The Influences of Growth, Maturational Timing and Physical Activity on Adult Bone Structural Strength at the Proximal Femur

A Thesis Submitted to the College of Graduate Studies and Research

In Partial Fulfillment of the Requirements for the Doctoral Degree

In the College of Kinesiology, University of Saskatchewan

Saskatoon, Saskatchewan, Canada

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Dedication

This dissertation is dedicated to my family, my loving wife, and my friends who have all supported me during the completion of this degree. You have all been there for the late nights, the early mornings, the triumphant successes and the heart breaking failures. Let's wrap this up together‼

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Glossary of Terms

Abbreviation	Full Terminology
aBMD	Areal bone mineral density
aBMDp	Areal bone mineral density peak
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
APHV	Age of peak height velocity
BA	Biological age
BMC	Bone mineral content
BR	Buckling ratio
CA	Chronological age
CSA	Cross sectional area
CSAp	Cross sectional area peak
CSMI	Cross sectional moments of inertia
Ct.Th	Cortical thickness
DXA	Dual energy x-ray absorptiometry
ED	Endosteal width
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
FM	Fat Mass
FSI	Femur strength index
HRpQCT	High resolution peripheral quantitative computed tomography
HSA	Hip structural analysis
Ht	Height
IT	Intertrochanter region of the proximal femur
LTM	Lean Tissue Mass
LTM%	Percent Lean Tissue Mass
MESm	Modeling minimum effective strain
MESp	Pathological minimum effective strain
MESr	Remodeling minimum effective strain
MLwiN	Multilevel modeling program for Windows
MRI	Magnetic resonance imaging
NN	Narrow neck region of the proximal femur
PA	Physical activity
PBMAS	Pediatric bone mineral accrual study
PHV	Peak height velocity
QCT	Quantitative computed tomography
QTL	Quantitative trait loci
S	Femoral shaft region of the proximal femur
SD	Standard deviation
SE	Standard Error
SPSS	Statistical package for social sciences
vBMD	Volumetric bone mineral density
WHO	World health organization

Wt	Weight
Z	Section modulus
Zp	Section modulus peak

Chapter One

Introduction, Literature Review, Objectives and Hypotheses

1.1 Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microstructural deterioration of the skeletal tissue causing increased bone fragility and fracture **[1]**. Osteoporosis related fragility is the leading cause of wrist, vertebral and hip fractures in individuals 60 years of age and older, but hip fractures have the most devastating consequences, as the morality rate drastically increases within the first year following a hip fracture incident **[2]**. This increased bone fragility is recognized as a important health concern **[3, 4]** and is acknowledged as a burden on the health care system **[5, 6]**, estimated to cost Canadians nearly $2 billion annually **[2]**. Given that approximately 2 million Canadians are currently living with this disease, affecting 1 in 4 women and 1 in 8 men over 50 years of age, the aging profile of the Canadian population will only further increase the costs associated with osteoporosis in the near future **[2, 6].**

Although the debilitating effects of osteoporosis often precipitate as a consequence of fractures in old age, osteoporosis is proposed to have pediatric antecedents **[7, 8].** As a result, preventative strategies have focused on identifying determinants of bone strength during the critical developmental periods of childhood and adolescence, with the notion that developing stronger bones earlier in life may have life-long effects. Childhood and adolescence represent a unique period, where the human body undergoes remarkable physiological and developmental changes that can dramatically alter an individual's bone strength **[7, 9]**. These childhood and adolescent changes occur in every healthy male and female, but when they occur and the magnitude of these changes is highly variable **[9-11]**. As a result, bone strength differences may be drastically affected by this biological variability and should be taken into consideration when assessing skeletal differences during the childhood and adolescent period. Age of peak height velocity (APHV) is a common maturational landmark, derived from individual growth measurements, that occurs in both males and females. The determination of APHV is widely utilized method in pediatric populations to controlling for the biological variation observed during childhood and

adolescence. Only by aligning individuals by a maturational benchmark, such as APHV, can comparisons be made and the unique determinants of bone strength identified during the critical childhood and adolescent period.

Given that bone strength, in the simplest form, can be defined as the ability of a bone to withstand fracturing, it is not surprising that its determinants are multifaceted. Traditionally, *in vivo* assessments of bone strength are determined using areal bone mineral density (aBMD). The positive association between aBMD and fracture prediction is well established **[12-17]** and, consequently, is the reason why aBMD remains the current gold standard for assessing osteoporosis, with an aBMD value less than or equal to -2.5 standard deviations from a normal health adult clinically defining osteoporosis **[2]**. Although aBMD remains a clinically relevant estimate of bone strength, it is only a single element. Bone strength is influenced not only by bone mass (aBMD), but also bone geometry, bone architecture, as well as the imposing loads acting on the bone **[18, 19]**. The investigation of geometric bone properties has recently garnered growing attention due to technological advancements. Investigations of geometric bone properties has been suggested to extend fracture prediction beyond that provided by aBMD, with bone geometry better able to differentiate between osteoporotic and non-osteoporotic individuals **[20-23].**

Hip structural analysis (HSA) is a unique method of estimating the geometric bone properties at regions within the proximal femur. Employing principles originally outlined by Martin & Burr **[24]**, the HSA procedure provides information about cross sections at three locations of the proximal femur: the narrow neck, intertrochanter, and femoral shaft **[25]**. HSA is a novel, reliable and precise method for estimating bone geometry and strength at the clinically significant proximal femur **[25, 26]** that extends the assessment of bone strength beyond the limited information provided by aBMD. Nonetheless, because of the novelty of these geometric assessments, it remains unsubstantiated how these geometric properties may contribute to the optimization and maintenance of bone strength.

Furthermore, there remains a paucity of information regarding how geometric bone properties develop from childhood and adolescence and into adulthood.

