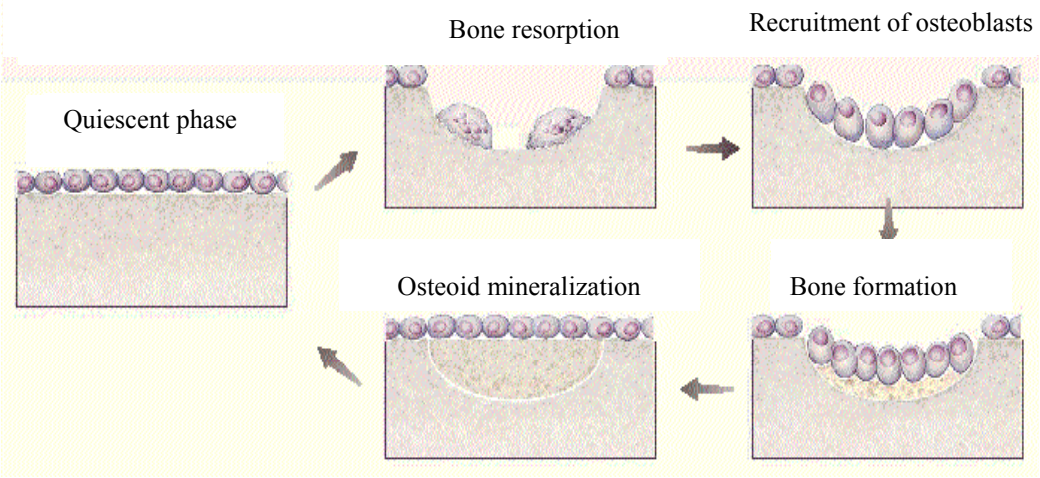


Figure 2.2 The bone remodeling process, involving the resorption of old bone by osteoclasts followed by formation of new bone my osteoblasts. Adapted from Manolagas.[9]

Bone Strength

The ability of bone to withstand mechanical strains is a function of both its material and structural properties. The material aspects of bone are made up of the organic and inorganic phases. The organic phase, consisting primarily of type 1 collagen, imparts tensile strength. The inorganic bone mineral aids in resisting compression. Collectively, the material properties of bone denote bone mass or BMC and areal bone mineral density (aBMD). Although bone mass is a single component of overall strength, it determines more than 80% of this parameter.[10] The geometric structure of bone is comprised of bone size, shape, cortical thickness, cross-sectional area, and trabecular architecture. Accruing bone on the periosteum increases bone strength for a given bone mass because an increase in bone strength is equal to the increase in bone radius raised to

the power of 4.[11] The geometrical changes in bone during growth are still under investigation. Growing bone must continually adapt its strength to the changes in body weight, bone length, and muscle forces that occur during pubertal growth.[12]

Bone Growth

Bone development occurs in two different realms, external growth of length and width, and internal growth as mineral is deposited and density increases. New bone is added on the exterior periosteum, increasing its size, or on the endosteum, increasing its density. The endosteal surface of bone is the inner bone layer and is subdivided into the trabecular, endocortical and intracortical surfaces.[13] In long bones, the zone between the diaphysis and the epiphysis, the metaphysis, is the main site of trabecular bone.[3] Trabecular bone growth is carried out by an increase in trabecular thickness and trabeculae hyperplasia that is attributable to remodeling with a positive balance.[13] In long bones, the epiphyses drive bone growth in length as endochondral ossification in the growth plates causes increased bone length.[14] During childhood development, longitudinal growth becomes restricted to the epiphyses after the diaphyseal shafts of long bones mineralize. Epiphysial growth in the limbs and all longitudinal growth conclude during the second decade of life.[15] Longitudinal bone growth parallels the pubertal growth spurt, contributing to peak height velocity and achieving adult stature. Bone growth in width is less understood than growth in length but is of great importance for bone stability. The increase in diaphyseal width of bone is stimulated by the periosteum, the outer covering of cortical bone. In a process called periosteal apposition, the growth in cross sectional size of bone occurs through the action of osteoblasts that deposit mineralized tissue on the periosteum.[14] Periosteal expansion is more dynamic

during childhood, a time when bone modeling is constant.[13] In adulthood, periosteal bone begins a cycle of resorption and formation, a characteristic of remodeling.[16-17] Overall, rapid bone growth occurs throughout early life, slows to a nadir in early childhood, and then peaks at puberty, after which periosteal growth is practically quiescent.[14]

Adolescent Development

Throughout the pubertal growth spurt, children vary widely in stature, body composition, rate of growth, and timing and tempo of biological maturation. Compared to other lifecycle stages, pubertal growth is associated with the most dramatic changes in sexual maturation, body composition and skeletal mass accumulation. At this stage of development, critical bone mass accrual and skeletal changes set the stage for determining lifelong bone strength and future risks for osteoporosis.[18,19] Although commonly regarded as a 'senile disease,' osteoporosis is becoming increasingly recognized as a pediatric concern.[20] Influential factors that contribute to developing osteoporosis take effect during growth, when achieving optimal bone mineral accretion is critical.[7,21,22] Understanding the determinants of bone mass accretion is of great importance to the strategy of optimizing peak bone mass accretion during growth in order to prevent osteoporosis fracture later in life.

Peak Bone Mass Accrual

During adolescence, bone mass accrual reaches its maximum velocity and the majority of adult bone mass is achieved,[23] while subsequent gains are at a slower rate.[24] By age 18 years, more than 90% of total body peak bone mass, theorized as the highest amount of bone mass present at the end of skeletal maturation, has been determined.[25]

The growth rate and timing of peak bone mass accretion is both site specific and unique for each individual. Studies have shown variance in bone growth and mineral accrual in the axial and appendicular bone sites related to pubertal stage. Bone growth at pre-puberty is typically faster in the legs and arms compared to the axial skeleton. Then, the spine accelerates in mineralization during early and midpuberty, and all three sites slow in development in late puberty.[26,27] Inconsistencies also occur between growth in size and bone mineralization with maximal bone growth preceding mineral acquisition at the onset of the pubertal growth spurt. Bass et al.[27] found that prepubertal girls (n=109) who had attained 80% of their adult height had only achieved 40% of their expected adult bone mass. Figure 3 is a graphic representation of the results from a six-year prospective analysis of 138 adolescents, which yielded evidence that the rate of bone mineral accrual peaks about eight months after peak rates of height velocity. This lag time can result in undermineralized peripubertal bone that is more vulnerable to fracture.[28] Matkovic et al.[29] reported from a cross-sectional study in 265 females, that aBMD and BMC remained consistent from the level attained by age 18 years until age 50 years. The evidence also suggests a usual age for peak aBMD of specific bone sites. For example, the earliest peak age recorded is for the hip, at 17 years, followed by the wrist, at 20 years, and then the spine, at 23 years. While the speed of peak bone mineral content velocity (PBMCV) and the age at which PBMCV is achieved varies within the population, attaining the greatest bone mass possible may likely protect one from bone loss and related fractures.

Because the largest gains in bone mineral accrue during the pubertal growth spurt, this developmental phase is a critical time for determining and, then, optimizing bone

growth determinants. Overall, the amount of bone mineral gained during pubertal growth is equivalent to the total amount one is estimated to lose throughout their entire adult years.[30]

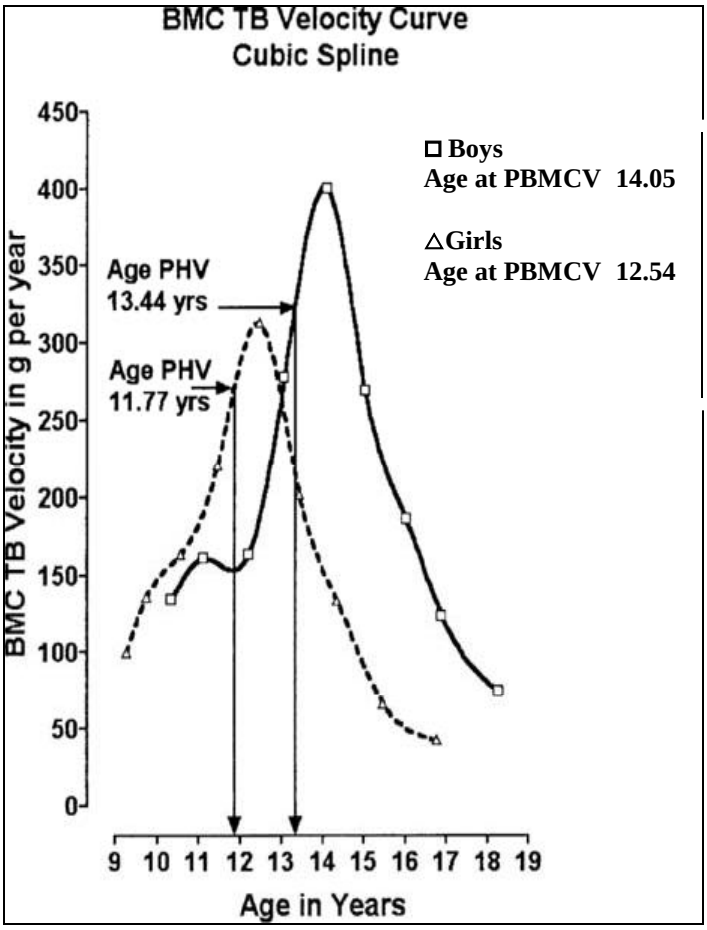


Figure 2.3. Ages at total body PBMCV for boys and girls. PHV is shown as a reference for pubertal maturation. Adapted from Bailey et al.[30]

In just the two years surrounding PBMCV 32% of adult lumbar spine BMC and 26% of adult total body BMC accrues.[28] Furthermore, during growth from age 6 to 16 years, a doubling of lumbar spine aBMD and a 2.5- and 3-fold gain in BMC in girls (n=793) and boys (n=761), respectively, has been reported.[31] Prospective analyses in adolescent females indicate that, by 12 years of age, approximately 80% of total adult bone mass has

accrued with a rate of mineralization that mirrors the progression of pubertal maturation, peaking at menarche and then slowing.[23, 32-35] For example, it is estimated that, by four years post-menarche, lumbar spine BMD has reached total adult level,[23,36] yet at other bone sites slow accumulations can carry on until age 20 years.[37]

Pubertal Changes in Body Composition

During the rapid growth period of puberty, maturational changes in body composition occur. In pubertal females, lean tissue mass decreases in exchange for gains in body fat, which dramatically affects body composition. At the average age of 12.5 years, adolescent girls enter their peak weight gain period, which results in approximately 18.3 pounds gained per year.[38] In pubertal females, lean tissue mass falls from approximately 80% to 74% of body weight and fat increases from approximately 16% to 27% of total body weight at full maturity. On average, pubertal females increase lean body mass by 44% and increase body fat by 120% during this peak growth period.[39]

In the United States, there have been remarkable increases in pediatric obesity, particularly during the pubertal years. The prevalence of obesity among children aged 6 to 11 years has more than doubled in the past 20 years, reaching 17% in 2006.[40] The most recent National Health and Nutrition Examination Survey (NHANES) reported that the obesity prevalence of adolescents 12 to 19 years of age is 17.6%,[41] more than triple the prevalence in 1976-1980. This unprecedented epidemic of obesity during childhood development and pubertal growth demands a thorough understanding of the relationship of fat mass to bone mineral accrual in growing and maturing children as literature has indicated that overweight may be associated with suboptimal bone growth and an increased risk of fractures.

Body Composition and Bone

Clinical studies have provided evidence that specific measures of body composition, such as total lean mass and fat mass may be more strongly correlated to bone mass than total body weight.[42] For example, lean muscle mass has been shown as a critical factor in bone development in children,[19,43] and fat mass has been shown as a major determinant of bone mineral measures in post-menopausal women.[44-45] Manzoni et al.[46] conducted a cross-sectional study of 115 children, aged 5 to 18 years and reported that lean mass was strongly correlated to total body BMC in children who are normal weight and those that are obese (r=0.94 and r=0.91, respectively). The University of Saskatchewan Pediatric Bone Mineral Study[30] (n=138) showed, prospectively, that peak lean mass velocity and peak BMC velocity are independently associated (r=0.05, P<0.001), implicating that muscle development drives bone development with muscle growth preceding bone growth.

Body composition variables can also have a significant impact on BMC accretion as body weight induces additional loads on the weight-bearing bones. Overall, evidence supports the relationship of lean mass and bone development; however, while previous research finds them weakly associated during childhood growth, the relationship of fat mass and bone has yet to be fully examined prospectively during the pubertal growth phase.

Bone Mineral Accrual Determinants

Although the majority of the variance in peak bone mass accrual is determined by genetics,[47] the World Health Organization identifies osteoporosis as a disease with modifiable risk factors that are key to prevention.[48] Individual risk of developing the

disease as an adult is significantly related to controlling these modifiable factors starting in childhood and throughout growth and development. Environmental influences of variances in bone mass accrual include physical activity, dietary intake, endocrine factors, and body composition characteristics.[49]

Genetic Determinants

Heritability greatly impacts peak bone mass as it affects bone size, calcium absorption efficiency, biomechanical stimulation response, and other processes. Heaney[8] suggested that, for skeletal development, reaching one's full genetic potential entails achievement of skeletal size and mass that were not restricted by inadequate nutrient supplies or insufficient mechanical loading. Research from the comparison of twins yields evidence that heredity explains 60 to 80% of the variability in peak bone mass accrual.[8,47] While the specific genes involved have yet to be determined, those that are related to body size, and thus bone size, are most significant. Candidate genes include those for growth hormone, IGF-1, their receptors, and their binding proteins as well as those for vitamin D receptors.[8] Discovery of polymorphisms in estrogen receptor genes and the binding site of collagen type 1 α1 genes make them likely sites of regulation as well.[50] Studies that have examined the relationship of various bone parameters and candidate genes have disparate conclusions, however, it is widely accepted that variation in bone phenotype is likely the result of polymorphisms. Polymorphisms create variation in phenotype through manipulation of transgenic interactions and gene-environment interactions, complicating the relationship of genetics and bone development.[51]

The development of peak bone mass is influenced by the interaction of heritable traits and environmental factors. Alterations in the modifiable factors associated with

peak bone mass accrual can, subsequently, modify gene expression. Heredity determines individual efficiency of utilizing and conserving nutrients necessary to support bone growth and maintenance. Comparing two individuals with suboptimal intakes, one may possess genes that maximize utilization of the nutrient and, thus, the ability to approach the ideal peak mass while the other utilizes the nutrient insufficiently. In this example, gene expression was manipulated through alteration in the environmental factor of dietary intake.[8] Research supports this example through the report that one-third of the variance in bone mass in healthy 24-year-old males is attributable to the variance in peak nocturnal growth hormone secretion, which is influenced by both heredity and environment.[52] Fulfillment of genetic potential is possible only when other factors, such as diet, physical activity, and hormone production are sufficient.

Dietary Intake

Adequate dietary intake and balanced nutrition provide the building blocks for bone mineral accrual.[8] Both micronutrients and macronutrients determine overall bone health and the potential to accrue one's maximum bone mass during pubertal growth. Adolescent calcium requirements have been extensively studied, yet the Dietary Reference Intake is only given as an Adequate Intake level and the interactions of this nutrient with others are still under investigation. Maintaining a positive calcium balance during growth is the best means of ensuring maximum calcium deposition in the osteoid matrix.[79] Many studies have shown the positive effects of calcium supplementation, in the forms of dairy products, fortified foods, or supplements, during the adolescent growth spurt on bone mass and density.[29,80-81] However, the positive effects were mostly seen in subjects with habitually low dietary calcium intakes. Micronutrients that modify calcium

metabolism or affect bone development include calcium, phosphorous, magnesium, copper, manganese, and zinc. Vitamins that impact calcium homeostasis and bone include vitamins D, C, and K.[8,79] According to 2002 NHANES data,[82] only 28% and 31% of females aged 9 to 13 years and 14 to 18 years, respectively, exceeded the adequate intake level for calcium, which is 1300 mg for these age groups.

Additionally, consumption of adequate macronutrients benefits bone development and is essential to the synthesis of bone matrix. Adequate calorie and dietary protein intake prevent protein-energy malnutrition, a condition that can impede bone formation.[8] Protein intake also affects bone development as it is required for the formation of the growth hormone and insulin-like growth factor 1 (IGF-1) amongst others.[79] NHANES data[82] from 2002 indicates that the average protein intake for girls aged 9 to 13 years and 14 to 18 years is 2 and 1.42 grams per kilogram body weight, respectively. Fruit and vegetable consumption has been found to significantly benefit bone mineral accrual in adolescent girls who meet or exceed the recommended daily allowance.[83] This association is regarded with caution, however, as adequate fruit and vegetable consumption can be markers of overall dietary quality and healthy lifestyle habits, which encompass other environmental influences of bone.