Regardless of the measures used to define bone strength, it is hypothesized that if an individual is able to develop greater bone strength during growth, one can prevent or delay the malicing effects of osteoporosis in old age. Although the accrual of bone mass is widely accepted to peak and plateau during the third decade of life **[27-31]**, only speculative cross sectional studies have suggested that geometric bone strength measures plateau contemporaneously **[32, 33]**. This implies that the first two decades of life represent a unique window of opportunity to maximize integral components of bone strength. During this period bone undergoes drastic changes, especially during the developmental periods of childhood and adolescence, where the capacity for bone adaptation may be critically influenced by non modifiable and modifiable factors. Adolescent pubertal timing has been documented to be one of these influential factors because of the link between pubertal hormones and bone development **[34-38]**. For example, estrogen is a pubertal hormone that exponentially increases during late childhood and early adolescence; it is estrogen that is proposed to alter the adaptability of bone in both males and females, increasing the sensitivity of bone to mechanical stimulation **[36, 39, 40]**. Thus, it is suggested that an earlier onset of puberty would expose individuals to estrogen sooner and provide bone strength benefits, both in terms of bone mass and geometric bone properties. Recent literature supports this supposition, observing that a natural early onset in puberty decreases fracture prevalence **[41]**, increases bone mineral mass development during adolescence **[42]** and in adulthood **[43-45]** and increases cortical volumetric BMD (vBMD) and cortical thickness at the distal radius in adult females **[45].** This literature, however, has focused primarily on cross sectional and retrospective data in female populations with emphasis on appendicular fracture sites. This has resulted in a scarcity of literature in male populations and at other clinically relevant fracture sites such as the proximal femur. To truly appreciate the effects of pubertal timing on geometric bone properties in both sexes, prospective

longitudinal studies using non invasive techniques able to assess geometric bone properties at clinically relevant fracture sites, such as the proximal femur, are necessary. This type of design would allow for the precise assessment of pubertal onset and document potential changes to geometric bone properties at a relevant fracture site over time in both sexes. Longitudinal prospective research in this area is therefore necessary to extend our understanding of the whether pubertal timing may influence the optimizing and/or maintenance bone strength in both sexes.

In addition to the potential benefits of an early pubertal onset on geometric bone properties, mechanical loading early in life may also provide additional enhancements **[46-49]**. According to the mechanostat theory **[50-52]**, bone adaptation is a response to mechanical function. As mechanical loads increase, bone adaptation is initiated to maintain bone strength within tolerable limits to prevent fracturing. Physical activity is often used as a model of mechanical loading because it places dynamic loads on the skeleton that are suggested to be essential in eliciting bone adaptation **[50-52].** Not surprisingly, physical activity has been documented to have positive effects on bone mass, geometry and architecture throughout life **[48, 53-65]**. Given that bones are undergoing vast alterations during childhood and adolescence, largely the result of growth, engaging in physical activity during this period is purported to be a unique opportunity where the benefits of mechanical loading on bone structural strength can be maximized **[66]**; however it remains unsubstantiated whether these benefits accrued during adolescence transfer to skeletal advantages in adulthood. It has been recently observed that individuals who participate in higher levels of physical activity during adolescence have greater bone mass in young adulthood **[57, 63, 67-72]**. Retired athlete models have also suggested that not only are benefits to bone mass maintained **[68, 69, 72]**, but geometric bone properties as well, with former athletes having greater cross sectional area **[73, 74]**. These data would suggest that childhood and adolescence physical activity is associated with skeletal benefits to bone mass and bone geometric properties; however, these conclusions are derived largely from retired athlete populations. Thus, these

conclusions may be confounded by the possibility of genetic predispositions or selection bias, which may not reflect those of a general healthy population. Further confounding these conclusion is the role of body composition on bone structural strength. Muscular forces produce the greatest physiological strain on the bone, thus predisposing individuals with greater muscle mass to further stimulate osteogenic responses. Additionally, muscle is a dense tissue that provides the body with additional mass, resulting in greater ground reaction forces, which, according to the mechanostat may be favorable to stimulate osteogenesis. Thus, the benefits observed in athlete models may be confounded by genetics and body compositional differences that may be favorable toward bone structural strength during childhood and adolescence. Whether the positive effects of early life physical activity on adult geometric bone properties are also observed in healthy non-athlete specific populations remains unsubstantiated. Therefore, investigations on the effects of childhood and adolescent physical activity on geometric bone properties in adulthood, in non-athlete specific populations are warranted to further justify the investment into early life physical activity **[69]**.

Therefore, the aim of this dissertation is to investigate the effects of growth, pubertal timing and early life physical activity on adult bone strength measures at the proximal femur. To achieve this aim, three studies will be conducted utilizing both longitudinal and cross sectional analyses of prospective, longitudinally collected data. *Study 1* will examine the longitudinal development of geometric bone measures at the proximal femur from childhood, through adolescence and into early adulthood, with the aim to identify whether a peak/plateau in bone geometry occurs and the contribution of the adolescent period towards the development adult bone structural strength. *Study 2* will then examine the relationship between pubertal timing and bone structural strength from adolescence into early adulthood, with the goal of determining whether the onset of puberty influences the development of bone structural strength at the proximal femur. Finally, *Study 3* will examine the influence of

adolescence physical activity on the development of adult bone structural strength at the proximal femur.

1.2 Review of Literature

To establish the conceptual framework for investigating the role of growth, maturation and physical activity on geometric bone structural strength, a discussion of the following topics is necessary: osteoporosis, bone health, growth and development, bone physiology, the factors that influence bone development, and the concept of bone strength. This chapter aims to summarize these relevant topics and concepts in order to develop a framework upon which the purpose of this dissertation will be established.

1.2.1 Osteoporosis and Bone Health

Osteoporosis is the most prevalent bone related disease in the world, estimated to afflict more than 200 million individuals worldwide **[75, 76]**. In Canada, approximately 5% of the population, mostly the elderly, suffer from this debilitating disease, with one in four women and one in eight men over the age of 50 affected by osteoporosis **[2]**. In 2011, the cost of treating osteoporosis in Canada was estimated at nearly $2 billion annually **[2].** The burden of this disease results in both direct medical cost, such as those from hospitalization and rehabilitative care, and indirect costs related to incidental health issues **[77]**. It is projected that by 2031, nearly a quarter of the Canadian population will be over the age of 65 and, as a result, it is expected that osteoporosis related health issue will cost the Canadian health care system an additional $4.5 billion annually. Not surprisingly, osteoporosis is thus recognized as an important Canadian health concern **[3, 4]** and acknowledged as a current and future burden on the health care system **[5, 6]**.

The major clinical consequence of osteoporosis is fracture. The deterioration of the bony tissue associated with osteoporosis results in increased skeletal fragility at most skeletal sites; however osteoporotic fractures are most common at the distal radius, lumbar spine and proximal femur **[78]**. Hip

fractures are of particular concern since they are associated with significant morbidity, loss of independence, and mortality **[3, 4]**. Individuals who experience a hip fracture have a 10-25% increase in mortality risk within the first year and a nearly 3 fold increase in future fracture risk **[2, 3, 77]**. Consequently, the prevention and treatment of osteoporosis related hip fractures are of major concern.

Osteoporosis is commonly diagnosed by the assessment of aBMD, a measure of bone areal density, using dual energy-x-ray absorptiometry (DXA). The World Health Organization (WHO) defines osteoporosis clinically as an aBMD score less than or equal to -2.5 standard deviations (SD) below the average mean for a healthy 23 year old adult **[79].** The assessment of aBMD remains the gold standard for diagnosing osteoporosis and determining fracture risk because of its strong association with bone strength (the concept of bone strength is discussed in detail in section *1.2.3 Bone Biology and Bone Strength*); however, the appreciation of whole bone strength and the concept of bone health are providing an alternative paradigm to assessing and treating osteoporosis. Osteoporosis was once characterized as a bone mass disease, but the WHO definition has been updated to acknowledge the role of micro-architectural deterioration to tissue arrangement and composition. As a result, the prevention and treatment strategies targeted towards osteoporosis and reducing fracture risk are not limited solely to increasing bone mass, but rather on developing and maintaining bone strength and bone health.

Bone health refers to the ability of the skeletal system to fulfill all the required roles necessary for proper human function. The skeletal system acts as a mineral reservoir, a site for erythropoiesis, protection for vital organs, and structural support for locomotion. Any hindrance to these functions impacts both an individual's bone health and overall health. For the purpose of this dissertation, bone health will refer to the bone's ability to fulfill the necessary demands for structural support. Logically then, osteoporosis is a detriment to bone health because of its deleterious effects on the structural properties of bone. Thus, it remains imperative to investigate how the structural properties of bone

develop and how potential external and internal factors influence these structural properties in order to better comprehend and develop strategies for preventing and treating osteoporosis and improving lifelong bone health.

Detriments to bone health resulting from osteoporosis often go unnoticed until a fracture incident has occurred in old age, which has lead to osteoporosis being characterized as disease of the elderly. Despite this characterization, it has been proposed that osteoporosis may have pediatric antecedents **[7, 8]**. Particular attention has been given to the periods of rapid growth and development, such as the adolescent growth spurt. During this period, the skeleton undergoes dramatic changes, unlike anything observed during the rest of the human lifecycle. As a result, the adolescent period is described as critical for proper bone strength development where nearly 25-35% of bone mineral is identified to be accrued **[7, 27, 53, 55, 66, 80]**. It has therefore been hypothesized that optimizing bone strength during these critical periods could have lifelong ramifications for bone strength and the prevalence of osteoporosis in old age.

In summary, the societal burden of osteoporosis related fractures is monumental. The need for effective preventative strategies is of upmost importance, with a focus on developing and maintaining bone strength and bone health. Finally, the adolescent period may be essential to these preventative strategies, as it may represent a period integral to the development of lifelong bone strength.

1.2.2 Growth and Development

With adolescence being described as a unique period that may be integral to bone development, it is first necessary to identify what makes this time so distinct. Imperative to this is a discussion of the concepts of growth, maturation and development as they are fundamental in characterizing the unique nature of the childhood and adolescence period.

1.2.2.1 Growth, Maturation and Development

The terms growth, maturation and development are often used synonymously, but each refers to specific biological activities **[9]**. Growth refers to an increase in the size of the body or any one of its parts **[9, 10]**. For a bone, the increase in bone size, length, cross sectional area or periosteal width can all be referred to as bone growth, and, as is evident by the assessment of stature, there can be a wide variation in the endpoint of growth. Maturation refers to the *tempo* and *timing* of the progress towards the mature adult state **[9]**. The timing refers to when specific maturational events occur (e.g. the specific time/age when ossification of the growth plates occurs). In contrast, the tempo refers to the rate at which maturation progresses (e.g. time/age when maximal statural growth occurs in adolescence as indexed by peak height velocity). The timing and tempo vary considerably between individuals, and the variation in progress over time implies variation in the rate of change **[9]**. The measure of growth is often used to determine maturity; however growth focuses on size at a given time point, while the maturity focuses on the process of attaining the adult mature state **[9]**. So, if skeletal maturity is determined by the achievement of a fully ossified skeleton, the growth of the epiphysis in long bones (i.e. the change in shaped) can be used as a maturity indicator, and provide information on the skeleton's progress towards a mature state. Development is a broader concept that is used in two distinct contexts: behavioural and biological. Behavioural development refers to the acquisition of behavioural competencies. This is the learning of appropriate behaviours dictated by society and as such is culture specific **[10]**. Biological development refers to the processes of differentiation and specialization. For bone, development can include the differentiation of mesenchymal cells to bone specific cells, as well as the alteration of bone shape due to specific loading patterns. It is important to recognize that the growth, maturation and development are interactive processes that occur simultaneously; however, their temporal patterns may not be contemporaneous **[9, 10]**.

1.2.2.2 Growth Curves

Describing the specific growth of different segments and body tissues, Richard Scammon **[81]** proposed that growth can be summarized in four patterns or growth curves: the General curve; the Neural curve; the Genital curve; and the Lymphoid curve (Figure 1.1). For the purpose of this dissertation focus will be on the general growth curve as it is pertinent to bone development.

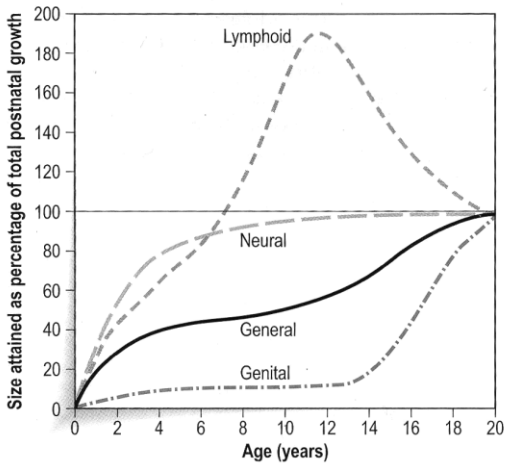


Figure 1.1: Scammon's curves of systemic growth depicting various tissue growths in size from childhood to adulthood. Modified from Malina et al **[9]**

The general growth curve describes the general growth pattern observed for stature, weight, and most external dimensions of the body. This curve is also representative of the growth pattern of most systems in the body including muscle and bone. The general growth curve follows an S shape or sigmoid pattern with four distinct phases **[9, 81]**. The first phase is characterized by rapid growth during infancy and early childhood. This is followed by a period of steady and constant growth during middle childhood. Similar to the first phase, the third phase is characterized by period of rapid growth during adolescence. Following adolescence the velocity of growth slows down with an eventual cessation of growth in late adolescence and early adulthood **[9]**. As is evident by Scammon's growth patterns, the periods of infancy and adolescence are times when rapid changes are occurring to size (growth) as we all as

maturational and developmental progression. The dramatic changes occurring at these periods make them unique 'windows of opportunity' which may play a vital role in overall growth.