While healthful dietary intake patterns benefit bone mineral accrual, some foods and nutrients are associated with less bone mineral accretion. The negative consequences of low nutrient dense beverage consumption have been shown in girls, but not boys, who drank either carbonated or non-carbonated, sugar-based beverages. A significant negative correlation was demonstrated between soft drink and milk consumption in both boys and girls, but negative correlations between low nutrient dense beverages and bone mineral

accrual were apparent in adolescent girls only.[18, 83] Other dietary components inhibit calcium absorption or increase calcium excretion. Oxalic acid, which is found in green leafy vegetables like spinach and kale, and phytic acid, found in some seeds, both inhibit absorption of calcium and, thus, its bioavailability. Urinary calcium excretion is accelerated with high sodium chloride intake and excessive protein intake. The interaction of nutrients and calcium are of importance so that dietary intake of calcium is increased if one's diet contains known absorption inhibitors or excretion accelerators so that positive calcium balance is maintained.[8,79]

Physical Activity

Bone provides means for locomotion and protects vital organs. Bone mass determines the integrity of the architecture of bone and is modified to manage the stress of mechanical loading and muscular activity to prevent fracture.[53] Various forms of movement and loading activities impact bone development both directly and indirectly. Previous research has indicated that high loading activities greatly impact bone mass acquisition prior to and throughout puberty.[54-55] Studies have also examined whether physical activity can contribute to the prevention of osteoporosis as evidenced by the enduring benefits on bone during growth.[56-57]

Frost established a theory for the response of bone to physical activity, proposing that a mechanical strain threshold, termed the minimum effective strain, directs the mechanical adaptations of bone.[58] Frost's mechanostat theory describes a feedback control system in which bone structure is maintained in a state such that normal daily mechanical stress does not exceed the minimum effective strain of bone.[59] Physical activity produces loading forces on bone, resulting in stress on bone above the minimum

effective strain. The increased mechanical resistance on bone stimulates bone modeling through osteoblast activation and increased bone mineralization.[24,53] In reference to the mechanostat theory, the osteogenic potential of physical activity is rooted in those actions that produce tensile, compressive, shear, bending, and torsion stress on bone.[60]

Physical activity can also impact bone acquisition indirectly. Exercise can enhance fat free muscle mass and increase muscle strength, allowing for greater force of muscular contractions in hypertrophied muscles at sites of bone attachments, stimulating bone growth.[43,61] A theoretical model developed by van der Meulen et al.[62] regarding long bone cross-sectional growth, suggested that morphological development is not merely age-related but is strongly determined by increased body mass and subsequently increased mechanical stimuli. Physical activity also enhances muscular vascularization, which concomitantly increases circulation of bone, amplifying its nutrient, hormone and oxygen supply.[1] Muscle stimulation may also act as a mechanobiological mediator in regulating adaptation of bone. Qin et al.[63] showed that changes in intramedullary pressure due to muscle contraction, via low magnitude, high frequency signaling, altered blood flow and fluid pressure in bone tissue, inducing bone formation. Using in vitro and in vivo models, Hamrick et al.[64] have investigated the endocrine aspect of muscles, such as the release of osteogenic growth factors called myokines. In a study by Liu et al.,[65] evidence suggest that myokines contribute to bone healing in a novel mouse model that follows the relocation of muscle-derived cells into the fracture callus. In this model, muscle is not only a source of mechanical stimuli for bone but also appears to act as an endocrine organ that indirectly regulates bone metabolism, secreting osteogenic factors through local paracrine and endocrine secretions.

The period of pubertal development offers a critical opportunity to maximize peak bone mass, offsetting future fracture risk, through physical activity. Research has shown that active children accumulate more bone mineral compared to inactive peers,[28,33] and initiating sports training before puberty results in greater BMD and bigger bones,[20,66,67] contributing to lower fractures risks. Reports from The Saskatchewan Bone Mineral Accrual Study[28] state 9% and 17% greater total body BMC in active boys and girls, respectively, and 18% greater lumbar spine BMC in highly active children compared to the least active group one year after PBMCV.[28] The potential influence on bone, however, depends on the type of activity, the intensity, the years of training, and the age at which training began.[20,68] Studies have shown high impact exercise is a superior mode of activity that can result in greater gains in bone mass than lower impact activity.[69,70] Activities that generate high ground reaction forces, such as gymnastics,[54,71,55] racket sports[66,67] and running[72] have the capacity to produce greater lumbar spine BMD gains in female athletes during prepubertal development and throughout adolescence. In a three year prospective analysis, Nurmi-Lawton et al.[54] found that female gymnasts sustained 24-51% greater BMC and 13-28% greater BMD during the pubertal years. High impact activities use body weight as the source of mechanical loading, which can produce much greater loads on weight-bearing bones than that produced from muscle contraction alone.[15] Youth sports that require high impact loading actions have been shown to increase peak BMD by as much as 20% compared to sedentary controls in the weight-loaded bones. Studies have shown variable skeletal response to activity related to different pubertal stages. Elevated estrogen levels in pubertal females enhance the sensitivity of bone to mechanical loading,[73] allowing the impact exercise to

synergistically contribute to peak bone mass accretion, leading to greater bone mass gains during the pubertal growth spurt.[20,66,67,74]

Physical activity prior to and during pubertal growth offers a valuable opportunity to maximize peak bone mass; however, the lasting impact of exercise on bone assumes that the changes in bone are sustained throughout growth and adulthood.[68] Scientific conclusions remain controversial as to whether the benefits of exercise training during pubertal growth persist into adulthood if training is discontinued. Retrospective analyses of retired athletes have reported both sustained benefits of greater bone mineral density[56,75] and bone mineral that is similar to age-matched, sedentary controls.[76,77] Gains in bone mineral content during exercise intervention trials have been shown to endure 1-8 years after cessation in pre- and early pubertal children.[57,78] Gunter et al.[57] followed participants of a seven month jumping intervention for eight years, at which point participants had sustained 1.4% greater BMC gains at the hip compared to controls. Longitudinal results from The Saskatchewan Bone Mineral Accrual Study[28] followed active and inactive adolescents into their third decade of life. The active males maintained an 8% greater adjusted total body BMC, reported an 11% greater adjusted total hip BMC and had 9% greater BMC of the femoral neck. Active adolescent females had 9% and 10% greater total hip and femoral neck BMC, respectively, at young adulthood while differences had diminished at the total body and lumbar spine sites.[68]

Physical activity can have a lasting impact on the rate of bone loss even in the adult years. For exercise to have a lasting effect, it should be dynamic, exceed a threshold intensity and strain frequency, detract from usual loading patterns of bones, and be

supported with adequate energy and nutrient intake.[15] However, the greatest gains in bone due to high impact loading are seen during growth in children and adolescents.

Hormones

The metabolic functions of the skeleton are affected by a variety of hormones that regulate bone growth and turnover. Paracrine hormones are secreted from cells and act on neighboring cells while autocrine hormones target the same cell from which they are secreted. The major systemic influences on the metabolic functions of bone are the calcium-regulating hormones, parathyroid hormone (PTH) and 1,25-dihyrdroxyvitamin D. PTH is secreted by the parathyroid gland when serum calcium concentration is below homeostatic levels, stimulating bone resorption. Increases in PTH with age may be linked to elevated bone turnover and bone loss seen in older populations. 1,25-dihydroxyvitamin D, the active circulating form of vitamin D, regulates intestinal calcium and phosphorous absorption. Among other metabolic functions, 1,25-dihydroxyvitamin D may also play a critical role in the differentiation of osteoblasts and osteoclasts as well as in the stimulation of bone formation and resorption.[84] Calcitonin affects the metabolic activities of bone through its potent inhibition of bone resorption, which is the reason for its use as a treatment medication for osteoporosis.

Other hormones are attributed with bone influencing abilities as evidence supports the impact of their imbalance or malfunction as detrimental to bone. These hormones are not secreted in response to fluctuating serum calcium levels yet they can either directly or indirectly impact bone by adjusting calcium metabolism. Examples of these hormones include estrogen, IGF-1, growth hormone, testosterone, and corticosteroids. Pubertal hormones are particularly critical as evidenced by the presence of osteopenia in

adolescents with abnormal pubertal development.[50] The sex steroid, estrogen, impacts bone both directly and indirectly and is involved in skeletal maturation and mineralization.[5] Estrogen can suppress bone resorption and helps regulate intestinal calcium absorption and secretion.[85-86] Estrogen can also modulate serum 1,25-dihydroxyvitamin D levels, renal calcium excretion, and the secretion of PTH.[87,88] IGF-1 and growth hormone are significant pubertal hormones that increase dramatically during development and are augmented by sex steroids. Growth hormone stimulates IGF-1 production in bone,[89] and IGF-1 mediates the effect of growth hormone on bone, resulting in increased bone growth and turnover via the activation of osteoblasts, collagen synthesis and longitudinal bone growth.[50] During maturational growth, the pattern of IGF-1 secretion mirrors that of bone mineral gain. During puberty, both IGF-1 and bone mineral content increase, reaching a peak similar in timing to peak height velocity, and then decrease with age.[90-91] The androgen, testosterone, is a sex steroid that stimulates bone growth. In males and females, androgen receptors found in growth plate osteoblasts mediate its anabolic effect on bone.[50,92] Corticosteroids, such as glucocorticoids, are produced in the adrenal cortex and can be detrimental to bone. Glucocorticoids inhibit bone formation, increase bone resorption, and decrease sex steroid synthesis. Glucocorticoids block intestinal calcium absorption,[93] activate osteoclasts[94] and disrupt the growth hormone/IGF-1 axis, inhibiting peak bone mass achievement.[95]

Bone and Muscle Development

The theory that muscle mass is the strongest determinant of bone development has been well established in both cross-sectional[46,96,97] and prospective[43,72,98,99] pediatric studies. Research conducted using dual energy x-ray absorptiometry (DXA), peripheral

quantitative computed tomography (pQCT), and axial quantitative computed tomography (QCT) have shown that muscle mass (reported as fat-free soft tissue (FFST) or lean mass) and/or muscle size (reported as muscle cross-sectional area (MCSA)) are either strongly related to and/or independently predict parameters of bone strength.

The strong association of bone and muscle development during growth is established, but ambiguity remains as to the timing of peak velocity of each in relation to the other. Interpretation of conclusions must be guided by recognition that lean mass is not equivalent to muscle mass just as BMC is not equivalent to bone size or strength.[100] In a longitudinal study of pubertal boys and girls comparing peak velocity of lean body mass accretion (PVLBM) and PBMCV, Rauch et al.[43] found that lean body mass accrual preceded peak BMC accrual. In a multiple regression model analyzing sex, peak height velocity and total body PVLBM as predictors of total body PBMCV, only PVLBM was independently associated with total body PBMCV ($r^2 = 0.50$; $P < 0.001$). This evidence is in agreement with Frost's mechanostat theory and the view that bone strength adjusts in response to muscle contraction during growth.[100]

In contrast to the report of Rauch et al.,[43] a prospective study by Xu et al.[100] revealed parameters of muscle development were delayed compared to bone growth. In the seven-year longitudinal study from prepuberty to early adulthood, Xu et al.[100] found that bone length and total bone cross sectional area of the tibia peaked one year before muscle cross-sectional area (mCSA), challenging the evidence from the study by Rauch et al.[43] However, cortical cross-sectional area, total body BMC, and cortical volumetric BMD peaked within one year following peak growth velocity of mCSA. In conclusion, if muscle force drives bone growth, the expectation is that peak growth velocity in mCSA,

an accepted surrogate for muscle strength, will precede growth velocities of bone strength determinants. Xu et al.[100] saw the opposite occur in the tibia: the peak growth of bone size preceded peak growth of muscle area at the tibia only.

Adiposity and Bone

Understanding the relationship of adiposity and bone development is of increasing concern due to the escalating prevalence of overweight and obesity among children and adolescents. Variances in body weight and body composition influence bone mass accrual. Studies have shown that, instead of total body weight, specific measures of body composition such as fat mass and percent body fat, are important indicators of bone mass, bone size[42, 101] and BMD[102] In adults, higher BMI or greater adiposity has been related to decreased risks of fractures and osteoporosis.[101,103,104] These findings have encouraged investigations to determine if the same beneficial effect of adiposity exists in youth. Recent literature indicates that being overweight or having greater percentage body fat during childhood or adolescence is correlated with lower than expected bone mass and increased incidence of fracture.[19,105,106] Taylor et al.[107] found that in children with BMI $\geq 95^{th}$ percentile, significantly more fractures were reported compared with non-overweight children. Frost's postulate is that increased fracture risks in obese children is related to greater impact force during a fall, lifestyle choices contraindicative to strong bones and/or excess adiposity that hinders optimal bone growth.[108]

Conversely, some investigations have concluded that obesity has either no effect on bone mass or is protective to bones and positively contributes to peak bone mass accrual.[96,97,109-110] The decline in fracture risk in obese adults supports these conclusions, however, the influence of above normal pediatric fat mass has yet to be fully investigated

over the longitudinal period of childhood and pubertal growth. The unprecedented prevalence of pediatric and adolescent obesity, in addition to the earlier onset of adult obesity with each increment of birth year, commands the exceptional gravity of understanding the implications of greater fat mass in childhood and adolescence for adult bone mass.[40,111]

Previous Research

Limitations of DXA Technology in Pediatric Bone Research

In research studies of childhood and adolescent bone growth, researchers have chosen to report different bone measurements. Technologies have been developed to estimate various surrogates of bone strength, including BMC, aBMD, and bone geometrical indices, but these parameters individually do not completely explain strength of the whole bone, although each is an important variable in bone research. Empirical studies of body composition have employed DXA technology, which is capable of assessing total body bone and soft-tissues, specifying the increments of the total body that are fat tissue, lean tissue, and bone mineral. DXA analyses produce two-dimensional bone images, which are credited with furthering scientific understanding of the impact of fat mass on BMD in adults as well as bone mineral accrual in children since its emergence in the 1990s. However, DXA provides areal, not volumetric BMD (vBMD), measures, which are difficult to interpret for the developing skeletons of children when adjustments must be made to account for body size, gender, and maturation.[112,113]

DXA lacks technology to assess geometrical properties of bone and is incapable of distinguishing cortical and trabecular components. To completely characterize whole bone strength, measurements of bone mineralization *and* geometrical properties must

both be accounted for.[114,115] Technology, such as QCT and pQCT has recently become available that produces 3-dimensional bone images. Overcoming the limitations of DXA, QCT and pQCT are able to assess vBMD and bone structural parameters, differentiate cortical from trabecular bone, and most notably, analyze muscle at particular bone sites. Researchers have encouraged that muscle size and strength should be considered in analyses of bone, regardless of the parameter reported, due to the strong association of lean mass and bone.[116,117] With the additional benefits of bone and muscle assessments available through pQCT and QCT, researchers are able to more accurately delineate adiposity as an independent determinant of bone mass accrual during pubertal growth.

Especially in pediatric bone investigations, most methods of assessing bone are imperfect due to the changes in skeletal growth.[118] For example, in DXA analyses, BMD is reported in bone mineral per unit area, but it is not a true volumetric density because only the length and breadth of the bone are measured, not the depth. Researchers supplement BMD with bone mineral apparent density (aBMD) and volumetric BMD (vBMD), which are measurements that adjust for body size and attempt to estimate true volumetric density. Overall, these measures assess how densely packed the bony material is within the periosteal envelope but inadequately account for changes in bone size. Also, researchers often erroneously report aBMD and BMC synonymously. Furthermore, Heaney[119] suggests that aBMD be avoided in assessments of bone in growing individuals or when measurements will be compared across different ethnicities or genders, reasoning that bone size and shape are not constant across groups or time. Using aBMD in such situations may cause error in detecting important differences, limiting the clinical significance of research findings. During periods of rapid growth, bones may

grow in size prior to mineral accrual, which may produce density measurements that are lower than expected or less than a previous measurement. On the other hand, BMC is consistent with geometrical properties of bone in pediatric populations. Bone mass is determined by the mineral content, thus making BMC a primary contributing factor in bone strength. Overall, BMC and bone area are of greater reliability in childhood and adolescent bone studies because these measurements account for bone size and detect significant changes that can be overlooked by measures of aBMD.[113, 120]

The following sections review the cross-sectional and prospective literature in which DXA, pQCT, and QCT have been employed to ascertain the influence of childhood and adolescent obesity on bone development.

Cross-Sectional DXA Studies

Cross-sectional pediatric studies of the relationship of fat and bone provide the foundation for further investigations of FFST, fat mass and total body BMD and BMC. Investigators have set out to answer whether body weight is negatively associated with bone growth in children and adolescents. A cross sectional study by Goulding, et al.[121] suggested that overweight and obesity in childhood and adolescence may prevent optimal BMC accrual, resulting in less than predicted bone mass, when corrected for body weight and lean mass, and increased susceptibility to childhood fractures.[122] In this study, white boys and girls aged 3 to 19 years were grouped according to BMI percentiles as normal weight (BMI <85th percentile), overweight (BMI >85th and <95th percentile) and obese (BMI ≥95 percentile). Expected BMC was predicted using stage wise regression of log-transformed BMC based on age and weight. The residual between observed and predicted values was used to calculate the ratio of observed-to-predicted BMC. Goulding, et al.[121]

showed lower than predicted BMC and bone area in overweight and obese subjects. The disparity between higher body weight and bone development suggests that adiposity is not advantageous for bone development and may be a factor in suboptimal BMC accrual. Weiler et al.[123] confirmed the conclusions of Goulding's study, finding that high percentage body fat in adolescent girls, aged 10 to 19 years, negatively impacts bone mineral content ($p = 0.003$), mineral content corrected to bone area ($p = 0.02$) and bone density ($p = 0.003$). In a more focused cross-sectional pediatric investigation, Goulding et al.[124] examined boys and girls aged 3 to 19 years concerning the impact of higher adiposity on bone mineral of the lumbar spine. Overweight and obese girls had 8% less and 12% less bone mineral in the lumbar spine, respectively, corrected for bone area, height, weight, and pubertal development. Obese boys had 13% less bone mineral in the lumbar spine corrected for the same covariates. The significance of this report is that teenagers with high adiposity may be supporting adult weight with a child's spinal BMC. Furthermore, Rocher et al.[125] found that in prepubertal obese children, the ratio of bone mass to total body weight was significantly lower ($P < 0.0001$) compared to controls, indicating that the skeleton is insufficiently mineralized to support the excess body weight. While obese children had higher crude values of bone mass and bone area, after adjusting for body weight or lean mass, the opposite trend was significant. Recently, Dimitri et al.[106] reported that obese children, particularly those with prior fracture(s), had lower total body BMC despite greater lean mass relative to height compared to non-obese children without fracture history. Overall, bone mass and size at the radius, spine and total body was positively related to lean mass adjusted to body-size and negatively related to fat mass. A primary bone abnormality, as defined by Crabtree,[126] exists when

total body lean mass corrected for body size is normal but BMC corrected for lean mass is reduced, as is the case with obese children in this study.

Other cross-sectional investigations[96,109-127,128,129] oppose these conclusions, contributing evidence to the contrary that childhood overweight is not linked to negative effects on bone or positively influences bone mass during growth. In examining which body composition components relate to total body BMD, Faulkner et al.[96] found that, in males and females aged 8 to 16 years, age, height and FFST were the strongest predictors, and no variance was accounted for by fat mass. Greater adiposity has also been observed to be a positive predictor of bone development. When children were grouped by percentage body fat into groups of <25%, 25-30%, and >30% body fat, Ellis et al.[130] found the group with the highest body fat percentage (>30%) had significantly greater ($P < 0.0005$) total body BMC relative to height compared to the normal fat group. Similarly, Leonard et al.[131] observed that 4- to 20- year-old boys and girls that were obese, based on BMI-for-age percentiles, achieved greater lumbar spine aBMD when corrected for height. El Hage, et al.[128] investigated adolescent males and females ages 14 to 16 years, and found that in females, both lean and fat mass positively related to whole body BMD, but only fat mass was positively associated to lumbar spine BMD. The conclusion of the study concerning females was that fat mass, rather than lean mass, is a strong determinant of whole body BMD in females.

The disagreements in conclusions of the cross-sectional studies discussed are founded mainly on the selection of confounders, body weight, height, or both, for their respective statistical analyses. When weight is controlled for in analyses, children with higher adiposity appear to have less bone mass than normal weight peers. Conversely,

adjusting for height yields results of bone mass in overweight and obese children that is similar to or greater than their normal weight peers.

Prospective DXA Studies

When prospective data have been adjusted for height, maturation, or FFST, DXA studies primarily support a positive relationship between changes in total body fat and total body BMC.[97,132] Young et al.[97] deduced that aBMD of the lumbar spine and hip was solely influenced by lean mass and that BMC of the total body was positively related to both lean mass and fat mass, independently. However, Young et al.[97] observed a shift from FFST to fat mass as the strongest predictor of total body BMC after the pubertal growth spurt in girls. Clark et al.[132] observed that fat mass in prepubertal children was a positive predictor of bone area and subsequent growth over the two years of participation. Furthermore, because adjustments for height did not significantly affect the relationship of fat mass and bone size, the researchers presumed that increased adipose tissue enhances the rate of periosteal apposition leading to increased diameter, rather than longitudinal, growth. On the other hand, as the female subjects entered early puberty, Tanner stage 3, the association of adiposity and bone size had evolved into a negative relationship, which suggests a pubertal transition in the effect of fat mass on bone mineral accrual during pubertal growth.[132] In a 6-year prospective study of females in late adolescence(aged 17 to 22 years), Petit et al.[133] compared subjects who gained weight and those whose weight remained stable. During the 6-year study, both groups experienced a similar increase in bone bending strength at the proximal femur, as measured by section modulus with DXA. An increase in subperiosteal width was apparent in the stable weight group but was absent in the weight gainers. Concomitantly,

the weight gainers experienced reduced bone strength relative to body weight as the study progressed.[133] Prospective DXA studies contribute to understanding the progression of the relationship of fat mass and bone. Disagreements in observations are, potentially, rooted in divergent maturation and age of subjects. As with cross-sectional analyses, prospective DXA data is corrected for various aspects of body composition and growth measurements, which must be considered in interpretations. Additionally, the limitations of DXA technology in detecting aspects of geometrical bone properties restrict researchers from determining how adiposity relates to and how pubertal growth shapes these parameters.

pQCT Studies

Peripheral and axial QCT technology has added significant assessment capabilities to bone and body composition research endeavors. Because bone strength cannot be measured in vivo, it is estimated from measurements of both geometrical (size and shape) and material (bone mineral content) qualities of bone through QCT and pQCT.[19] The three-dimensional results of a pQCT scan assess cortical bone area, bone cross-sectional area, cortical BMC, periosteal circumference, muscles cross-sectional area (MCSA), and strength-strain index (SSI).[19] Just as BMC and BMD are indirect measures of bone strength, SSI estimates bone strength and reflects the stress capacity of a bone or its ability to withstand loading forces without fracture. Bone strength is important to consider because seemingly insignificant changes in bone size and shape can impact bone strength even without a change in bone mass.[137] Ducher et al.[134] observed inferior cortical bone structural characteristics of the radius in overweight children compared to normal weight children, aged 7 to 10 years. This conclusion has remarkable

clinical implications in that the distal radius is the site with the highest incidence of fracture in prepubertal and pubertal children.[135] In a QCT analysis adjusted for lean mass, Janicka et al.[136] found total fat mass was a negative predictor of lumbar spine trabecular density as well as cortical bone size of the femoral midshaft. Pollock et al.[19] looked at the effects of adiposity on bone strength indices in late adolescent females aged 18 to 19 years. In the study, subjects of primarily non-Hispanic Caucasian ethnicity were divided into normal- and high-fat groups based on body fat percentage. The normal fat group had a body fat percentage of < 32% whereas the high-fat group had a body fat percentage of ≥ 32%. Subjects in the high-fat group showed lower bone area, lower BMC, lower periosteal circumference (bone size), and lower strength-strain indices than their normal-fat counterparts (see Figure 4). The evidence from this study suggests that higher adiposity is not advantageous and might actually be harmful to adolescent skeletal growth.[138]

Other studies connecting adiposity and adolescent bone development examining the effects of fat on bone tissue and mass accrual have contradicting deductions regarding the fat-bone relationship. For example, Fricke et al.[139] found a positive association between total fat mass and trabecular vBMD of the radius, even when controlling for MCSA, in children and adolescents. The mixed evidence in studies that account for muscle-related properties suggest that at non-weight bearing sites, adiposity negatively impacts bone size and geometrical properties, instead of density. Significant research investigating the impact of varying body compositions on bone development suggests an important relationship exists between body fat and bone. However, our understanding of the coinciding events of bone accrual and fat accumulation during pubertal growth

remains incomplete for lack of studies following children from childhood throughout puberty, and into late adolescent years.

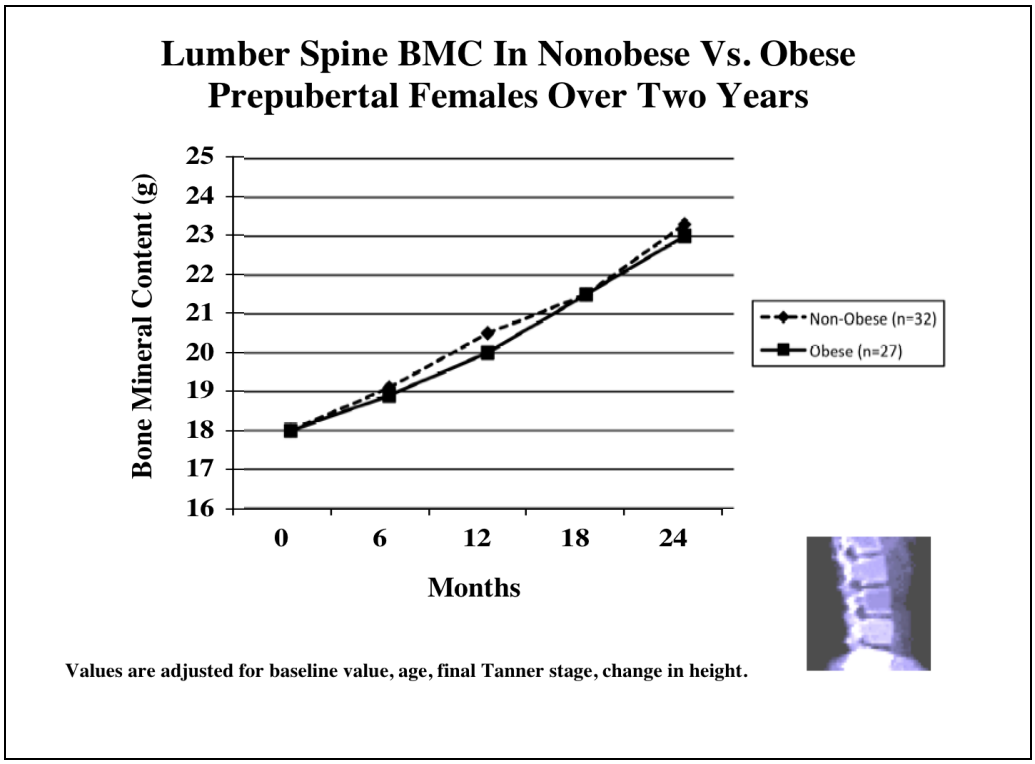


Figure 2.4. Changes in total body BMC(g) in non-obese and obese prepubertal females over 2 years.[138]

Summary

Peak bone mass accrual during the period of growth from childhood through adolescence is influenced primarily by genetics but is also affected by environmental elements such as diet and physical activity. Body composition variables including lean mass, body weight and height are the results of the interaction of genetics and environment and contribute greatly to BMC accrual. The degree of influence that fat mass has on BMC accrual and peak bone mass is less understood. Previous investigations have corrected for different covariates, and some have determined fat mass to be

protective while others conclude it is disadvantageous to bone development. Empirical evidence from childhood and adolescent studies is equivocal and lacks long-term prospective studies. To our knowledge, no studies to date have analyzed, prospectively, body fat accumulation and BMC accrual during the growth period that encompasses childhood to post-menarche adolescence.

References

1. Rodan G. Introduction to bone biology. Bone 1992;13:s3-s6.

2. Heaney R, ed. Bone Biology in Health and Disease: A Tutorial. In: Shils ME, Olson JA, Shike M, Ross AC. (ed.) Modern Nutrition in Health and Disease, 9th ed. . Baltimore: Williams and Wilkins; 1999.

3. Khan KM, McKay HA, Kannus P, Bailey D, Wark J, Bennell K. Physical Activity and Bone Health. Champaign, IL: Human Kinetics; 2001.

4. Baron R. Anatomy and Ultrastructure of Bone. In: Favus MJ, ed. Primer on the Metbolic Bone Diseases and Disorders of Mineral Metabolism. 4th edition ed. New York: Lippincott Williams & Wilkins; 1999:3-10.

5. Einhorn T. The Bone Organ System: Form and Function. San Diego, CA: Academic Press; 1996.

6. Ham A. Histology. 7th ed. Philadelphia (PA): Lippencott; 1974.

7. Parfitt AM. The two faces of growth: benefits and risks to bone integrity. Osteoporos Int 1994;4:382-98.

8. Heaney RP, Abrams S, Dawson-Hughes B, et al. Peak Bone Mass. Osteoporosis International 2000;11:985-1009.

9. Manolagas SC, Jilka RL. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. N Engl J Med 1995;332:305-11.

10. Rizzoli R, Bonjour JP. Determinants of peak bone mass and mechanisms of bone loss. Osteoporos Int 1999;9 Suppl 2:S17-23.

11. Turner CH, Burr DB. Basic biomechanical measurements of bone: a tutorial. Bone 1993;14:595-608.

12. Orwoll ES. Toward an expanded understanding of the role of the periosteum in skeletal health. J Bone Miner Res 2003;18:949-54.

13. Parfitt AM, Travers R, Rauch F, Glorieux FH. Structural and cellular changes during bone growth in healthy children. Bone 2000;27:487-94.

14. Rauch F. Bone accrual in children: adding substance to surfaces. Pediatrics 2007;119 Suppl 2:S137-40.

15. Borer KT. Physical activity in the prevention and amelioration of osteoporosis in women : interaction of mechanical, hormonal and dietary factors. Sports Med 2005;35:779-830.

16. Frost H. Structural adaptions to mechanical usage (SATMU): 1. Redefining Wolff's Law: The bone modeling problem. Anat Rec 1990;226:403-13.

17. Balena R, Shih MS, Parfitt AM. Bone resorption and formation on the periosteal envelope of the ilium: a histomorphometric study in healthy women. J Bone Miner Res 1992;7:1475-82.

18. Whiting S, Healey A, Psiuk S, Mirwald R, Kowalski K, Bailey DA. Relationship between carbonated and other low nutrient dense beverages and bone mineral content of adolescents. Nutr Res 2001;21:1107-15.

19. Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, D. LR. Is adiposity advantageous for bone strength? A pQCT study in adolescent females. Am J Clin Nutr 2007;86,1530-8.

20. Vicente-Rodriguez G. How does Exercise Affect Bone Development during Growth? Sprots Medicine 2006;7:561-9.

21. Bailey DA. Prevention of Osteoporosis: A pediatric concern. Lifestyle Medicine (Rippe, J M, ed) 1999:578-84.

22. McKay HA, Bailey DA, Mirwald RL, Davison KS, Faulkner RA. Peak bone mineral accrual and age at menarche in adolescent girls: a 6-year longitudinal study. J Pediatr 1998;133:682-7.

23. Bonjour JP, Theintz G, Buchs B, Slosman D, Rizzoli R. Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. Journal of Clinical Endocrinology and Metabolism 1991;73 (3):555-63.

24. Bailey DA, Faulkner RA, McKay HA. Growth, physical activity, and bone mineral acquisition. Exercise and Sport Science Reviews 1996;24:233-66.

25. 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. J Clin Invest 1994;93:799-808.

26. Bradney M, Karlsson MK, Duan Y, Stuckey S, Bass S, Seeman E. Heterogeneity in the growth of the axial and appendicular skeleton in boys: implications for the pathogenesis of bone fragility in men. J Bone Miner Res 2000;15:1871-8.

27. Bass S, Delmas PD, Pearce G, Hendrich E, Tabensky A, Seeman E. The differing tempo of growth in bone size, mass, and density in girls is region-specific. J Clin Invest 1999;104:795-804.

28. 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. J Bone Miner Res 1999;14:1672-9.

29. 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. J Nutr 2004;134:701S-5S.

30. Bailey DA. The Saskatchewan Pediatric Bone Mineral Accrual Study: bone mineral acquisition during the growing years. Int J Sports Med 1997;18 Suppl 3:S191-4.

31. Kalkwarf HJ, Zemel BS, Gilsanz V, et al. The bone mineral density in childhood study: bone mineral content and density according to age, sex, and race. J Clin Endocrinol Metab 2007;92:2087-99.

32. LLoyd T, Rollings N, Andon MB, et al. Determinants of bone density in young women. I. Relationships among pubertal development, total body bone mass, and total body bone density in premenarchal females. Journal of Clinical Endocrinology and Metabolism 1992;75 (2):383-7.

33. Slemenda CW, Reister TK, Hui SL, Miller JZ, Christian JC, Johnston Jr. CC. Influences on skeletal mineralization in children and adolescents: Evidence for varying effects of sexual maturation and physical activity. The Journal of Pediatrics 1994;125:201-7.

34. Theintz G, Buchs B, Rizzoli R, et al. Longitudinal monitoring of bone mass accumultation in healthy adolescents: Evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. Journal of Clinical Endocrinology and Metabolism 1992;75 (4):1016-65.

35. Glastre C, Braillon P, David L, Cochat P, Meunier PJ, Delmas PD. Measurement of bone mineral content of the lumbar spine by dual energy x-ray absorptiometry in normal children: Correlations with growth parameters. Journal of Clinical Endocrinology and Metabolism 1990;70:1330-3.

36. Abrams SA, Copeland KC, Gunn SK, Gundberg CM, Klein KO, Ellis KJ. Calcium absorption, bone mass accumulation, and kinetics increase during early pubertal development in girls. J Clin Endocrinol Metab 2000;85:1805-9.

37. Thomas KA, Cook SD, Bennett JT, Whitecloud TS, 3rd, Rice JC. Femoral neck and lumbar spine bone mineral densities in a normal population 3-20 years of age. J Pediatr Orthop 1991;11:48-58.

38. Barnes HV. Physical growth and development during puberty. Med Clin North Am 1975;59:1305-17.

39. Frisch R. Fatness, puberty, and fertility: the effects of nutrition and physical training on menarche and ovulation. In: Brooks-Gunn J, Peterson AC, eds. New York: Plenum Press; 1983.

40. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. Jama 2006;295:1549-55.

41. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. Jama 2002;288:1723-7.

42. Hamrick M, Della-Fera, MA., Baile, CA., Pollock, NK., Lewis, RL. Peripheral Body Fat as a Regulator of Bone Mass: Experimental Evidence from Animal Models. Unpublished Manuscript.

43. Rauch F, Bailey DA, Baxter-Jones A, Mirwald R, Faulkner R. The 'muscle-bone unit' during the pubertal growth spurt. Bone 2004;34:771-5.

44. Galvard H, Elmstahl S, Elmstahl B, Samuelsson SM, Robertsson E. Differences in body composition between female geriatric hip fracture patients and healthy controls: body fat is more important as explanatory factor for the fracture than body weight and lean body mass. Aging (Milano) 1996;8:282-6.

45. Reid IR, Ames R, Evans MC, et al. Determinants of total body and regional bone mineral density in normal postmenopausal women--a key role for fat mass. J Clin Endocrinol Metab 1992;75:45-51.

46. Manzoni P, Brambilla P, Pietrobelli A, et al. Influence of body composition on bone mineral content in children and adolescents. American Journal of Clinical Nutrition 1996;64:603-7.

47. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults. A twin study. J Clin Invest 1987;80:706-10.

48. Organization WH. Statement by Dr. Gro Harlem Brundtland, Director General of the World Health Organization on World Health Day 2002.

49. McCormick RK. Osteoporosis: integrating biomarkers and other diagnostic correlates into the management of bone fragility. Altern Med Rev 2007;12:113-45.

50. Soyka LA, Fairfield WP, Klibanski A. Clinical review 117: Hormonal determinants and disorders of peak bone mass in children. J Clin Endocrinol Metab 2000;85:3951-63.

51. Peacock M, Turner CH, Econs MJ, Foroud T. Genetics of osteoporosis. Endocr Rev 2002;23:303-26.

52. Russell-Aulet M, Shapiro B, Jaffe CA, Gross MD, Barkan AL. Peak bone mass in young healthy men is correlated with the magnitude of endogenous growth hormone secretion. J Clin Endocrinol Metab 1998;83:3463-8.