Although the general pattern of growth is very similar between individuals, there is considerable individual variability in the overall size attained and the rate of growth at different ages and between sexes **[9, 10]**. This variability is evident in the growth of the body as a whole as well as to specific segments and tissues. This is reflected in Figure 1.2. where we see two adolescent males both at the same chronological age (years from birth), but differing considerably in physical maturity (biological age). The adolescent male on the left is an early maturer, and it is apparent because of his increased muscular, statural height and the appearance of secondary sex characteristics. The boy on the right is a late maturer and it becomes evident by their contrasting physiques that despite their similar chronological age, they differ in biological age. It thus becomes clear that this biological variability becomes a major concern when drawing definitive conclusions on the independent effects of internal or external factors on changes to health outcomes **[9-11]**. Therefore, the process of normal growth and biological maturation must be considered in order to identify the independent effects of internal or external factors on health outcomes especially during periods of rapid growth **[11]**. To control for biological maturation an assessment of maturity is required, and its incorporation into research methodologies is necessary **[11]**.

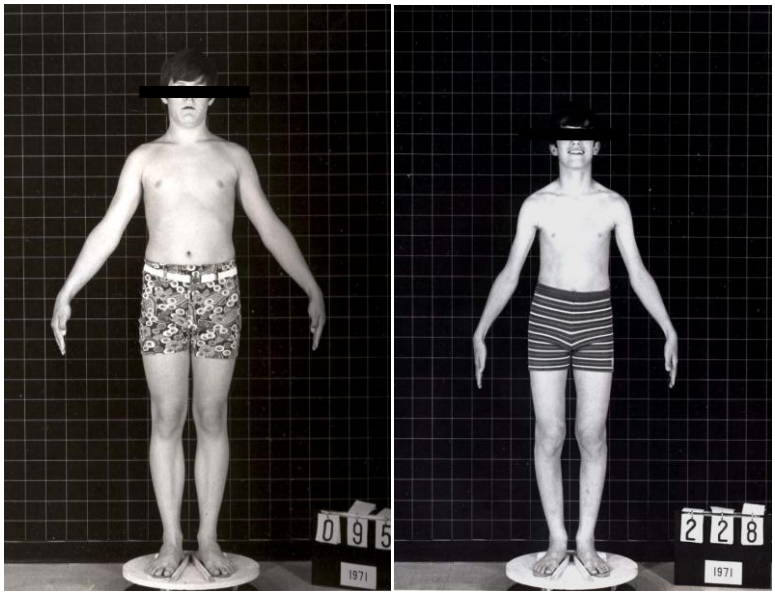


Figure 1.2: Photographs of two males from the Saskatchewan Growth and Development Study depicting the biological variation that is apparent during growth. Both males were assessed at the same chronological age (14 years of age), but the male on the left is more advanced in terms of biological age. This is evident by his increased stature, musculature, and the appearance of secondary sex characteristics. The male on the left could be described as an early maturer, while the male on the right as a late maturer.

1.2.2.3 Longitudinal Study Design

Individual changes in growth and development can only be studied using the same individuals measured repeatedly over a period of time. This type of design is referred to as a longitudinal design. Typically, cross sectional designs are a more attractive option for investigating growth because they can be carried out quickly, can include a large number of participants and can be quite cost effective**[9]**. Unfortunately, cross sectional designs only provide a snap shot of the variation in growth and they provide little information about individual growth patterns **[10]**. This may result in spurious conclusions being drawn that are ill considerate of growth or developmental changes that may be taking place contemporaneously. Unlike cross sectional designs, longitudinal designs assess the same individuals over time, allowing for the entire growth pattern of each individual to be ascertained. This allows for both the variation in timing and tempo of growth to be considered, especially in pediatric populations. Additionally, with the recent mandate to determine antecedents of health, longitudinal designs can

assess patterns of development that can truly determine links between early life factors and adult health.

Despite longitudinal designs having the advantage of assessing within-individual variance and assessing changes over time, they are expensive to operate, have issues of participant retention and often require sophisticated statistically analyses to accurately interpret the data **[10]**. Regardless of the disadvantages, to assess growth and development, whether it is for bone or muscular development, longitudinal designs are necessary.

1.2.2.4 Maturity Indicators

Maturity indicators are used to assess maturity within individuals to make comparisons between individuals and are often based on definable and sequential changes that are characteristic of the progression of the body from immaturity to maturity. There are several maturity indicators that may be utilized, each with their own intrinsic benefits and limitations **[11]**. The most commonly used methods employ one or more of the following: (i) menarcheal status (prospective or retrospective recall of the age of onset of the first period); (ii) secondary sex characteristics (visual inspection and categorization of gentialia, breast and/or pubic hair development); (iii) skeletal age (visual inspection and categorization of bones imaged from hand wrist radiographs); and somatic characteristics (identification of morphological landmarks). Of all of these only skeletal age and somatic characteristics can be aligned to make sex comparisons because they are the only indicators that share common timing of events in both sexes; however since no radiographs were available for the studies to be presented in this thesis, the somatic characteristic of the age of attainment of peak linear growth, also known as age of peak height velocity (APHV) was chosen as the index to assess maturity.

1.2.2.4.1 Age of Peak Height Velocity

The age of peak height velocity (APHV) is a somatic maturity indicator derived from landmarks on the height growth curve. APHV is the most commonly used somatic maturity indicator in longitudinal studies of childhood and adolescence**,** but other somatic milestones, such as age of initiation of PHV and cessation of growth have also been used **[9]**. An example of a typical height velocity curve and the somatic maturity landmarks derived from this curve for males and females is depicted in Figure 1.3.

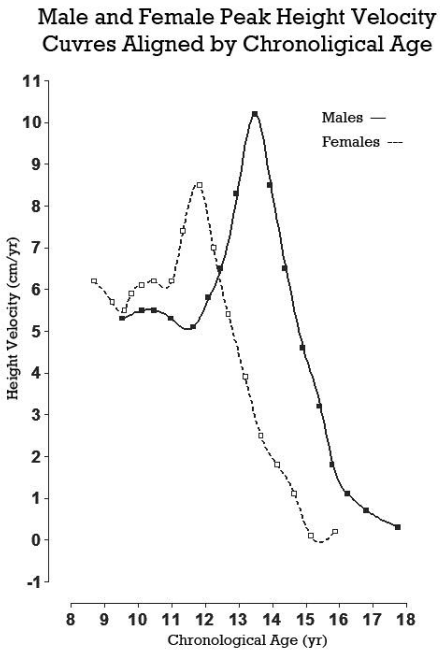


Figure 1.3: Male and female height velocity curves. Data derived from participants from the University of Saskatchewan's Pediatric Bone Mineral Accrual Study