53. Frost HM. Vital biomechanics: Proposed general concepts for skeletal adaptations to mechanical usage. Calcif Tissue Int 1988;42:145-56.

54. Nurmi-Lawton JA, Baxter-Jones AD, Mirwald RL, et al. Evidence of sustained skeletal benefits from impact-loading exercise in young females: a 3-year longitudinal study. J Bone Miner Res 2004;19:314-22.

55. Helge EW, Kanstrup IL. Bone density in female elite gymnasts: impact of muscle strength and sex hormones. Med Sci Sports Exerc 2002;34:174-80.

56. Pollock NK, Laing EM, Modlesky CM, O'Connor PJ, Lewis RD. Former college artistic gymnasts maintain higher BMD: a nine-year follow-up. Osteoporos Int 2006;17:1691-7.

57. Gunter K, Baxter-Jones AD, Mirwald RL, et al. Impact exercise increases BMC during growth: an 8-year longitudinal study. J Bone Miner Res 2008;23:986-93.

58. Frost H. Bone "mass" and the "mechanostat": a proposal. Anat Rec 1987;219:1-9.

59. Frost HM. The role of changes in mechanical usage set points in the pathogenesis of osteoporosis. J Bone Miner Res 1992;7:253-61.

60. Heinonen A. Biomechanics. In: Khan K, McKay H, Kannus P, al. e, eds. In: Phsyical activity and bone health. 1st ed. Champaign (IL): Human Kinetics; 2001:23-34.

61. Frost HM. The Utah paradigm of skeletal physiology: an overview of its insights for bone, cartilage and collagenous tissue organs. J Bone Miner Metab 2000;18:305-16.

62. van der Muelen M, Beaupre GS, Carter DR. Mechanobiologic influeces in long bone cross-sectional growth. Bone 1993;14:635-42.

63. Qin YX, Lam H. Intramedullary pressure and matrix strain induced by oscillatory skeletal muscle stimulation and its potential in adaptation. J Biomech 2009;42:140-5.

64. Hamrick M, McNeil, P, Patterson, S. Role of muscle-derived growth factors in bone formation. J Musculoskelet Neuronal Interact 2010;10:64-70.

65. Liu R, Peacock, L., Schindeler, A., Little, DG. Myogenic progenitors contribute to ectopic bone formation in open fracture repair. J Bone Miner Res 2009;24:SA0228.

66. Kannus P, Haapasalo H, Sankelo M, et al. Effect of starting age of physical activity on bone mass in the dominant arm of tennis and squash players. Annals of Internal Medicine 1995;123:27-31.

67. Haapasalo H, Kannus P, Sievanen H, et al. Effect of long-term unilateral activity on bone mineral density of female junior tennis players. Journal of Bone and Mineral Research 1998;13(2):310-9.

68. Baxter-Jones AD, 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;43:1101-7.

69. Fuchs RK, Bauer JJ, Snow CM. Jumping improves hip and lumbar spine bone mass in prepubescent children: a randomized controlled trial. J Bone Miner Res 2001;16:148-56.

70. Heinonen A, Sievanen H, Kannus P, Oja P, Pasanen M, Vuori I. High-impact exercise and bones of growing girls: a 9-month controlled trial. Osteoporos Int 2000;11:1010-7.

71. Laing E, Massoni J, Nickols-Richardson S, Modlesky C, Lewis R. A prospective study of bone mass and body composition in female adolescent gymnasts. Medicine and Science in Sports and Exercise 2001;33:s146.

72. Duncan CS, Blimkie CJ, Cowell CT, Burke ST, Briody J, Howman-Giles R. Bone mineral density in adolescent female athletes: relationship to exercise type and muscle strength. Med Sci Sports Exerc 2002;34:286-94.

73. MacKelvie KJ, Khan KM, McKay HA. Is there a critical period for bone response to weight-bearing exercise in children and adolescents? a systematic review. Br J Sports Med 2002;36:250-7.

74. Bailey DA, Martin AD, McKay MA, Whiting S, Mirwald R. Calcium accretion in girls and boys during puberty: a longitudinal analysis. J Bone Miner Res 2000;15:2245-50.

75. Bass S, Pearce G, Bradney M, et al. Exercise before puberty may confer residual benefits in bone density in adulthood: Studies in active prepubertal and retired female gymnasts. Journal of Bone and Mineral Research 1998;13:500-7.

76. Karlsson MK, Johnell O, Obrant KJ. Is bone mineral density advantage maintained long-term in previous weight lifters? Calcif Tissue Int 1995;57:325-8.

77. Magnusson H, Linden C, Karlsson C, Obrant KJ, Karlsson MK. Exercise may induce reversible low bone mass in unloaded and high bone mass in weight-loaded skeletal regions. Osteoporos Int 2001;12:950-5.

78. McKay H, Smith E. Winning the battle against childhood physical inactivity: the key to bone strength? J Bone Miner Res 2008;23:980-5.

79. Cromer B, Harel Z. Adolescents: at increased risk for osteoporosis? Clin Pediatr (Phila) 2000;39:565-74.

80. 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. Am J Clin Nutr 2008;87:455-62.

81. Rozen GS, Rennert G, Dodiuk-Gad RP, et al. Calcium supplementation provides an extended window of opportunity for bone mass accretion after menarche. Am J Clin Nutr 2003;78:993-8.

82. Moshfegh AG, J; Cleveland, L. What We Eat in America, NHANES 2001-2002: Usual Nutrient Intakes form Food Compared to Dietary Reference Intakes. In: U.S. Department of Agriculture ARS, ed.; 2005.

83. Whiting SJ, Vatanparast H, Baxter-Jones A, Faulkner R, Mirwald R, Bailey DA. Factors that Affect Bone Mineral Accrual in the Adolescent Growth Spurt. The Journal of Nutrition 2004:696S-700S.

84. Raisz LG. Physiology and pathophysiology of bone remodeling. Clin Chem 1999;45:1353-8.

85. Liel Y, Shany S, Smirnoff P, Schwartz B. Estrogen increases 1,25-dihydroxyvitamin D receptors expression and bioresponse in the rat duodenal mucosa. Endocrinology 1999;140:280-5.

86. Draper CR, Edel MJ, Dick IM, Randall AG, Martin GB, Prince RL. Phytoestrogens reduce bone loss and bone resorption in oophorectomized rats. Journal of Nutrition 1997;127:1795-9.

87. Riggs BL, Khosla S, Melton LJ, 3rd. A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. J Bone Miner Res 1998;13:763-73.

88. Prince RL. Counterpoint: estrogen effects on calcitropic hormones and calcium homeostasis. Endocr Rev 1994;15:301-9.

89. Bikle DD, Harris J, Halloran BP, Currier PA, Tanner S, Morey-Holton E. The molecular response of bone to growth hormone during skeletal unloading: regional differences. Endocrinology 1995;136:2099-109.

90. Rosen CJ. Serum insulin-like growth factors and insulin-like growth factor-binding proteins: clinical implications. Clin Chem 1999;45:1384-90.

91. Lofqvist C, Andersson E, Gelander L, Rosberg S, Blum WF, Albertsson Wikland K. Reference values for IGF-I throughout childhood and adolescence: a model that accounts simultaneously for the effect of gender, age, and puberty. J Clin Endocrinol Metab 2001;86:5870-6.

92. Abu EO, Horner A, Kusec V, Triffitt JT, Compston JE. The localization of androgen receptors in human bone. J Clin Endocrinol Metab 1997;82:3493-7.

93. Klein RG, Arnaud SB, Gallagher JC, Deluca HF, Riggs BL. Intestinal calcium absorption in exogenous hypercortisonism. Role of 25-hydroxyvitamin D and corticosteroid dose. J Clin Invest 1977;60:253-9.

94. Hahn TJ, Halstead LR, Teitelbaum SL, Hahn BH. Altered mineral metabolism in glucocorticoid-induced osteopenia. Effect of 25-hydroxyvitamin D administration. J Clin Invest 1979;64:655-65.

95. Pantelakis SN, Sinaniotis CA, Sbirakis S, Ikkos D, Doxiadis SA. Night and day growth hormone levels during treatment with corticosteroids and corticotrophin. Arch Dis Child 1972;47:605-8.

96. Faulkner KG, Cummings SR, Black D, Palermo L, Gluer C-C, Genant HK. Simple measurement of femoral geometry predicts hip fracture: The study of osteoporotic fractures. Journal of Bone and Mineral Research 1993;8 (10):1211-7.

97. Young D, Hopper JL, Macinnis RJ, Nowson CA, Hoang NH, Wark JD. Changes in body composition as determinants of longitudinal changes in bone mineral measures in 8 to 26-year-old female twins. Osteoporos Int 2001;12:506-15.

98. Baxter-Jones AD, Eisenmann JC, Mirwald RL, Faulkner RA, Bailey DA. The influence of physical activity on lean mass accrual during adolescence: a longitudinal analysis. J Appl Physiol 2008;105:734-41.

99. Schoenau E, Neu CM, Beck B, Manz F, Rauch F. Bone mineral content per muscle cross-sectional area as an index of the functional muscle-bone unit. J Bone Miner Res 2002;17:1095-101.

100. Xu L, Nicholson P, Wang Q, Alen M, Cheng S. Bone and muscle development during puberty in girls: a seven-year longitudinal study. J Bone Miner Res 2009;24:1693-8.

101. Reid IR. Relationships among body mass, its components, and bone. Bone 2002;31:547-55.

102. Whiting SJ. Obesity is not protective for bones in childhood and adolescence. Nutr Rev 2002;60:27-30.

103. Reid IR. Relationships between fat and bone. Osteoporos Int 2008;19:595-606.

104. Premaor MO, Pilbrow L, Tonkin C, Parker R, Compston J. Obesity and Fractures in Postmenopausal Women. J Bone Miner Res 2009.

105. Goulding A, Jones IE, Taylor RW, Manning PJ, Williams SM. More broken bones: a 4-year double cohort study of young girls with and without distal forearm fractures. J Bone Miner Res 2000;15:2011-8.

106. Dimitri P, Wales J, Bishop N. Fat and Bone in Children - Differential Effects of Obesity on Bone Size and Mass According to Fracture History. J Bone Miner Res 2009.

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

108. Frost HM. Obesity, and bone strength and "mass": a tutorial based on insights from a new paradigm. Bone 1997;21:211-4.

109. Young D, Hopper JL, Nowson CA, et al. Determinants of bone mass in 10- to 26-year-old females: a twin study. Journal of Bone and Mineral Research 1995;10(4):558-67.

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

111. Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. Jama 2004;291:2847-50.

112. Gordon C, Bachrach L, Carpenter TO, Karsenty G, Rauch F. Bone health in children and adolescents: a symposium at the annual meeting of the Pediatric Academic Societies, May 2003. Curr Probl Pediatr Adolesc Health Care 2004;34:226-42.

113. Petit MA, Beck TJ, Kontulainen SA. Examining the developing bone: What do we measure and how do we do it? J Musculoskelet Neuronal Interact 2005;5:213-24.

114. Wainwright S, Biggs W, Currey J, Gossline J. Mechanical design in organisms. London: Edward Arnold; 1976.

115. Rauch F, Schonau E. Skeletal development in premature infants: a review of bone physiology beyond nutritional aspects. Arch Dis Child 2002;86:F82-F5.

116. Rauch F, Schoenau E. The developing bone: slave or master of its cells and molecules? Pediatr Res 2001;50:309-14.

117. Klein GL, Fitzpatrick LA, Langman CB, et al. The state of pediatric bone: summary of the ASBMR pediatric bone initiative. J Bone Miner Res 2005;20:2075-81.

118. Faulkner RA, Bailey DA. Osteoporosis: a pediatric concern? Med Sport Sci 2007;51:1-12

119. Heaney RP. BMD: the problem. Osteoporos Int 2005;16:1013-5.

120. Rockell JE, Williams SM, Taylor RW, Grant AM, Jones IE, Goulding A. Two-year changes in bone and body composition in young children with a history of prolonged milk avoidance. Osteoporos Int 2005;16:1016-23.

121. 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. Int J Obes Relat Metab Disord 2000;24:627-32.

122. Goulding A, Grant AM, Williams S. Bone and body composition of children and adolescents with repeated forearm fractures. J Bone Miner Res 2005;20:2090-6.

123. Weiler HA, Janzen L, Green K, Grabowski J, Seshia MM, Yuen KC. Percent body fat and bone mass in healthy Canadian females 10 to 19 years of age. Bone 2000;27:203-7.

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

125. Rocher E, Chappard C, Jaffre C, Benhamou CL, Courteix D. Bone mineral density in prepubertal obese and control children: relation to body weight, lean mass, and fat mass. J Bone Miner Metab 2008;26:73-8.

126. Crabtree NJ, Kibirige MS, Fordham JN, et al. The relationship between lean body mass and bone mineral content in paediatric health and disease. Bone 2004;35:965-72.

127. 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;36:568-76

128. El Hage RC, CB. Jacob, C. Jaffre, C. . Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys. European Journal of Applied Physiology 2009:759-64.

129. El Hage RJ, C. Moussa, E. Benhamou, CL. Jaffre, C. Total body, lumbr spine and hip bone mineral density in overweight adolescent girls: decreased or increased? J Bone Miner Metab 2009;27:629-33.

130. 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:S274-7.

131. Leonard MB, Shults J, Wilson BA, Tershakovec AM, Zemel BS. Obesity during childhood and adolescence augments bone mass and bone dimensions. Am J Clin Nutr 2004;80:514-23.

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

133. Petit MA, Beck TJ, Hughes JM, Lin HM, Bentley C, Lloyd T. Proximal femur mechanical adaptation to weight gain in late adolescence: a six-year longitudinal study. J Bone Miner Res 2008;23:180-8.

134. 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;90:1104-11.

135. Khosla S, Melton LJ, 3rd, Dekutoski MB, Achenbach SJ, Oberg AL, Riggs BL. Incidence of childhood distal forearm fractures over 30 years: a population-based study. Jama 2003;290:1479-85.

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

137. Seeman E, Delmas PD. Bone quality--the material and structural basis of bone strength and fragility. N Engl J Med 2006;354:2250-61.

138. Pollock N, Bredas V, Laing E, Baile C, Hamrick M, Lewis R. Body fat is negatively associated with bone strength and geometric indices of the tibia and radius in adolescent females. J Bone Miner Res 2006;21:S377.

139. Fricke O, Land C, Semler O, et al. Subcutaneous fat and body fat mass have different effects on bone development at the forearm in children and adolescents. Calcif Tissue Int 2008;82:436-44.

CHAPTER 3

A PROSPECTIVE ANALYSIS OF BODY FAT AND BONE MINERAL ACCRUAL DURING PUBERTAL GROWTH

Harris, K.L., Laing, E.M., Hall, D.B., Baile C.A., Lewis R.D. 2009. To be submitted to the American Journal of Clinical Nutrition.

A Prospective Analysis of Body Fat and Bone Mineral Accrual During Pubertal Growth

Harris, K.L.[1], Laing, E.M.[1], Hall, D.B.[2], Pollock N.K.[3], Baile C.A., Lewis R.D.[1]

[1]Department of Foods and Nutrition, The University of Georgia, Athens, GA, USA
[2]Department of Statistics, The University of Georgia, Athens, GA, USA
[3]Department of Pediatrics, Prevention Institute, Medical College of Georgia, Augusta, GA, USA

Running Title: OBESITY, ADOLESCENT, FEMALE, PUBERTY, BMC ACCRUAL, BODY COMPOSITION

Emails:
harrisk5@uga.edu
emonk@uga.edu
danhall@uga.edu
rgildea@uga.edu
NPOLLOCK@mail.mcg.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 effects of fat mass on bone mineral accrual from early childhood to adolescence are unclear. The purpose of this prospective investigation was to determine the effects of fat mass accumulation on changes in bone mineral content (BMC) in young females (N=191; aged 4 to 8 years at baseline), over a period of up to 11 years. Total body, lumbar spine, total proximal femur, and forearm BMC, as well as total body percent fat and fat mass, were determined by dual-energy X-ray absorptiometry. A mean annual increment (MAI), given as the slope of the regressions of each subject's repeated measurements over time, was calculated for each subject and for each measurement site. Pearson and partial correlations were calculated between the MAI for BMC at each bone site and the MAI for either percent body fat or fat mass. Partial correlations were corrected for the MAIs in total body lean mass and height. Both percent body fat and total body fat mass, respectively, were positively correlated to BMC at the total body ($R=0.42$, $p<0.0001$ and $R=0.69$, $p<0.0001$), lumbar spine ($R=0.23$, $p=0.003$ and $R=0.47$, $p<0.0001$), non-dominant total proximal femur ($R=0.38$, $p<0.0001$ and $R=0.60$, $p<0.0001$), and non-dominant forearm ($R=0.37$, $p<0.0001$ and $R=0.61$, $p<0.0001$). When corrected for height and total body lean mass, the relationships between lumbar spine BMC and total body percent fat ($p=0.99$) or fat mass ($p=0.552$) attenuated. Significant associations remained at the other bone sites even when height and total body lean mass were accounted for in the analyses. In summary, this longitudinal analysis of females ranging from early childhood through adolescence indicates a significant positive impact of adiposity on BMC accrual. It is unclear why adiposity is favorably impacting BMC accrual in this study, but examination of body fat distribution, structural and geometric bone parameters

and assessment of hormonal factors, such as adipocytokines, warrant further consideration.

Key words: Fat Mass, Percent Body Fat, Puberty, Bone Mineral Content Accrual

Introduction

A high body weight is associated with greater bone mass, and substantial evidence supports the belief that fat-free soft tissue (FFST) is the most important component of body composition with respect to bone mineral accrual during pubertal growth. Depending on the methodologies employed, fat mass has been reported to influence bone mineral content (BMC) accrual both negatively[1-2] and positively.[3-4] Our understanding of the fat-BMC relationship is complicated by the fact that fracture rates, in the past decade, have been higher in obese children than non-obese peers.[1-5] Whether or not fat mass impacts bone mineral accrual in growing children, as well as the degree of its influence, remains unclear. With the exponential increase in childhood and adolescent obesity rates[6] it is imperative to establish if excess body fat has detrimental influences on bone mineral accrual during growth.

Findings from cross-sectional studies that utilized dual energy X-ray absorptiometry (DXA)-derived areal bone mineral density (aBMD) or BMC in overweight youth have been mixed. Evidence of less than expected BMC at the total body[7,8] and lumbar spine[9] and a lower ratio of BMC to total body weight in obese vs. normal weight children[7] supports the theory that a higher level of body fat is unfavorable for the growing skeleton. Moreover, a negative association between BMC and fat mass has been shown in obese children with previous fractures.[2] Conversely, several studies suggest that fat mass positively impacts both BMC and aBMD and that

overweight and obese children have greater bone mass compared to normal weight peers when adjusting for height, maturation, or lean mass.[11-14] The statistical adjustments for either body weight, height, maturation, and/or fat-free mass and the use of aBMD as the primary outcome variable, may likely have contributed to these discrepant findings with respect to fat and bone. In addition, the synonymous reporting of aBMD and BMC may lead to misinterpretation of important differences among growing children. Instead, BMC and bone area, which account for bone size and detect significant changes that can be overlooked by measures of aBMD alone, are of greater reliability in childhood and adolescent bone studies.[15-16] Furthermore, the cross-sectional designs of these studies limit the observation of changes in body composition and bone mineral accrual before, during, and after the maturational period.

Few prospective studies exist in the literature that examine fat and bone gains during growth. Two DXA investigations support a positive association between changes in total body fat mass and total body BMC accrual when data are unadjusted or are corrected for height, maturation, or lean mass.[17,18] Despite following subjects through the maturational period, the conclusions of Young et al.[17] are primarily supported by measures of aBMD, limiting the scope of implications for growing children. To date, the only BMC data reported have been at the total body, which showed significant relationships with fat mass when height was accounted for during the growth- and post-growth phases.[17] Similarly, the prospective data from Clark et al.[18] report only total body (less head) BMC data, which showed positive relationships with fat mass in peripubertal children, aged ~10 years. Additionally, with only a two year follow up period, the narrow age range of this study may have limited the potential to assess the

effects of pubertal maturation stages in relation to fat mass. Pubertal maturation may influence the effects of fat mass and bone if the positive relationship between fat mass and BMC becomes negative in more mature females.[18,19] The evidence of these studies is again limited by the absence of observation throughout the full maturational spectrum. Therefore, it is crucial to extend the period of observation from childhood throughout the pubertal growth phase and into post-menarche ages to collect evidence that explores the theory that pubertal maturation affects the degree and/or direction of influence of adiposity on BMC.

Few prospective studies have examined multiple ethnicities or have accounted for repeated measurements of BMC at multiple anatomical sites, including both weight-bearing and non-weight bearing bones. The conclusions of previous investigations have been confounded by inadequate follow up periods as well as results of aBMD instead of or as higher precedence to BMC. To our knowledge, this study is the first to observe subjects over an 11-year time period from ages as young as 4 to as old as 19 years that has assessed both body composition and BMC accrual throughout the maturational period. The primary objective of this project was to determine if excess body fat accumulation impacts BMC accrual positively or negatively in females during growth.

Materials and Methods

Participants

Female children, aged 4 to 8 years at the onset of the study, from Athens, Georgia, USA participated in this investigation. Participants were healthy, reporting no history of conditions known to influence bone metabolism and were originally recruited for a two-year prospective study investigating the influence of artistic gymnastics

participation on changes in bone.[20] Recruitment for the original study occurred from fall 1997 to summer 2000, and all testing was completed at the Bone and Body Composition Laboratory at The University of Georgia. The present study included annual participant follow-up measurements up to 11 years since baseline. The ethnicity (Hispanic or Latino, non-Hispanic or Latino) and race (American Indian or Alaska Native, Asian, black or African American, Native Hawaiian or other Pacific Islander, white, or any combination of the above) of participants were categorized based on parent identification according to the National Institutes of Health Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research.[21] The ethnic distribution of the sample from the originally recruited sample (N = 204) was 64% white, 27% black, 3% Asian, 2% Hispanic, 1% Indian, and 3% other (i.e. biracial).[20] Follow up measurements were provided by 129 of these participants at 3 years (80 white, 30 black, 8 Asian, 1 Hispanic, 2 Indian, 3 Caucasian/African American, 3 Caucasian/Asian, 1 Caucasian/Hispanic, 1 Caucasian/American Indian); 95 at 4 years (65 white, 20 black, 5 Asian, 1 Hispanic, 1 Caucasian/African American, 2 Caucasian/Asian, 1 Caucasian/American Indian); 52 at 5 years (42 white, 3 black, 2 Asian, 1 Hispanic, 1 Indian, 2 Caucasian/African American, 1 Caucasian/Asian); 17 at 6 years (16 white, 1 black); 7 at 7 years (6 white, 1 black); 44 at 9 years (30 white, 6 black, 1 Asian, 1 Hispanic, 1 Indian, 1 Caucasian/African American, 2 Caucasian/Asian, 1 Caucasian/Hispanic, 1 Caucasian/American Indian); 12 at 10 years (8 white, 3 black, 1 Caucasian/African American); and 15 at 11 years since baseline (14 white, 1 black). Prior to follow-up appointment, each participant and guardian completed informed assent and consent forms, respectively. The University of Georgia Institutional Review Board for Human Subjects approved the study protocol.

Data Collection

Data collection transpired between October 1997 July 2009. The original study design called for a staggered enrollment of 4 subject cohorts, fall (September-November) winter (December-February), spring (March-May), and summer (June-August). All repeat measurements occurred during the same season that they were originally recruited and measured. The procedures for data collection included anthropometric measurements, bone scans, health history questionnaires, as well as physical activity and three-day diet questionnaires.

Anthropometric Measures

Anthropometric measures were conducted according to the Anthropometric Standardization Reference Protocol.[22] Each subject, wearing light clothing and no shoes, was weighed on a calibrated double beam balance scale measuring to the nearest 0.25 kg (Fairbanks Scales, Kansas City, MO). Height was measured to the nearest 0.1 cm on a wall-mounted standiometer (Novel Products, Rockton, IL). Anthropometric measurements from girls 6-10 years of age (n=10) who were measured twice by the same person within a 2-week period in our laboratory, were used to calculate single measure intraclass correlation (ICC) coefficients. The intraclass correlation coefficient is an index of the rating reliability of a single technician. The ICC (R-value) and the test-retest CV (%) values for height and weight were 0.99, 0.4% and 0.99, 1.4%, respectively. To determine body mass index (BMI) percentiles for each subject, BMI (in kg/m^2) values were plotted on BMI-for-age charts.[23]

Sexual Maturation

At each annual measurement, participants were assessed for pubertal maturation, according to stages of breast development (stages 1-5), by a physician using the criteria described by Tanner.[24] In our laboratory, the calculation of a single measure ICC for physician-assessed breast and pubic hair development in 6 to 10-year old girls (n=10) revealed a perfect agreement (R=1.0) when evaluated by the same physician twice within a two-week period. Sexual maturation was self-assessed following year two and was determined using the same modified version of the Stages of Sexual Development.[24] Subjects indicated their sexual maturation from a photographic representation that depicts breast development stages. An 86% agreement has been shown for self-assessment of breast development and physician evaluation of sexual maturity.[25] The following interpretation criteria have been suggested to identify pubertal stage: pre-pubertal (1: no development), early-pubertal (2-3: some development), and post-pubertal (4-5: after menarche).[26]

Bone Mineral Content and Body Composition

Dual energy X-ray absorptiometry was used to analyze both BMC and total body composition measurements. BMC (g) of the total body, lumbar spine, non-dominant total proximal femur and radius was measured using the Hologic QDR-1000W instrument (DXA; QDR-1000W, Hologic Inc., Waltham, MA). The lumbar spine analysis was performed using DXA Low Density Spine Software version 4.74 (Hologic Inc.), whereas DXA Pediatric Whole Body Analysis software version 5.73 (Hologic Inc.) was employed to analyze fat mass (g), percent fat, and fat-free soft tissue mass (g). At follow up, the lumbar spine and non-dominant total proximal femur were assessed using the Hologic QDR-1000W instrument. Measures of body composition and total body BMC were made

using the Hologic Delphi A (DXA; Delphi A, Hologic Inc., Waltham, MA) instrument. Analysis of the total body at follow up was performed using the DXA Pediatric Whole Body Analysis Software (version 12.4). DXA has been highlighted by Goran as an accurate method to assess body composition and skeletal mass in pediatric populations.[27] The DXA scan quality was ascertained via a daily calibration procedure using the provided manufacturers' standard phantoms. To calibrate the DXA machines, a lumbar spine phantom containing calcium hydroxyapatite and epoxy embedded in a Lucite cube was scanned (Hologic c-caliber anthropometric spine phantom, model DPA-QDR-1). The CV observed in our laboratory from 365 scans of the spine phantom on the Hologic 1000-W instrument over 5 years was 0.27%. A CV of 0.36% was observed in our laboratory from 648 scans of the spine phantom on the Delphi-A instrument over 3 years. The test-retest measurements for aBMD using the Hologic 1000-W instrument are: total body, 1.2%; lumbar spine, 1.3%; total proximal femur, 1.6%; and forearm, 2.1%. Quality control of soft tissue measurements were assured by weekly calibrations with a three-step soft tissue wedge, which contain varying thickness levels of aluminum and Lucite that have been calibrated to stearic acid (100% fat) and water (8.6% fat; Hologic, Inc.). In our laboratory, the percent fat single measure ICC and CV in 5 to 8-year old girls (n=10) scanned twice on the Hologic 1000-W instrument by the same technician within a 1-week period were R=0.99 and 2.0%, respectively. One-way random effects model, intraclass correlation coefficients were calculated in females (n = 10), aged 18-30 years, scanned twice using the Delphi-A instrument over a 7-day period for fat mass, fat-free soft tissue mass, and percentage body fat (all $R \geq 0.87$) and for BMC of the whole body, lumbar spine, and total proximal femur (all $R \geq 0.98$).

Dietary Intake

Dietary intake was assessed using a 3-day diet record completed by subjects and their parent at home for 2 weekdays and 1 weekend day. All data entries were analyzed by the same individual using Food Processor Nutrition and Dietary Analysis System (Version 7.9; ESHA Research, Salem, OR, USA). Diet records included time, type, and amount of food eaten, along with preparation method. Specifically, the food diary inquired about calcium-fortified foods and beverages consumed each day as well as nutritional supplements. The researcher subsequently added intake from fortified foods as well as calcium and vitamin D supplements to the dietary intake data. This method has been shown to be valid and reliable for estimating energy and nutrient intakes in children.[28-30] Accounts of nutrient intakes from 3-day diet records have been shown to correlate highly with direct observation (R = 0.78-0.94) in 9- to 10- year old females. In a study of late adolescents and young adults, 3-day diet records agreement score means ± standard deviation were 81 ± 15%.[31] Using a one-way random effects model, average measure ICCs were calculated in our laboratory for estimated dietary intake of energy (R = 0.47), calcium (R = 0.71), and vitamin D (R = 0.94) in female children 6-10 years of age (n = 10), who completed 3-day diet records twice in a 2-week period.

Physical Activity

Physical activity was assessed subjectively with a modified version of a questionnaire developed by Slemenda et al.[32] Trained researchers administered the questionnaire by interviewing the participant and her parent(s). Parents and participants were asked to indicate the participant's activity level compared to their peers on a 5-point Likert scale. The responses on the questionnaire numerically indicate varying levels of

physical activity compared to the subject's peers: 1 = inactive, 2 = below average, 3 = average, 4 = above average, and 5 = very high.

Statistical Analyses

Statistical Analysis Software version 9.1 (SAS, Cary, NC) was used to analyze the data, and statistical significance was considered for a P value ≤ 0.05. Descriptive statistics (mean ± SD) were calculated for all variables. A paired sample means t-test was used to analyze the change in variables over time in a sample of 81 subjects who had both a baseline pre-menarche measurement and a post-menarche measurement. To account for multiple measures over time and varying lengths of time between follow up visits for each participant, a "mean annual increment" (MAI) was estimated for each variable for each subject. Subsequent correlations and partial correlations between the estimated MAIs were computed. The MAI is the average yearly increase that a subject experienced over the range of years studied and is given by the slope of a regression of the subject's responses, taken repeatedly over time, by age. The variables for which a MAI was computed include BMC of the total body, lumbar spine, total proximal femur, and forearm, total percent body fat, kilograms of fat mass, kilograms of lean mass, and height in centimeters. Subsequent correlations and partial correlations between the estimated MAIs were computed. Pearson correlations were computed between the MAIs of both percent body fat and fat mass and each of the BMC variables (total body, spine, hip, and forearm). Partial Pearson correlations were computed between the MAIs for both percent body fat and fat mass and each of the BMC variables, controlling for the MAIs for height and lean mass.

Two different DXA instruments were used during the course of this study. The Hologic 1000-W (Hologic, Inc.) was used for scans obtained at earlier time points while the Delphi-A was used at later measurement occasions to obtain whole body data only, due to whole body software malfunction in the Hologic 1000-W. For measurements obtained using the Delphi-A, values that would have been obtained had the Hologic 1000-W been available, were calculated and assigned. These calculations were completed by developing a regression equation to predict Hologic-generated values from measurements obtained using the Delphi-A. This equation was derived from a separate dataset comprised of participants similar in age and ethnicity to the dataset of interest containing measurements from both machines for each subject. We assumed that the Hologic 1000-W and Delphi-A measurements are related to each other via a simple linear regression model:

(1) $$z_j^H = \alpha_0 + \alpha_1 z_j^D + e_j \text{ , } j = 1, \ldots, n,.$$

By fitting model (1) to the calibration dataset, we obtained a prediction equation that predicts the 1000-W measurement for a subject with a Delphi-A value. Using this predication equation, the 1000-W measurement for a subject i at measurement occasion j from the study sample of interest is represent by y_{ij}, where i = 1,…, N and j = 1, …, T. For most subjects, the last measurement occasion (the Tth) incorporates a Delphi-A in place of a 1000-W measurement, which is represented by y^D_{iT} instead of y^H_{iT}. At this occasion, we used the prediction equation calculated from fitting model (1) to the calibration dataset to obtain

(2) $$\hat{y}_{iT}^H = \hat{\alpha}_0 + \hat{\alpha}_1 y_{iT}^D.$$

If all measurements at all occasions were available from the 1000-W alone, the best fit

model for estimated the MAI is

(3) $$\hat{y}_{iT} = \beta_{i0} + \beta_{i1}age_{ij} + \varepsilon_{ij}.$$

where β_{i1} represents the MAI for the i^{th} subject, and ε_{ij} s is the individual error terms assumed to have mean = 0 and a suitable variance structure. A non-constant error variance fits this model for most of the variable of interest, so we assumed a flexible error variance as $var(\varepsilon_{ij}) = \sigma^2 \varepsilon^{\varphi age_{ij}}$ where φ and σ^2 are parameters to be estimated. Because $\hat{y}_{iT} = yi_T + \delta_{iT}$ and y_{iT} satisfies model (3), we have

(4) $$\hat{y}_{iT} = y_{iT} + \delta i_T = \beta_{i0} + \beta_{i1}\, age_{iT} + \varepsilon_{iT} + \delta_{iT},$$

and can estimate the MAIs for each subject using the model

(5) $$\omega_{ij} = \beta_{i0} + \beta_{i1}\, age_{ij} + u_{ij}\ ,\ i = 1, \ldots, N,\ j = 1, \ldots, T,$$

where $\omega_{ij} = y_{ij}$ and $u_{ij} = \varepsilon_{ij}$ if $j < T$ if we have an actual 1000-W measurement or $\omega_{ij} = \hat{y}_{ij}$ and $u_{ij} = \varepsilon_{ij} + \delta_{ij}$ if we have a Delphi-A measurement. Model (5) was fit to the sample data with the prediction error variance $var(\delta_{ij})$ estimated from the calibration data set. MAI estimates, $\beta_{i1,}$ from the fitted model were estimated for each variable of interest for each subject. Because each data point is actually an estimated slope from a separate regression model, the error of the estimated slopes was accounted for in correlation analyses by weighting by the MAI standard errors. The resulting correlations reflect associations between annual growth rates of the variables of interest and quantify how closely related rates of change in fat are to rates of bone mineral accrual controlling for measures of maturity, lean mass and height.

Results

Participant Characteristics

Participant characteristics at pre-menarche and post-menarche maturation stages are presented in **Table 1.** This study collected data from subjects followed over 11 years from as young as 4 to as old as 19 years of age. Eighty-one subjects (57 white, 16 black) provided measurements at both baseline and post-menarche. The average number of years after baseline at which the post-menarche measurement occurred was 7.9 years. Both height and weight increased significantly as did BMI, and the average BMI-for-age percentile was between the 5^{th} and $<85^{th}$ percentile both at pre- and post-menarche. Although BMI-for-age percentile did not change significantly during maturation, there were more subjects that would be classified as overweight and obese based on the BMI-for-age percentile cutoffs at post-menarche compared to pre-menarche. Percent body fat increased significantly and the number of subjects that are obese, based on ≥ 32 percent body fat, was greater at post-menarche than pre-menarche. During pubertal growth, BMC at all four sites significantly increased. **Figure 1** shows the BMC accrual for the spine, total proximal femur, and forearm during growth in 37 subjects with measurements at pre-, early-, and post-pubertal stages as determined by breast development stages. Dietary intake of calcium did not differ from pre-menarche to post-menarche. However, the adequate intake (AI) level of calcium for girls in the post-menarche age range is 500 mg greater than the AI for pre-menarche ages. Thus, subjects at post-menarche consumed about 400 mg on average less than the AI. Average vitamin D intake significantly decreased from pre- to post-menarche, but the average intake level at post-menarche was still adequate.