To obtain the age of PHV whole year height velocity increments are plotted and a mathematical curving fitting procedure is used to identify the age when maximum velocity occurs **[10]**. To understand how yearly height velocities are obtained a working example, in conjunction with a detailed description is provided in Appendix B. Females typically reach peak height velocity at around 12 years of age, while males achieve PHV at 14 years of age. There is still great variability within these ages as females have been documented to range between approximately 10 to14 years of age and males between 12 to 16 years of age **[9]**. Once the APHV is obtained individuals can be aligned by biological age rather than

chronological age. In contrast to chronological age (CA), biological age (BA) is determined as the number of years away from APHV rather than years from birth. Thus, at APHV biological age is equal to zero. For example, an individual who is tested at CA of 12.0 years, and has an APHV of 14.2 years, will have a BA of -2.2 years at 12.0 years, a BA of 0.0 at 14.2 years and a BA of +2.2 at 16.4 years. In addition to being able to align individuals at the same maturation age, individuals can also be classified into maturational groupings. Since the APHV occurs, on average, close to 12 years of age in females and 14 years of age in males with a standard deviation of approximately 1 year[9], individuals can be classified as either early or late maturers if they fall one year outside of these ages **[82]**. Another unique feature of using APHV as a maturity indicator is the ability to make sex comparisons. Because APHV is a common maturational landmark that occurs in both sexes, individuals can be compared along a collective characteristic. The main disadvantage of using APHV as a maturity indicator is that its assessment is dependent on time series data, which limits its practical utility to longitudinal study designs; however Mirwald and colleagues **[83]** have addressed this concern with sex-specific predictive APHV regressions. Derived from stature measures, weight and CA, APHV can be predicted within ± 1 year in adolescent populations. This method of assessment makes APHV an accurate, quick, non-invasive and practical maturity indicator which can also be incorporated into cross sectional designs**[10]**.

1.2.3 Bone Biology and Bone Strength

Above, the concepts of growth, maturation and development have been discussed, along with the outlining of the various maturity indicators that may be employed to control for biological variation. The proceeding section will include a discussion of basics of bone biology to establish the impact of bone structural properties on bone health and osteoporosis. It is important to note that maturation stage has a significant independent effect on both bone biology and bone strength particularly as bone grows.

Thus, the proceeding section is dedicated to this task and will cover topics related to the bone composition, modelling and remodelling, and general bone development.

1.2.3.1 Bone Tissue and Composition

Bone is a highly specialized tissue with unique attributes that enable it to serve several functions including structural support, movement, protection for vital organs, hematopoesis, and the maintenance of mineral homeostasis. Bone is a composite material, consisting of rigid minerals encased by flexible proteins. The mineral component of bone consist of calcium and phosphate, arranged in a complex matrix of organic and inorganic compounds forming the mechanically rigid, load bearing bone mineral crystal hydroxyapatite **[84]**. The hydroxyapatite structure provides mechanical stability and serves as the body's mineral reservoir for calcium and phosphate ions. Human bones are composed of approximately 60% hydroxyapatite **[85, 86]** [1]. The remainder consists of connective protein and space. Collagen is the connective protein that binds the hydroxyapatite, allowing the minerals to be organized in a matrix of flexible fibrous protein. Although other proteins help strengthen the bone matrix, collagen's triple helical molecular structure serves as the building block for the matrix's fiber network **[84]**. The combination of the rigid hydroxyapatite along with pliable collagen scaffolding gives bone its unique viscoelastic properties which enable it to withstand compressive and tensile stresses and bending and torsional moments.

There are two main classifications of human bones: flat bones, such as the mandible and skull, and long bones, such as the radius and femur. For the purpose of this dissertation, emphasis will be placed on the long bone types. Long bones are comprised of cortical and trabecular bone. Cortical bone, also known as compact bone, comprises approximately 80% of the adult skeleton and is characterized by its dense and

[1] This is a total body approximation. The mineral composition varies depending on site and function **[86]** . For example, the ossicles require greater stiffness necessary for the fidelity of sound transmission and are composed of approximately 90% mineral **[85]**.

solid macroscopic structure **[86]**. Cortical bone is primarily found in the shaft of long bones and forms the outer shell surrounding trabecular bone. As a result of cortical bone's dense composition and high volume fraction, cortical bone is favored at sites where structural rigidity, rather than flexibility, is required **[85]**. This is also why cortical bone is the common site for muscle and tendon attachments. The more metabolically active trabecular bone, also known as cancellous bone, is composed of rods and fused plates that give trabecular bone its distinctive honey comb like appearance **[86]**. In contrast to the more rigid cortical bone, the sponge-like structural design allows trabecular bone to act more like a spring, absorbing more energy by deforming **[85]**. Not surprisingly, trabecular bone is found at sites, such as the vertebral body, where a flexible structure is favoured. As well as helping maintain structural integrity, trabecular bone also serves as a surface for mineral exchange.

Although cortical and trabecular bone may differ in composition and structure, they are formed, maintained and remodeled by the same three types of cells: osteoblasts, osteoclasts, and osteocytes. The osteoblasts are matrix producing cells that regulate bone mineralization. The osteoblasts are highly enriched with alkaline phosphatase and secrete type I collagen and other bone matrix proteins essential for bone formation **[87]**. Osteoclasts are part of the monocyte/macrophage family and are the exclusive bone resorptive cells **[88]**. Because osteoclasts are responsible for bone resorption, they also play an active role in regulating the release of calcium and phosphate. Finally, nearly 90% or all bone cells in the human body are osteocytes. Osteocytes are former osteoblasts that have been embedded into the bone matrix during bone formation. Despite their relative 'inactivity', osteocytes are believed to coordinate the spatial and temporal recruitment of cells for bone formation and resorption **[89-92]**. In addition, even though the bone matrix isolates each osteocyte, they are able to interact with one another through an elaborate network of dendritic processes that are proposed to enable osteocytes to act as the bone's mechanosensory cells, helping to detect mechanical loading and regulate the process of bone modeling and remodeling **[89, 91, 93]**.

1.2.3.2 Bone Modeling and Remodeling

Bone is a dynamic tissue that undergoes significant turnover in comparison to other bodily organs **[89]**. Each of the 213 bones in the human body is sculpted and constantly renewed by the processes of modeling and remodeling **[86]**. Bone modeling is the process by which bones are shaped and reshaped by the independent actions of osteoblasts and osteoclasts **[86]**. This process is predominantly observed during growth, where there is an addition of new bone. Bone modeling is unique from remodeling in that bone formation is not tightly coupled to bone resorption **[86]**. During bone modeling, bone is selectively added or removed from existing surfaces with the goal of optimizing bone strength **[94]**. This process alters the bone size, shape, and spatial orientation of a long bone's cross section by selectively inhibiting or activating cellular activity at the resorptive or appositional surfaces **[94]**. The sites of resorption and apposition in long bones are often the periosteal and endosteal surfaces (Figure 1.4). Alterations at the periosteal and endosteal surfaces are often region specific and can result in significant improvements to geometric bone properties, through a process called macromodeling, where a macroscopic piece of bone increases in growth without any alteration in its basic figure **[95]**.. In addition, the trabecular bone may undergo minimodeling, where the trabeculae align and shift their orientation in line with the loading forces **[95]**. Both types of modeling are responsible for the strengthening of developing bone.