The Influence of Fat Mass and Percent Body Fat on BMC

Table 2 illustrates the R-values for the change in BMC MAIs at the total body, lumbar spine, proximal femur, and forearm and change in percent body fat MAI. At each of the four skeletal sites, there was a statistically significant positive correlation between BMC accrual and gain in percent body fat. This significant and positive correlation remained at the total body, proximal femur, and forearm when the data were weighted for the MAIs for total body lean mass and height. Conversely, when the change in MAI for BMC of the spine and that of percent body fat were weighted for the MAI for total body lean mass and height, the association was no longer statistically significant (P = 0.9902).

Table 3 depicts the R-values for the changes in BMC MAIs at the total body, lumbar spine, proximal femur, and forearm and the change in total body fat mass MAI. At each of the four skeletal sites, there was a significant and positive correlation between BMC accrual and gain in total body fat mass. This significantly positive correlation remained at the total body, proximal femur, and forearm when the data were weighted for the MAIs in total body lean mass and height. Conversely, when the change in MAIs for BMC of the spine and total body fat mass were weighted for the MAIs in total body lean mass and height, the association was no longer statistically significant (P = 0.5520).

Discussion

The current study is one of the few prospective reports assessing bone mineral changes in females followed from the pre-pubertal years into post-menarche. The primary finding was that with advancing age and pubertal maturation, females who gain greater amounts of body fat also accrue more BMC. This was true for the hip and forearm even

after correcting for gains in height and lean mass. While some childhood bone studies suggest that fat is not advantageous for bone, this study does not support that hypothesis.

Two other prospective studies have examined the impact of fat on BMC gains in children and adolescents but both have focused on total body BMC only. Our results are aligned with those of Young et al.[17] who reported that fat mass is a strong independent predictor of total body BMC both during pubertal growth and in the post-puberty phase. The findings of Clark et al.[18] also show that fat mass positively predicts total body BMC, but only in prepubertal females. In the later stages of maturation, fat was inversely related to total body BMC accrual and thus, seems dependent on maturation.[18] While we did not correct for maturation or breast stage in our statistical approach, correcting for changes in height is a reliable approach to account for maturational changes.[33]

The positive associations of mean annual increments in fat mass or body fat percent with BMC of the total body, total proximal femur, and forearm suggest that adiposity is not detrimental to bone mineral accrual and may in fact benefit skeletal development. Our data support the cross-sectional findings of Ellis et al.,[11] Timpson et al.,[34] and Leonard et al.[35] who reported that total body BMC or regional BMC were associated with higher fat mass after adjusting for several variables, including height, in the statistical models. However, Leonard et al.[35] found greater vertebral BMD in obese children which disagrees with our results of no relationship between fat mass gains and BMC accrual of the spine. The results of our study also oppose the previous conclusions that fat is not related to[3] or is disadvantageous for the growing skeleton.[2,36] Moreover, the reports that obese and overweight children are at higher risk of fracture[5,37,38] are not supported by our results. Instead, the higher incidence of fracture in obese children may

be related to greater magnitude of force during a fall, increased postural sway, or impaired balance.[39] The discrepancies between our conclusions and previous reports may be due to variance in statistical methods (i.e. adjusting for height vs. weight) and the criteria used to distinguish obese from non-obese (i.e. BMI percentile vs. percent body fat). Inconsistencies among the bone sites used to report bone mineral data also contribute to disparate results.

It is unclear why greater fat gains led to greater BMC accrual at most bone sites. There are several possible mechanisms for the positive relationship of fat and bone mineral, including weight bearing vs. non-weight bearing sites, bone type, and endocrine factors. Our analyses included measurements of both weight-bearing (i.e. total proximal femur) and non-weight bearing (i.e. non-dominant forearm) bones. Percentage body fat and total fat mass were positively correlated to BMC accrual in bones that are load-bearing as well as those that are not. Furthermore, the four bone measurement sites included varying compositions of cortical and trabecular bone. Our study reports no significance between changes in fat mass or percentage body fat and changes in lumbar spine BMC after adjusting for lean mass and height. One explanation may be the differences in bone composition between the lumbar spine and other bone sites. For example, the non-dominant forearm and total proximal femur consists mainly of cortical bone whereas the lumbar spine, contains mostly trabecular bone.[40] Trabecular bone is more metabolically active than cortical bone, and when mechanically stressed, trabecular bone adapts primarily by enhanced mineralization where cortical bone mainly increases in size.[41,42] However, the spine of obese children has been shown to be undermineralized relative to body weight,[9] which was not adjusted for in our analyses. Furthermore, whole

body fat mass may be less predictive of trabecular bone than site-specific fat stores. We did not assess the effects of different distributions of fat mass on bone (i.e. central vs. appendicular adiposity) so we were unable to ascertain whether the spine is affected by trunk fat whereas the other bone sites are affected by appendicular fat mass.

Fat may influence bone accrual through the action of the adipocyte secretion of adipokines.[43] Excess adipose tissue may also enhance bone mineral accrual indirectly by increasing estrogen and altering the timing of pubertal events[44] or by initiating puberty earlier in obese children than their normal-weight peers.[45] Although serum estradiol was not assessed in the current study, if obesity stimulates puberty earlier, estrogen exposure may be prolonged and bone mineral accrual may be initiated earlier, resulting in greater BMC gains during growth. Bone growth may also be stimulated by adiposity through the production of leptin, which has been shown to positively predict bone area and bone area growth in 8 to 13 year-old girls.[46] Leptin amplifies skeletal growth by upregulating the expression of insulin-like growth factor-1[47] and osteoblast differentiation[48] thereby increasing both linear growth and bone mass.

Quality control issues arise when conducting a long-term study. This prospective study is strengthened by the consistency in lab technicians from baseline testing who scanned and analyzed for body composition and BMC using the same DXA instrument. Also, subjects and subjects' parents completed the same dietary intake and physical activity questionnaires from baseline at each follow-up appointment, thus minimizing confusion and non-compliance errors. A limitation of the current study is the need to utilize two different DXAs. At the last measurement occasion, the Delphi-A instrument was used for whole body bone and body composition measurements in place of the

original Hologic 1000-W instrument. However, prior to the switch, a separate dataset of individuals scanned on both instruments was used to derive a conversion equation that predicts a Hologic 1000-W measurement from that obtained using the Delphi-A instrument. Therefore, measurements at the last follow-up were corrected for the substitution in instruments.

This prospective study measures only the material properties of bone and did not use pQCT to measure structural changes. The implications of this investigation are limited by the absence of three-dimensional assessments for indices of bone strength, which are quantified by pQCT. Ultimately, the implications of our study relate to fracture prevention via increased bone strength, which is a function of both material and structural properties. Fat may affect structural parameters differently than material properties.[36] For example, despite increased mineralization in relation to excess fat mass, there may be less endocortical or periosteal apposition in response to greater fat gains, which would negatively impact bone strength.[36] Our investigation opposes the theory that higher adiposity is not beneficial to bone or that it detracts from maximum bone development.[49] However, in the present investigation, pQCT measurements were not collected at baseline, thus eliminating prospective evaluation using this technology. Furthermore, the current study did not compare variables by race because the sample size of African Americans became too small to detect significant differences. However, one study found a race-dependent, negative relationship between total trunk weight and abdominal fat mass in Caucasians and African Americans. In African American children, intra-abdominal adipose tissue was inversely associated with BMC, but in Caucasian children, subcutaneous abdominal adipose tissue was inversely associated to BMC. Our

population, while mixed in race, was predominantly Caucasian, which may have skewed results if intra-abdominal fat mass in African Americans was exerting a negative influence on bone mineral gains. On the other hand, in a sample of Caucasians and African American boys and girls aged 7 to 12 years analyzed only for the effects of percent body fat on bone, there was no association found between race/ethnicity and BMC,[50] which supports our decision to include all races in the analyses.

In summary, greater gains in fat mass were associated with greater bone mineral accrual at the hip and forearm over the course of pubertal maturation. Future studies are needed to address the differential effects of structural changes, fat and its distribution, and race on bone mineral content accrual.

Table 3.1. Participant characteristics at pre-menarche and post-menarche.[1]

	Pre-menarche[2]	Post-menarche[2]
Age & Anthropometry		
Age	6.5 ± 1.6	14.6 ± 2.2*
Height (cm)	120.1 ± 11.9	163.5 ± 7.7*
Weight (kg)	25.0 ± 7.7	61.6 ± 14.2*
BMI (kg/m^2)	17.0 ± 2.5	23.0 ± 4.8*
BMI-for-age percentile	67.2 ± 27.0	68.3 ± 28.0
Overweight prevalence (%)[3]	30%	38%
Obese prevalence (%)[3]	11%	16%
Tanner Stage[4]	1.0 ± 0.27	3.5 ± 1.0*
Body Composition[5]		
Fat Mass (kg)	6.58 ± 3.93	20.3 ± 10.5*
Lean Mass (kg)	16.8 ± 4.2	39.5 ± 6.41*
Body Fat %	25.5 ± 7.81	31.2 ± 9.04*
Obese prevalence (%)[6]	23%	38%
Total Body		
BA (cm^2)	1244 ± 261	2382 ± 291*
BMC (kg)	853 ± 264	2206 ± 376*
aBMD (g/cm^2)	0.68 ± 0.07	0.92 ± 0.90*
Lumbar Spine		
BA (cm^2)	30.6 ± 5.09	57.6 ± 7.22*
BMC (kg)	17.4 ± 5.0	55.4 ± 13.6*
aBMD (g/cm^2)	0.56 ± .07	0.94 ± 0.14*
Total Proximal Femur		
BA (cm^2)	16.2 ± 4.2	31.8 ± 4.9*
BMC (kg)	10.2 ± 3.8	30.7 ± 6.6*
aBMD (g/cm^2)	0.61 ± .08	0.94 ± 0.14*
Forearm		
BA (cm^2)	6.39 ± .49	13.5 ± 1.96*
BMC (kg)	2.34 ± .81	6.75 ± 1.48*
aBMD (g/cm^2)	0.36 ± .05	0.50 ± 0.06*
Dietary Intake		
Energy (kcals)	1627 ± 32	1729 ± 431
Protein (g)	54.5 ± 3.9	69.8 ± 14.9*
Calcium (mg)	861 ± 42	875 ± 444
Vitamin D (µg)	9.97 ± .66	6.68 ± 9.12**
Physical Activity[7]	3.59 ± .77	3.28 ± 0.76***

[1]All values are mean ± SD. n = 81 for both pre-menarche and post-menarche.

* $p < 0.0001$, ** $p = 0.014$, *** $p = 0.006$

[2] Pre-menarche = prior to menses at baseline; post-menarche = after menses at follow-up.

[3] BMI classification as determined by CDC growth charts for children.[51] BMI >85th percentile = overweight. BMI >95th percentile = obese.

[4] Maturation stages determined by breast development as defined by Tanner.[24] Pre-pubertal = breast stage 1; early-pubertal = breast stage 2 or 3 and 3.3 ± 1.5 years from baseline; post-pubertal = breast stage 4 or 5 and 7.9 ± 2.6 years from baseline.

[5] Measurements assessed using DXA.

[6] Classification of body fat percent based on amounts of body fat associated with cardiovascular disease risks.[52] Body fat percent ≥ 32% = obese.

[7] Physical activity level assessed by parent questionnaire developed by Slemenda et al.[53] (1 = inactive, 2 = below average, 3 = average, 4 = above average, and 5 = very high).

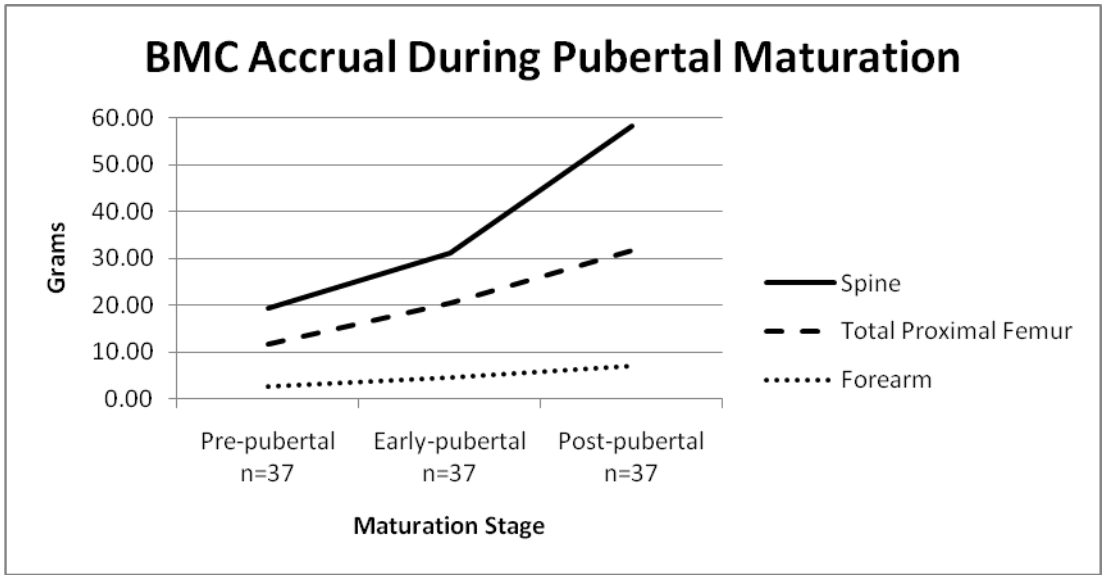


Figure 3.1. BMC accrual at pre-, early-, and post-pubertal maturation stages. Repeated measurements of 37 subjects captured BMC at the spine, total proximal femur, and forearm during each pubertal stage. Maturation stages were determined by self-report of breast development according to the pubertal stages as defined by Tanner.[24]

Table 3.2. Correlations (R-values) relating mean annual increment (MAI) of bone mineral content (BMC) to MAI for percent body fat in females over a period of up to 11 years.[1]

Skeletal Site	% Body Fat	
	Pearson[†]	**Pearson Partial**[‡]
TBBMC[2]	0.42225	0.52031
P-value	<0.0001	<0.0001
SPIBMC[3]	0.22852	0.00095
P-value	0.0026	0.9902
TPFBMC[2]	0.37905	0.35291
P-value	<0.0001	<0.0001
FRMBMC[2]	0.36849	0.31771
P-value	<0.0001	<0.0001

[1] Correlations and partial correlations for percent body fat (% body fat) and total body bone mineral content (TBBMC), lumbar spine bone mineral content (SPIBMC), total proximal femur bone mineral content (TPFBMC), and forearm bone mineral content (FRMBMC).

[2] Correlations are based on n = 172

[3] Correlations are based on n = 171

[†] The R value explains the association between the MAI for BMC of the total body, lumbar spine, proximal femur, or forearm and the MAI for percent body fat. This association illustrates the relationship of changes in BMC at each skeletal site and changes in percent body fat up to 11 years.

‡ The R value explains the association between the MAI for BMC of the total body, lumbar spine, proximal femur, or forearm and the MAI for percent body fat, accounting for the effects of MAIs for height and lean mass.

Table 3.3. Correlations (R-values) relating mean annual increment (MAI) of bone mineral content (BMC) to MAI for total body fat mass in females over a period of up to 11 years.[1]

Skeletal Site	Total Body Fat (kg)	
	Pearson[†]	**Pearson Partial**[‡]
TBBMC[2]	0.69017	0.65296
P-value	<0.0001	<0.0001
SPIBMC[3]	0.47449	0.04607
P-value	<0.0001	0.5520
TPFBMC[2]	0.59568	0.31039
P-value	<0.0001	<0.0001
FRMBMC[2]	0.60826	0.33974
P-value	<0.0001	<0.0001

[1] Correlations and partial correlations for total body fat mass (kg) and total body bone mineral content (TBBMC), lumbar spine bone mineral content (SPIBMC), total proximal femur bone mineral content (TPFBMC), and forearm bone mineral content (FRMBMC).

[2] Correlations are based on n = 172

[3] Correlations are based on n = 171

[†] The R value explains the association between the MAI for BMC of the total body, lumbar spine, proximal femur, or forearm and the MAI for percent body fat. This association illustrates the relationship of changes in BMC at each skeletal site and changes in total body fat mass up to 11 years.

‡ The R value explains the association between the MAI for BMC of the total body, lumbar spine, proximal femur, or forearm and the MAI for total body fat mass, accounting for the effects of mean annual increments in height and lean mass.

References

1. Goulding A, Jones IE, Taylor RW, Manning PJ, Williams SM. More broken bones: a 4-year double cohort study of young girls with and without distal forearm fractures. J Bone Miner Res 2000;15:2011-8.

2. Dimitri P, Wales J, Bishop N. Fat and Bone in Children - Differential Effects of Obesity on Bone Size and Mass According to Fracture History. J Bone Miner Res 2009.

3. Faulkner KG, Cummings SR, Black D, Palermo L, Gluer C-C, Genant HK. Simple measurement of femoral geometry predicts hip fracture: The study of osteoporotic fractures. Journal of Bone and Mineral Research 1993;8 (10):1211-7.

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

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

6. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. Jama 2006;295:1549-55.

7. 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. Int J Obes Relat Metab Disord 2000;24:627-32.

8. Weiler HA, Janzen L, Green K, Grabowski J, Seshia MM, Yuen KC. Percent body fat and bone mass in healthy Canadian females 10 to 19 years of age. Bone 2000;27:203-7.

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

10. Rocher E, Chappard C, Jaffre C, Benhamou CL, Courteix D. Bone mineral density in prepubertal obese and control children: relation to body weight, lean mass, and fat mass. J Bone Miner Metab 2008;26:73-8.