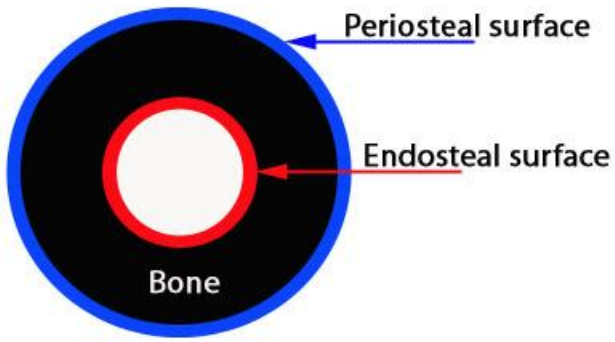


Figure 1.4: Depiction of the location of the endosteal surface (inner surface) and the periosteal surface (outer surface) in a long bone

Bone remodeling, although similar to modeling, is a distinct and unique process. Remodeling follows an activation, resorption then formation sequence **[94].** This sequence of events consists of four distinct cycles: activation, resorption, reversal, and formation. During the activation stage there is recruitment of the osteoclast precursors allowing for the infiltration of the bone lining cell layer and the fusion of pre-osteoclasts **[86]**. How the sites are selected for activation is unclear, but there is some evidence to support that target sites require tissue repair **[96].** The pre-osteoclasts adhere to the bone matrix and form a sealing zone which provides a unique environment in which the bone resorption phase takes place. Before resorption can occur, osteoclast maturation must precede with the aid of local cytokines such as RANKL, interleukins -1 and -6, and systemic hormones parathyroid hormone (PTH) and 1, 25 – dihydroxyvitamin D_3 (Vit-D) **[94]**. Specific proton pumps allow H^+ ions to be transferred into the sealing zone allowing the osteoclast to effectively dissolve the mineral matrix and digest the organic bone matrix leaving a saucer-shaped cavity called Howship's lacunae **[94]**. The resorption phase ends with self inflicted osteoclast cellular death (apoptosis). This apoptosis is followed by reversal. During reversal, the Howship's lacunae are filled with osteocytes and pre-osteoblasts that were liberated from the resorption of the bone matrix. The most important element of the reversal stage is the release of coupling signals that summon osteoblast activity to the resorptive cavity **[86]**. The coupling signals determine osteoblast proliferation and amount of growth factors released. Without these coupling mechanisms, remodeling would result in a net loss of bone **[86]**. With increasing age, the remodeling process eventually results in a net loss, as the coupling mechanisms do not fully replace the bone that has been resorbed **[89, 94]**. Finally, the formation phase is initiated, where the osteoblasts synthesize the organic matrix by triggering the mineralization of calcium and phosphate ions found in the extra cellular matrix. As the formation phase continues, osteoblasts are incorporated into the newly formed matrix as osteocytes. The osteocytes remain in constant contact whilst in the matrix by means of gap junction enabling them to transmit information to one another when necessary. Before the completion

of bone formation, the osteoblasts endure one of three fates: they are either (i) incorporated in matrix (becoming osteocytes), (ii) remain on surface as bone lining cells, or (iii) undergo apoptosis. The majority of osteoblasts undergo apoptosis, but the osteoblasts impregnated in matrix and on the surface will play a role in future remodeling cycles **[94]**.

1.2.3.3 Proximal Femur Bone Growth

The proximal femur is classified into a subgroup of bone types known as long bones, and the method of growth at the proximal femur is similar to growth observed in other long bones in the body. Growth in length of the proximal femur is due to changes that occur at epiphyseal discs, also known as the growth plates. The femur has three epiphyseal disk regions: two located near the proximal end and one located distally (Figure 1.5). The epiphyseal discs are primarily composed of cartilaginous tissue called condrocytes and as they proliferate they increase linear bone growth. The condrocytes proliferating and maturing cause the condrocytes to change shape from round to flat, which become layered forming hypertrophied condrocyte cell columns **[97]**. These hypertrophied condrocytes secrete hormones and proteins that attract bone marrow blood vessels, along with osteoblasts and osteoclasts between the condrocyte cell columns. The infiltration of the blood vessels and bone cells helps to mineralize the cartilage and remodel the newly formed cartilage into bone tissue **[97, 98]**. This process is known as endochondral ossification. This process continues throughout childhood and adolescence, resulting in the increase in bone length and stature, but sees a programmed senescence in adulthood, resulting in complete epiphyseal ossification and fusion. Although the causes of the epiphyseal fusion is highly debated, intrinsic mechanisms within the growth plate appear to direct this senescence **[99]**.

In conjunction to this linear bone growth, the proximal femur also undergoes growth in size and shape during childhood and adolescence, through the redistribution of mass by a process previously mentioned called modeling. At the periosteal surface, osteoblasts expand the periosteal shell by forming

new cortical bone. Contemporaneously, osteoclasts at the endosteal surface resorb bone expanding the medullary cavity. These processes reshape the inner and outer surfaces of the bone, positioning the cortical shell further away from the center of the bone and preventing the cortex from becoming excessively thick and heavy **[98]**.

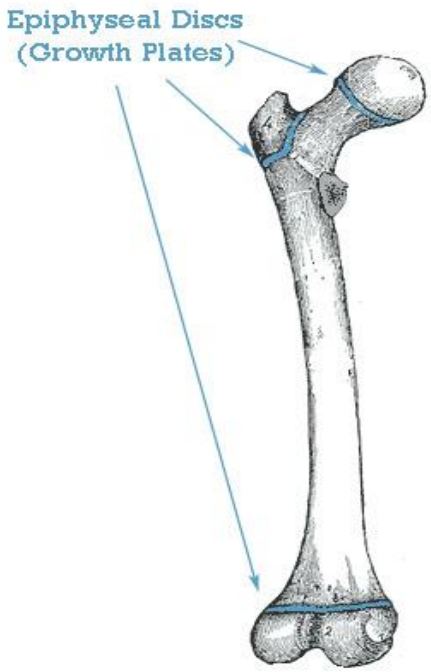


Figure 1.5: Illustration from the posterior side of the left femur. The three major growth plates of the femur are depicted in blue, with two visible at the proximal femur and one visible at the distal region. Image modified from **[100]**