11. 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:S274-7.

12. 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;36:568-76.

13. El Hage RJ, C. Moussa, E. Benhamou, CL. Jaffre, C. Total body, lumbr spine and hip bone mineral density in overweight adolescent girls: decreased or increased? J Bone Miner Metab 2009;27:629-33.

14. El Hage RC, CB. Jacob, C. Jaffre, C. . Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys. European Journal of Applied Physiology 2009:759-64.

15. Rockell JE, Williams SM, Taylor RW, Grant AM, Jones IE, Goulding A. Two-year changes in bone and body composition in young children with a history of prolonged milk avoidance. Osteoporos Int 2005;16:1016-23.

16. Petit MA, Beck TJ, Kontulainen S. Examining the developing bone: What do we measure and how do we do it? J Musculoskelet Neuronal Interact 2005;5:213-24.

17. Young D, Hopper JL, Macinnis RJ, Nowson CA, Hoang NH, Wark JD. Changes in body composition as determinants of longitudinal changes in bone mineral measures in 8 to 26-year-old female twins. Osteoporos Int 2001;12:506-15.

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

19. Petit MA, Beck TJ, Hughes JM, Lin HM, Bentley C, Lloyd T. Proximal femur mechanical adaptation to weight gain in late adolescence: a six-year longitudinal study. J Bone Miner Res 2008;23:180-8.

20. Laing EM, Wilson AR, Modlesky CM, O'Connor PJ, Hall DB, Lewis RD. Initial years of recreational artistic gymnastics training improves lumbar spine bone mineral accrual in 4- to 8-year-old females. J Bone Miner Res 2005;20:509-19.

21. USHHS. NIH Policy and Guidelines on The Inclusion of Women and Minorities as Subjects in Clinical Research. In: Research OoE, ed.; 2001.

22. Lohman TG, Roche AF, Martorell R. Anthropometric Standardization Reference manual. Champaign, IL: Human Kinetics; 1988.

23. Kuczmarski RJ, Ogden CL, Guo SS, et al. 2000 CDC Growth Charts for the United States: methods and development. Vital Health Stat 11 2002:1-190.

24. Tanner J. Growth and Adolescence. 2nd ed. Oxford: Blackwell Scientific Publications; 1962.

25. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics 1980;66:918-20.

26. Slemenda CW, Reister TK, Hui SL, Miller JZ, Christian JC, Johnston Jr. CC. Influences on skeletal mineralization in children and adolescents: Evidence for varying effects of sexual maturation and physical activity. The Journal of Pediatrics 1994;125:201-7.

27. Goran M. Measurement Issues Related to Studies of Childhood Obesity: Assessment of Body Composition, body Fat Distribution, Physical Activity, and Food Intake. Pediatrics 1997;supplement:505-17.

28. Taylor RW, Goulding A. Validation of a short food frequency questionnaire to assess calcium intake in children aged 3 to 6 years. Eur J Clin Nutr 1998;52:464-5.

29. Bergman EA, Boyungs JC, Erickson ML. Comparison of a food frequency questionnaire and a 3-day diet record. Journal of American Dietetic Association 1990;90(4):1431-3.

30. Crawford PB, Obarzanek E, Morrison J, Sabry ZI. Comparative advantage of 3-day food records over 24-hour recall and 5- day food frequency validated by observation of 9- and 10-year-old girls. J Am Diet Assoc 1994;94:626-30.

31. Krantzler NJ, Mullen BJ, Schutz HG, Grivetti LE, Holden CA, Meiselman HL. Validity of telephoned diet recalls and records for assessment of individual food intake. Am J Clin Nutr 1982;36:1234-42.

32. Slemenda CW, Miller JZ, Hui SL, Reister TK, Johnston Jr. CC. Role of physical activity in the development of skeletal mass in children. Journal of Bone and Mineral Research 1991;6:1227-33.

33. Garn SM, Haskell JA. Fat thickness and developmental status in childhood and adolescence. AMA J Dis Child 1960;99:746-51.

34. Timpson NJ, Sayers A, Davey-Smith G, Tobias JH. How does body fat influence bone mass in childhood? A Mendelian randomization approach. J Bone Miner Res 2009;24:522-33.

35. Leonard MB, Shults J, Wilson BA, Tershakovec AM, Zemel BS. Obesity during childhood and adolescence augments bone mass and bone dimensions. Am J Clin Nutr 2004;80:514-23.

36. Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, D. LR. Is adiposity advantageous for bone strength? A pQCT study in adolescent females. Am J Clin Nutr 2007;86:1530-8.

37. Goulding A, Jones IE, Taylor RW, Williams SM, Manning PJ. Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. J Pediatr 2001;139:509-15.

38. Goulding A, Grant AM, Williams S. Bone and body composition of children and adolescents with repeated forearm fractures. J Bone Miner Res 2005;20:2090-6.

39. Goulding A, Jones IE, Taylor RW, Piggot JM, Taylor D. Dynamic and static tests of balance and postural sway in boys: effects of previous wrist bone fractures and high adiposity. Gait Posture 2003;17:136-41.

40. Riggs BL, Wahner HW, Seeman E, et al. Changes in bone mineral density of the proximal femur and spine with aging. Differences between the postmenopausal and senile osteoporosis syndromes. J Clin Invest 1982;70:716-23.

41. Rico H, Gonzalez-Riola J, Revilla M, Villa LF, Gomez-Castresana F, Escribano J. Cortical versus trabecular bone mass: influence of activity on both bone components. Calcif Tissue Int 1994;54:470-2.

42. Ducher G, Prouteau S, Courteix D, Benhamou CL. Cortical and trabecular bone at the forearm show different adaptation patterns in response to tennis playing. J Clin Densitom 2004;7:399-405.

43. Pollock NK, Dong, Y., Gutin, B., and Hamrick, M.W. Lower bone mass and adiponectin in overweight adolescents with cardiovascular risk factors. J Bone Miner Res 2009;24.

44. Fisch R.C. RR. Height and weight at menarche and a hypothesis of menarche. Arch Dis Child 1971;46:695-701.

45. Klein K.O. LK, de Lancey E, Brown JM, Considine RV, Hassink SG. Effect of obesity on estradiol level, and its relationship to leptin, bone maturation and BMD in children. J Clin Endocrinol Metab 1998;83:3469-75.

46. Matkovic V, Ilich JZ, Skugor M, et al. Leptin is inversely related to age at menarche in human females. J Clin Endocrinol Metab 1997;82:3239-45.

47. Maor G. RM, Segev Y., Phillip M. Leptin acts as growth factor on the chondrocytes of skeletal growth centers. J Bone Miner Res 2002;17:1034-43.

48. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. Endocrinology 1999;140:1630-8.

49. Pollock N, Bredas V, Laing E, Baile C, Hamrick M, Lewis R. Body fat is negatively associated with bone strength and geometric indices of the tibia and radius in adolescent females. J Bone Miner Res 2006;21:S377.

50. Casazza K, Thomas O, Dulin-Keita A, Fernandez JR. Adiposity and genetic admixture, but not race/ethnicity, influence bone mineral content in peripubertal children. J Bone Miner Metab 2010.

51. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. Adv Data 2000:1-27.

52. Fitnessgram Reference Guide - Body composition assessment. The Cooper Institute, 2001. (Accessed September 9, 2005, at http://www.cooperinst.org/shopping/PDF%20single/BodyCompositionAssessment.pdf.)

53. Slemenda CW, Christian JC, Williams CJ, Norton JA, Johnston Jr CC. Genetic determinants of bone mass in adult women: A reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. Journal of Bone and Mineral Research 1991;6(6):561-7.

CHAPTER 4

SUMMARY AND CONCLUSIONS

Soft tissue masses, in particular ft mass and fat free soft tissue, have the potential to influence the degree and timing of peak bone mineral accrual. The positive influence of lean mass on bone mineralization during growth is well established,[1-2] but the relationships between fat and bone mineral accrual are still unclear. The conflicting findings may be due to differences in the body composition parameters assessed (i.e. BMI vs. fat mass) and the statistical values used as covariates (i.e. height vs. weight) in the analyses. Furthermore, only a few studies have prospectively examined the effects of increasing adiposity on BMC accrual through pubertal growth.

The aim of the present study was to prospectively evaluate the relationship of body fat accumulation and bone mineral accrual during pubertal maturation. The primary finding of our investigation was that greater body fat gains in young females were associated with greater BMC accrual at the hip and forearm, but not the spine, compared to those with lower body fat accumulation, after adjusting for fat-free mass accrual and gains in height. Participants were followed throughout maturation from pre- to post-menarche, spanning the ages of approximately 4 to 8 years at baseline to approximately 13 to 19 years at the last measurement occasion. Our results show that BMC at all four bone sites increased during pubertal growth as did lean mass, fat mass, and percent body fat. While the prevalence of obesity, defined by ≥ 32 percent body fat,[3] increased from

23% to 38%, average BMI-for-age percentile was not significantly different.. Mean dietary intake of calcium, while not significantly different from average intake at pre-menarche, was inadequate for the age range of post-menarche participants. Similarly, vitamin D intakes were significantly lower at post-menarche compared to pre-menarche. Although not significantly different between pre- and post-menarche, energy intake may have been under-reported. Physical activity significantly decreased during maturation from childhood to adolescence, which may be related to increased obesity prevalence.

The positive correlations between body fat and BMC accrual of the hip and forearm indicate that increased accumulation of body fat during pubertal maturation is not detrimental to bone mineralization and that the skeleton responds positively to adiposity and weight in the form of fat mass. The different BMC response of the forearm and hip vs. the spine to fat mass may be related to the different composition of bone at these sites. Also, the mineralization of the spine may be more dependent on total body weight or central adiposity. Our study is limited by lack of statistical adjustment for weight and the absence of body fat distribution assessments. It is possible that fat may be affect structural parameters differently than material properties.[4] For example, despite increased mineralization in relation to excess fat mass, there may be less endocortical or periosteal apposition in response to greater fat gains, which would negatively impact bone strength.[4]

Our observation showing a positive relationship between body fat and bone mineral accrual supports the conclusions of previous cross-sectional[5,6] and prospective[7] studies. Furthermore, our study does not show changes in body fat and BMC is maturation dependent, which disagrees with prior reports of fat mass becoming a

negative predictor of BMC as maturation progresses.[8, 9] While we did not correct for maturation or breast stage in our statistical approach, correcting for changes in height is a reliable approach to account for maturational changes.[10] Overall, the present study contributes to understanding the extent of the relationship of increased adiposity and skeletal mineralization throughout the entire maturational spectrum, from pre- to early- to post-pubertal stages.

Few prospective investigations have explored the relationship between changes in body composition and bone mineral accrual, and executing these longitudinal studies presents unique challenges. In this study, participants were voluntarily invited back for follow-up measurements, and the number of subjects returning decreased with each new measurement occasion. It is important to note that self-selective attrition may have introduced bias into the sample, whereby those subjects who returned frequently and provided measurements far beyond baseline may have health conscious parents or may be driven to adopt better health practices vs. those who dropped out soon after baseline may have been less healthy and less inclined to return for assessments of their growth. Quality control is another concern in longitudinal data collection and analyses. In our laboratory, the instruments used to measure and analyze body composition and bone mineral were operated by the same technician, were calibrated according to the manufacturers' instructions, and were assessed for inter-rater reliability, which was reported previously. However, over the course of our study, the whole body software used at baseline became dysfunctional, resulting in substitution with a different, more contemporary DXA instrument to obtain total body bone and body composition measurements. A conversion equation derived from a separate sample of individuals scanned on both instruments

corrected for the substitution. Future studies that consider our observations regarding the challenges of this prospective investigation will be better designed to minimize these potential sources of error.

In conclusion, these longitudinal data in females followed from pre- to post-pubertal maturation indicate that greater gains in adiposity are associated with greater BMC in the hip and forearm, and suggest that fat mass has a positive impact on bone mineral accrual. Future research is needed to elucidate the influence of fat mass on structural properties of bone and to determine the influence of different distributions of fat on the relationship between fat and bone at various skeletal sites.

References

1. Rauch F, Bailey DA, Baxter-Jones A, Mirwald R, Faulkner R. The 'muscle-bone unit' during the pubertal growth spurt. Bone 2004;34:771-5.

2. Manzoni P, Brambilla P, Pietrobelli A, et al. Influence of body composition on bone mineral content in children and adolescents. American Journal of Clinical Nutrition 1996;64:603-7.

3. Fitnessgram Reference Guide - Body composition assessment. The Cooper Institute, 2001. (Accessed September 9, 2005, at http://www.cooperinst.org/shopping/PDF%20single/BodyCompositionAssessment.pdf.)

4. Pollock NK, Laing EM, Hamrick MW, Baile CA, Lewis RD. Does Adiposity Affect Bone Strength Indices in Young Adult Black Females? J Bone Miner Res 2008;In Press (Abstract).

5. 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:S274-7.

6. El Hage RC, CB. Jacob, C. Jaffre, C. . Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys. European Journal of Applied Physiology 2009:759-64.

7. Young D, Hopper JL, Macinnis RJ, Nowson CA, Hoang NH, Wark JD. Changes in body composition as determinants of longitudinal changes in bone mineral measures in 8 to 26-year-old female twins. Osteoporos Int 2001;12:506-15.
8. Clark EM, Ness AR, Tobias JH. Adipose tissue stimulates bone growth in prepubertal children. J Clin Endocrinol Metab 2006;91:2534-41.

9. Petit MA, Beck TJ, Hughes JM, Lin HM, Bentley C, Lloyd T. Proximal femur mechanical adaptation to weight gain in late adolescence: a six-year longitudinal study. J Bone Miner Res 2008;23:180-8.

10. Garn SM, Haskell JA. Fat thickness and developmental status in childhood and adolescence. AMA J Dis Child 1960;99:746-51.

APPENDICES

APPENDIX A

ASSENT AND CONSENT FORMS

CONSENT FORM (PARENT)

I _____ agree to give consent for my child, _____, to participate in the research study titled "Bone Response to Gymnastics Training in Young Girls," which is being conducted by Dr. Richard D. Lewis and Dr. Emma Laing of the Department of Foods and Nutrition at the University of Georgia. Dr. Lewis and Dr. Laing may be reached in room 279 Dawson Hall at 542-4901 or 542-4918. I understand that the participation of my daughter is completely voluntary. I can withdraw consent at any time without penalty and have the results of the participation, to the extent that which it can be identified as my child's, removed from the research records, or destroyed.

The following points have been explained to me:
1) The reason for the research is to study the impact of physical activity on bone and growth in children. The benefits that my daughter can expect from participation are the assessment of bone health (bone mineral density), body composition (percentage of body fat and nonfat tissue), diet and growth. In addition, my daughter's gymnastics or other activity classes will be paid for the duration of her participation in the study (up to $65/quarter during the first two years of the study and $100/year thereafter). Payments will be distributed only if all testing sessions are completed for a given time point. A brief summary of the testing and payment schedule is provided in the table below. If my daughter does not complete a testing session, she will be removed from the study. All measurements are being used for research purposes only, not medical purposes. However, if abnormalities are found in any measure, I will be notified and my daughter will be referred to an appropriate health care professional.

2) The procedures are as follows:
   a) Testing will be conducted at 10 different time points (the first five, each 6 months apart: months 0, 6, 12, 18 and 24) and (the last five, each 12 months apart: 36, 48, 60, 72 and 84). At 0, 12, 24, 36, 48, 60, 72 and 84 months three different testing sessions (Session 1, Session 2, Session 3 and Session 4) will be required, whereas, only Session 3 will be required at 6 and 18 months (See table).

Time point (months)	Sessions to be Completed	Payment
0	1,2,3,4	Up to $65/quarter
6	3	Up to $65/quarter
12	1,2,3,4	Up to $65/quarter
18	3	Up to $65/quarter
24	1,2,3,4	$520 minus payments at 0, 6, 12, and 18 months
36	1,2,3,4	$100
48	1,2,3,4	$100
60	1,2,3,4	$100
72	1,2,3,4	$100
84	1,2,3,4	$100
		Total = $1,020

b) My daughter will fast the night before Session 1. On the day of testing for Session 1, my daughter and I will arrive in the Sports Nutrition Lab in Dawson Hall at the scheduled time. Prior to any testing or participation, a consent form will be read to me and an assent form will be read to my daughter. After which, the researcher and I will sign the consent form and the researcher and my daughter will sign the assent form. During the reading of the consent and assent forms, my daughter and I will be briefed and familiarized with the testing procedures that will be used during the study (15 minutes). My daughter and I will be given the opportunity to reread the consent and assent forms and ask any questions that we may have about the study. Each phase of the study will be explained to my daughter and me throughout testing, and my daughter can withdraw from the study at any time. Prior to any testing, my daughter and I will be walked through all procedures. My daughter, a female chaperone and I will walk to a private room where a Gynecologist/Obstetrician will assess my daughter's pubic hair and breast development, to determine her level of sexual maturation.

My daughter will be walked to the female restroom and will collect a urine specimen in private. A trained pediatric phlebotomist will then draw approximately 20 mL of blood from my daughter, after which she will be given a snack (15-20 minutes). If a blood sample cannot be obtained after two attempts, no further attempts will be made. A Research Assistant will then familiarize my daughter and me with the use of an accelerometer (an instrument used to assess physical activity) and completion of physical activity diaries. My daughter will wear the accelerometer at each time point for three days. Session 1 will require approximately 90 minutes. Upon completion of Session 1, my daughter and I will be scheduled for Session 2, Session 3 and Session 4.

c) For Session 2, my daughter and I will arrive at University Health Services. To assess bone age, an X-ray of the hand/wrist will be conducted by a trained radiologic technologist (10 minutes including waiting time).

d) For Session 3, my daughter and I will arrive at the Sports Nutrition Lab. I will answer questions regarding demographic information and medical history and my daughter will complete questionnaires dealing with her body shape perception, diet and physical activity (approximately 30 minutes). I understand that while information about diet and

eating habits are being obtained, none of the researchers are clinical psychologists and the information alone cannot be used to accurately diagnose an eating disorder. Dr. Patrick O'Connor in the Department of Exercise Science has experience to provide an accurate interpretation of my daughter's answers on the body image questionnaires. If there are any concerns with my daughter's responses, I will be informed and my daughter will be referred to a mental health professional in the Athens area.

e) After completion of the questionnaires, my daughter's height, sitting height, leg length and weight, and my height will be measured. She will also have her bone mineral density and body composition measured using a bone/body composition analyzer (DXA). These measurements will require approximately 60 minutes, which includes a small break in between each scan (four scans total). I understand that a trained laboratory technician or graduate assistant under the supervision of Dr. Richard D. Lewis will conduct all measurements.

3) The discomforts or stresses that may be faced during this research are minor discomfort from blood draws, urine collection and sexual maturation ratings. If undue discomfort or stress occurs, my daughter may decide to discontinue the testing at any time.

4) I understand that the only foreseen risk to my daughter is exposure to a small amount of radiation when assessing body composition and bone mineral density with DXA and bone age with X-rays. The scans will give a total maximum radiation dose of 7.5 mR per testing session. This dose is very small, as radiation doses from a dental bite-wing film are 334 mR, environmental background is 3.5 mR/week, and chest x-ray films are about 25-40 mR for 2 standard films. Thus, the exposure per session is 19-30% of standard chest x-rays. In the event that information from any scan is lost or unusable, no additional scans will be performed.

5) The results of my daughter's participation will be confidential and will not be released in any identifiable form without my daughter's prior consent and mine unless required by law. While the body image questionnaires will be administered to my daughter in private, I will be informed if there are any concerns with my daughter's responses. My signature on this form authorizes the use of my daughter's data in-group analyses, which may be prepared for public dissemination, without breaching my own or my daughter's confidentiality. To accomplish this, my daughter will be assigned a four digit subject participation code which will be used on all data collected during my participation and my daughter's participation in this research. A master list with my name, my daughter's name and corresponding code number will be kept separate from testing data and locked at all times.

6) The investigator will answer any further questions that my daughter or I may have about this research, either now or during the course of the project.

I understand the procedures described above. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

Richard Lewis/Emma Laing	_____	_____
Name of Researcher	Signature	Date

Telephone: 542-4901
Email: rlewis@fcs.uga.edu

_____	_____	_____
Name of Parent or Guardian	Signature	Date

Please sign both copies, keep one and return one to the researcher.

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

Assent Form

I _____ agree to take part in a study about bone health and growth.

I do not have to be in this study if I do not want to be. I have the right to leave the study at any time without giving any reason, and without penalty.

I will have pictures taken of my bones. During one set of pictures I will lie on a table for approximately one hour. I will take short breaks between the different pictures that are taken. During another set of pictures I will place my arm on a box for about 5 minutes.

I will have a blood sample taken from my arm. I will also have my height measured against a wall and my weight measured on a scale.

I will answer questions about the activities that I participate in, the foods that I eat and how I perceive the shape of my body. Some of the questions may cause me to be uncomfortable. I may skip any question that I do not wish to answer.

I will wear a little pouch during two weekdays and one weekend day. The pouch will measure how much I move around.

My parent and I will write down what I eat during two weekdays and one weekend day.

My answers and any information about me will be kept confidential. This means that the researchers will not use my name. It also means that my responses to questions and any information about me will not be shared with anyone else. If the researchers feel that my health may be in danger, some of my answers will be shared with my parent.

If you have any questions or concerns you can always ask me or call my teacher, Dr. Richard Lewis at the following number: 542-4901.

Sincerely,

Emma Laing, Ph.D.
Department of Foods and Nutrition
University of Georgia
279 Dawson Hall

I understand the project described above. My questions have been answered and I agree to participate in this project. I have received a copy of this form.

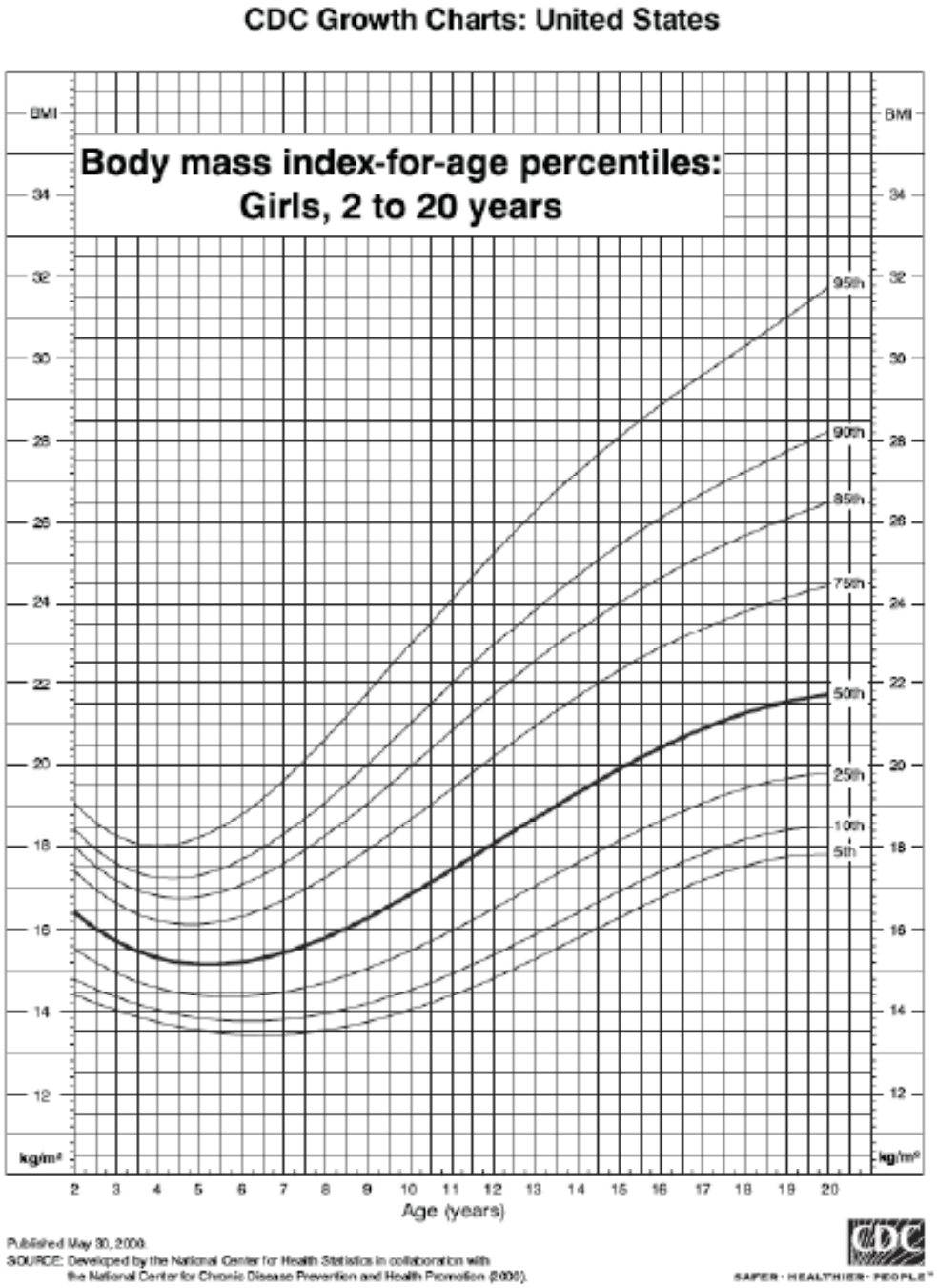
_____
Signature of the Participant/Date

Please sign both copies, keep one and return one to the researcher.

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

APPENDIX B

BMI CHART



APPENDIX C

THREE-DAY DIET RECORD

DIRECTIONS FOR KEEPING A 3-DAY DIET DIARY

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

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

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

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

4) Each food that you ate.

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

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

3. It is important to describe each food you eat in detail.
For example:
Write down brand names for each food you ate if you know them.

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

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

If you ate a casserole or a salad, write down the foods 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?

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

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

DAY 1 OF THE DIET DIARY

ID: _____ **CHECKED BY:** _____

DATE: _____ **DAY OF THE WEEK:** _____

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

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

_____

_____

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

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

DAY 2 OF THE DIET DIARY

ID: _____ **CHECKED BY:** _____

DATE: _____ **DAY OF THE WEEK:** _____

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

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

_____

_____

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

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

DAY 3 OF THE DIET DIARY

ID: _____ **CHECKED BY:** _____

DATE: _____ **DAY OF THE WEEK:** _____

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

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

_____

_____

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

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

APPENDIX D

DEMOGRAPHIC QUESTIONNAIRE

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

GENERAL INFORMATION QUESTIONNAIRE

Demographic Data:

I am going to ask you some questions about your age, family and education. Your mother or father can help you answer.

1. What is your date of birth? Month_____ Day _____ Year_____
2. What is your age? Years _____ Months _____
3. What was your birth weight? _____ Pounds _____ Ounces
4. Gender: (Circle One) Female Male
5. What is your grade in school?_____
6. How do you describe yourself? (Circle One or More: Mixed racial heritage should be indicated by checking more than one category)

Ethnicity: Hispanic or Latino
Non-Hispanic or Latino

Race: American Indian or Alaska Native
Asian
Black or African American
Native Hawaiian or other Pacific Islander
White
any combination of the above

7. Do you live with your parents? (Circle One) YES NO

   7a. If no, with whom do you live? _____

8. Do you have any brothers or sisters? (Circle One) YES NO

   8a. If yes, list ages of: _____Years (Brother) _____Years (Sister)
   _____Years (Brother) _____Years (Sister)
   _____Years (Brother) _____Years (Sister)

8b. If yes, do they participate in sports? (Circle One) YES NO

8c. If yes, list the sport and gender of sibling.

Sport_____(Brother or Sister)

Sport_____(Brother or Sister)

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

9. Do you have a twin brother or sister? (Circle One) YES NO

10. What is your mother's height and weight?_____Feet_____Inches_____Pounds

11. What is your father's height and weight?_____Feet_____Inches_____Pounds

12. What is your parents' income? (Circle One)

Less than $9,999
$10,000 - $19,999
$20,000 - $29,999
$30,000 - $39,999
$40,000 - $49,999
$50,000 - $59,999
$60,000 - $69,999
$70,000 - $79,999
$80,000 - $89,999
$90,000 - $99,999
Over $100,000

13. What is your mother's occupation?_____

14. What is your father's occupation?_____

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

GENERAL INFORMATION QUESTIONNAIRE

Health Data

Now, I am going to ask you to respond to a few questions about your health. I am the only one that will know how you answer these questions, so please be honest with your answers.

1. Have you gained or lost any weight (≥ 10 pounds) in the last 3 months? (Circle One)

   YES NO

   1a. If yes, how much? +_____ pounds OR -_____pounds

2. Have you had any height changes in the past 3 months? (Circle One) YES NO

   2a. If yes, how much? _____ feet _____ inches

3. How would you rate your present health? (Circle One)

   Poor Fair Good Excellent

4. Have you started your menstrual cycles? (Circle One) YES NO If so, what date?___

   4a. Are your menstrual cycles regular? YES or NO; *circle one*

   4b. If not, how long have they been irregular? _____

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

   5a. How old were you when you began using birth control pills? _____

   5b. How long have you been using them? _____

   5a. What periods of time did you stop using birth control pills? _____

   (Please give dates, if applicable)

6. Do you have any diseases or illnesses? (Circle One) YES NO

   6a. If yes, what diseases? _____

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

7. Are you taking any medications either prescribed by a doctor or over-the-counter (self-prescribed)? (Circle One) YES NO

7a. If yes, what medications? _____Amount per day _____

_____Amount per day _____

_____Amount per day _____

8. Do you currently smoke cigarettes? ___ YES or NO; *circle one*

8a. If yes, on the average, about how many cigarettes a day do you smoke?

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

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

_____months or years.

Those were some difficult questions to answer because the questions were so private. I want to assure you again that I am the only person who knows how you answered these questions. Thank you for being so honest with your answers.

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

GENERAL INFORMATION QUESTIONNAIRE

Nutrition Data:

These next questions are about your diet and eating habits. Try to think about how you eat.

1. Do you eat three meals per day? (Circle One) YES NO

   1a. If no, why not? _____

2. Do you eat snacks during the day? (Circle One) YES NO

   2a. If yes, how many snacks per day do you eat? _____ snacks per day

3. Are you following a special kind of diet? (Circle One) YES NO

   3a. If yes, what kind of diet? _____

4. Do you take any vitamin or mineral supplements or any "nutrition pills"? (Circle One) YES NO

   4a. If yes, what kind? _____Amount per day _____

   _____Amount per day _____

   _____Amount per day _____

5. Have you ever been on a diet to lose weight? (Circle One) YES NO

   5a. If yes, what kind of a diet was it? _____

   5b. How old were you when you were on this diet?_____ years _____months

   _____years_____months

6. Have you ever eaten a large amount of food and then vomited to get rid of the food? (Circle One) YES NO

   6a. If yes, how old were you? _____ years _____ months

   _____ years _____ months

7. Were you breastfed as an infant? (Circle One) YES NO

   7a. If yes, for how many months (exclusive)? _____

   7b. If yes, for how many months (supplemented with formula)?_____

Thank you for answering all of those questions. You did really well, and I appreciate you being so truthful with your answers.

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

GENERAL INFORMATION QUESTIONNAIRE

Physical Activity

The next questions that I will ask you are about your physical activity such as P.E. and exercise. There are no right or wrong answers, so please answer these questions the best that you can.

1. How would you rate your physical activity level? (Circle One)
   - Inactive
   - Below average
   - Average
   - Above average
   - Very high

2. Do you have any health problems that limit your activity? (Circle One) YES NO
   - 2a. If yes, what health problem? _____

3. Do you exercise or do physical activity regularly (not including P.E. class)? (Circle One) YES NO
   - 3a. If yes, how often? _____ hours per day/week/month (Circle One)

4. Do you participate in P.E. at school? (Circle One) YES NO
   - 4a. If yes, how often? _____ hours per day/week/month (Circle One)

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

   _____

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

GENERAL INFORMATION QUESTIONNAIRE

Bone Health Data:

The next questions have to do with your bones and your family's bones.

1. Does anyone in your family (including your parent's, grandparents, aunts, uncles, cousins) have osteoporosis or "humpback"? (Circle One) YES NO

   1a. If yes, who? _____

2. Has anyone in your family (including your parents, grandparents, aunts, uncles, cousins) had a hip or wrist fracture? (Circle One) YES NO

   2a. If yes, who? _____

3. Have you ever had a bone fracture or broken bone? (Circle One) YES NO

   3a. If yes, which bone(s)? _____

   3b. If yes, how old were you? _____years _____months

   3c. If yes, in what type of circumstance did the fracture take place? _____

   3d. If yes, how was the fracture treated (casting, medication, rest, etc.)?_____

   _____

4. Have you ever been told by a doctor that you have bone disease?
   (Circle One) YES NO

   4a. If yes, what disease? _____

   4b. If yes, how old were you? _____years _____ months

APPENDIX E

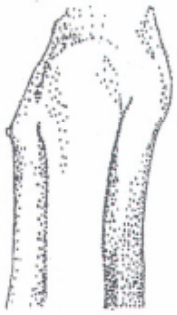
TANNER STAGES OF SEXUAL MATURATION

Lewis, Richard D.

SEXUAL MATURATION QUESTIONNAIRE (GIRLS)

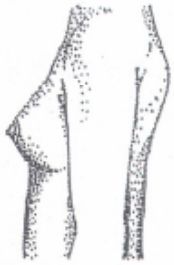
Subject ID#: _____
Date: _____

We need to find out what stage of sexual development you are in. Please look at the pictures and circle the one that looks most like you now.

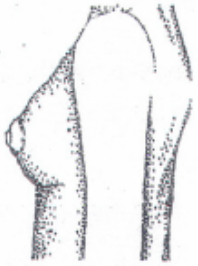


Stage 1: Elevation of papilla only.

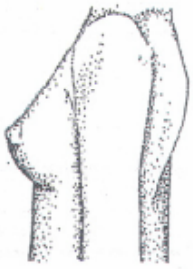
Stage 2: Elevation of breast and papilla as small mound, areola diameter enlarged.



Stage 3: Further enlargement without separation of breast and areola.



Stage 4: Secondary mound of areola and papilla above the breast.



Stage 5: Recession of areola to contour of breast.

Thank you for answering this question. Please send this questionnaire back to the researcher in the stamped envelope provided.

J. Tanner (1962) Growth and Adolescence, 2nd ed. Blackwell Scientific Publications, Oxford.

APPENDIX F

ANTHROPOMETRIC DATA RECORDING SHEET

Bone, Growth and Dietary Intakes in 4-8 Year Old Girls

(UGA BONE STUDY)

Participant Information Sheet
Anthropometrics/DXA

Subject ID: _____ timepoint: _____ Visit Date: _____

Race/Ethnicity: _____ Sex: _____

DOB: Month _____ Day _____ Year _____

Weight (kg):	_____ Measure 1	_____ Measure 2	_____ Average of 1 and 2
Height (cm):	_____ Measure 1	_____ Measure 2	_____ Average of 1 and 2
Sitting Ht (cm):	_____ Measure 1	_____ Measure 2	_____ Average of 1 and 2

BMI (g/cm^2): _____

Length of Radius (cm): _____ **R** or **L** handed (circle one)

DXA operator use

- ❑ Total Body
- ❑ Lumbar Spine
- ❑ Proximal Femur
- ❑ Radius

Scan date: _____
Completed by: _____
initials of operator

Comments:

_____
_____
_____
_____
_____
_____
_____
_____