1.2.3.4 Long Bone Strength

Bone strength is the ability of a bone to withstand fracturing. A fracture occurs when the external forces applied to a bone exceed its strength **[19, 101]**. The strength of a bone is dependent on the intrinsic properties of the bone material, the amount of bone material present, and the spatial arrangement of this material **[19]**. In healthy humans, the bone materials are relatively fixed, consisting of hydroxyapatite and collagen, but the composition of this material can vary based on site and age **[86, 102]**. This can result in vastly different bone strengths when comparing, for example, the radius in

childhood to the proximal femur in adulthood. Bone material composition is often estimated by determining the degree of tissue mineralization. Tissue mineralization is the amount of bone mineral incorporated into the bone matrix. Although there are currently no imaging technologies that can assess tissue mineralization directly, it is estimated from tissue level bone mineral content (BMC) and BMD measures. BMC is an estimate of the total amount of bone mineral encased within a bone while BMD is a measure of the amount of bone mineral per unit area or volume, and both are a reflection of not only the degree of mineralization but also the porosity of the bone tissue **[98, 103]**. From a mechanical perspective, the material composition (mineralization measures) can provide valuable information about the maximum stress a bone can withstand **[104]**, and, not surprisingly, cadaveric studies have shown that BMC and BMD provide good predictive power in predicting proximal femur ultimate failure loads **[105-108].** Although the ultimate failure load provides relevant insight into bone fracturing and strength, the clinical application of these tests remains controversial, as the location and speed of load application, alongside the lack of soft tissue support may not parallel *in vivo* bone fracturing episodes. Currently, aBMD, derived from dual energy x-ray absorptiometry, is the clinical measure for defining osteoporosis and there is strong evidence to suggest it is one of the best epidemiological predictors of osteoporotic hip fractures **[12-17]**; however, at the individual level, aBMD poorly predicts hip fractures **[17].** This limitation of bone mineral measures is a result of its overlapping connection to geometric and architectural properties of bone strength **[101]**. Although the bone mineral measure provides insightful information about bone strength, it is ultimately the whole bone that fails when a fracture occurs; therefore the arrangement of the bone material into a mechanically competent structure is a major determinant of bone strength **[98]**.

Since long bones mainly undergo compressive and tensile stresses, the structure must be arranged appropriately to withstand and resist these forces. Thus, overall bone strength can be estimated by assessing its ability to resist compressive and bending stresses and for variables that influence the

bone's compressive and bending strength can be referred to as bone structural strength measures. Compressive stresses are dependent on the intensity of the force applied and the area in which the load is distributed, while bending stresses are determined by the magnitude of deflection **[19, 104, 109]**. The ability to resist compressive and bending stresses are largely determined by the dimensions of the bone, and under specific loading condition, the stresses are entirely determined by geometric measures **[110]**. To appreciate the importance of geometric measures on whole bone's strength it is pertinent to understand how geometric measures such as cross sectional area (CSA), a measure of total bone area in a cross section, cross sectional moment of inertia (CSMI), a measure of the distribution of mass, and section modulus (Z), the maximum distance of material distribution from the neutral axis, contribute to long bone's compressive and bending strengths. In mathematical terms, compressive and bending stress can be represented by the following mechanical equations.

$$\sigma_c = P/A \quad (1.1)$$

where σ_c is the compressive stress, P is the force applied, and A is the area **[104]**

and

$$\sigma_b = My / CSMI \quad (1.2)$$

where, σ_b is the bending stress, M is the bending moment, *y* is the distance from the center of mass in the cross section, and CSMI is the cross section moment of inertia **[25, 104, 109]**

Therefore, given these mathematical relationships, to resist compressive forces, the long bone structure must either reduce the force applied or increase the area onto which the force is distributed. CSA is a measure of the total area in a cross section, and because of the direct inverse relationship between area and compressive stress, bone CSA can be employed as a measure of bone compressive strength **[104]**.

As noted in equation 1.2, to resist bending, the distance from the center of mass and the CSMI are very important because the bending moments are unlikely to be altered by the long bone itself. Since long bones are basically hollow cylinders, CSMI can be estimated with the following formula:

$$CSMI = \pi/4 * (R_o^4 - R_i^4) \quad (1.3)$$

where R_o is the outer radius and R_i is the inner radius **[25, 104, 109]**

Here, it becomes evident that CSMI is altered greatly by changes in the outer diameter rather than the inner diameter. It also becomes evident that the further away the mass is distributed from the neutral axis (the axis in a cross section where the stress or stains amount to zero), the greater the contribution to bending strength. As a result, CSMI often serves as a gauge of bending strength **[19, 104]**; however, because bending stresses are greatest at the furthest point from the neutral axis **[104]**, a superior measure of bending strength is section modulus. Section modulus (Z) is the ratio of material distribution over the maximum distance from the neutral axis and is traditionally represented by the formula:

$$Z = CSMI/y_{max} \quad (1.4)$$

where y_{max} is the maximum distance from neutral axis **[25, 104, 109]**

Section modulus is a more accurate measure of bending strength because it takes into account not only the distribution of mass (CSMI) but the maximum distance this mass is distributed. Since Z incorporates the maximum distance from the neutral axis to the outer surface, the maximum bending stress for a given bending moment can be written as,

$$\sigma_{max} = My_{max} / CSMI, \quad (1.5)$$

or simplified as

$$\sigma_{max} = M/Z \quad (1.6)$$

where, σ_{max} is the maximum bending stress, M is the bending moment, and Z is the section modulus **[110]**

This formula indicates that for a given bending moment, the bending stress experienced by a long bone, and resultant bending strength, is inversely associated with Z **[104, 110, 111]**. Thus, in a long bone, Z is a structural estimate of the bone's maximal bending strength and an indicator of overall bone strength.

Not surprisingly, when CSA and Z are used to predict failure load at the proximal femur, they are shown to have better agreement with material testing than femoral bone mineral mass **[25, 105, 112, 113].** Beck and colleagues **[25]**, using hip structural analysis (HSA), mechanically tested the breaking strength of 20 cadevaric femora identifying that HSA geometric bone measures predicted breaking strength better than DXA aBMD. Similar results are also reported using magnetic resonance imaging (MRI) and quantitative computed tomography (QCT). Manske et al **[112]** reported that cortical CSA, assessed by magnetic resonance imagining, had the greatest association with failure load at the femoral neck, explaining 46% of the variance in failure load. Using quantitative computed tomography, Manske et al further observed that femoral neck total CSA and cortical CSA combined to account for 69% of the variance in failure load compared to the 45% and 11 % explained by cortical BMC and BMD, respectively. More recently, Hansen and colleagues **[105]** predicted the mechanical compressive strength of 31 cadaveric femora using high resolution peripheral computed tomography (HRpQCT). They observed that geometric bone volume and femoral neck cortical thickness independently contributed to the prediction of mechanical compressive strength beyond that of DXA derived aBMD alone **[105]**. Geometric properties have also been reported to predict *in vivo* fracture risk [20, 22, 114-117]. HSA's buckling ratio and femur strength index (FSI), calculated using CSA and Z geometric measures, both significantly predicted hip fractures in adults but only provided approximately 1-2% additional predictive power when compared to aBMD **[114-116]**. Similarly, the works of Kaptoge et al. **[20]** and LaCroix et al. **[22]** report that geometric parameters at the proximal femur, derived from the assessment of CSA and Z, predict incident hip fractures as well as conventional aBMD at the femoral neck in both sexes. These findings emphasize that long bone strength can be estimated and derived from measures of geometric

CSA and Z at the proximal femur. While the potential of geometric properties for predicting fracture risk remains unsubstantiated in a clinical setting, their evaluation provides insight into the actions of external and internal stresses on hip fractures **[14]** Thus, investigating the influencing factors on geometric properties such as CSA and Z may be of clinical importance for determining fracture risk, improving bone strength and helping to better predict and prevent future fractures.

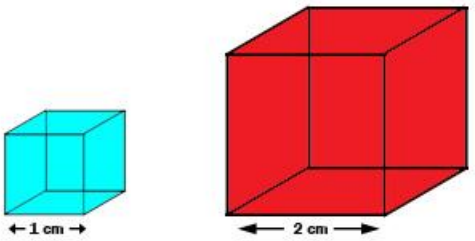
1.2.4 Assessing Bone Properties

There are numerous techniques and methods for assessing the various properties and components of bone. To discuss them all would be beyond the scope of this dissertation. Instead, focus will be directed on imaging techniques that assess *in vivo* bone properties at the clinically relevant proximal femur. The following section will concentrate on discussing the advantages and disadvantages associated with dual energy x-ray absorptiometry (DXA), quantitative computed tomography (QCT), magnetic resonance imaging (MRI), and hip structural analysis (HSA) in assessing the properties of bone.

1.2.4.1 Dual Energy X-Ray Absorptiometry (DXA)

Dual energy x-ray absorptiometry (DXA) is currently the most widely and readily available clinical tool for assessing bone. DXA has been employed as a clinical tool since the late 1980's **[118]** and has been adopted by the World Health Organization (WHO) as the pertinent tool for defining osteoporosis. DXA technology incorporates the principle of differential tissue attenuation, and by using two x-ray beams with differing peak kilovoltage (30-50keV and >70keV) **[118]** it is able to provide a two-dimensional projection image of a scanned region of interest, which commonly includes the whole body, lumbar spine, and proximal femur **[119]**. DXA offers a variety of valuable measures related to the assessment of bone and body composition. Specifically, DXA estimates bone mineral content (BMC, grams [g]), areal bone mineral density (aBMD, g/cm^2), lean tissue mass (LTM, g), and fat mass (g) of the total body and segmented regions of the appendicular skeleton. Areal BMD is the most commonly used measure from

DXA assessments, and aBMD of the hip and spine have been shown to be good indicators of fracture risk with DXA measured fracture risk increasing nearly 3-fold per standard deviation in hip aBMD **[120]**. DXA has proven to be a reliable and precise technique for estimating bone mineral and soft tissue composition, with coefficients of variations reported between 0.6-2% aBMD, 1.2-4% for BMC, 0.5-2% for LTM and 2-4% for FM **[121]**. Additionally, because of its low levels of ionizing radiation **[95]**, approximately 10-30 microsievert (μSv) for a proximal femur scan **[118, 122]**, which is less than the exposure found in a Trans-Canadian flight from Toronto to Vancouver **[123]**, it is a suitable tool for both pediatric and adult assessments. Furthermore, a DXA scanning procedure requires no special preparation from the participant, proving to be a painless and rapid method of assessment **[95]**. Although DXA remains a clinically relevant tool for assessing bone due to its ease of use, precision and reliability, it is not without its limitations. First, DXA is limited to a 2 dimensional (2D) plane of assessment. As a result, DXA is unable to assess bone properties in three dimensions, providing no information related to bone geometry and architecture. The 2-D image also does not allow for the assessment of cortical and trabecular bone, making DXA insensitive to potential morphological changes within the differing types of bone. Moreover, DXA can only estimate aBMD rather than true vBMD. Consequently, aBMD is more susceptible to inaccuracies related to bone size and orientation, often overestimating fracture risk in individuals with a small body frame**[118]**. For example, if two bones are made of the same material, but vary in size, the larger bone will have a greater aBMD (see Figure 1.6)



Measure	Blue Box	Red Box
Total Bone Mineral Density	1 g/cm³	1 g/cm³
Total Bone Mineral Content	1 g	8 g
Bone Area	1 cm²	4 cm²
Areal Bone Mineral Density	1 g/cm²	2 g/cm²

Figure 1.6: Demonstration of the size dependency of areal bone mineral density (aBMD) as measured by dual energy x-ray absorptiometry (DXA). Even though both boxes are composed of the same material, the red box has a greater aBMD. Modified from Khan et al **[95]**

1.2.4.2 Quantitative Computed Tomography (QCT)

Quantitative Computed Tomography (QCT) is as imaging technique that was introduced prior to DXA, but never gained the same prominence **[118]**. Unlike DXA, QCT uses a single x-ray source that rotates around an individual in conjunction with the detector plate. A bone mineral hydroxyapatite phantom is required to calibrate the machine and convert the machine's Hounsfield units into a vBMD measure **[19, 118, 124]**. A clear advantage of QCT is its ability to offer a three-dimensional image of a scanned region of bone. This allows QCT to provide cortical and trabecular bone separation, along with an estimation of bone geometry. Although QCT is typically used for lumbar spine assessments, proximal femur assessments have also been reported. A typical QCT proximal femur scan takes between 3-5 minutes and is able to provide estimates of CSMI, CSA, Z and vBMD **[125]**. Despite the clear advantages of QCT, the clinical impact remains relatively small compared to DXA **[118, 122]**. Additionally, QCT scan are associated with a high radiation dose (60 – 2900 μSv)**[19, 118, 124]**,which has reduced its practicality in longitudinal and pediatric studies.

1.2.4.3 Magnetic Resonance Imaging (MRI)

Recent advances in MRI technology have resulted in substantially enhanced MRI bone imaging. MRI technology uses pulses of radiowaves to differentiate tissues within the body. Each tissue has a distinct

magnetic resonance or vibration frequency which is detected by a resonance scanner. This information is then recorded by a computer to create an image of a three-dimensional shape. MRI technology allows for a variety of imaging sequences including multi-slice, oblique, spin-echo, and inversion recovery **[126]**. Spin-echo image sequencing remains the most commonly used for muscle and bone assessments **[126]**. Because MRI is a non-ionising method it is an attractive technique for longitudinal assessments of three dimensional bone properties in pediatric and adult populations. Measures derived from MRI have also been demonstrated to have strong correlations to bone histology and biomechanical strength testing *in vitro* **[127-129]**, displaying MRI's potential for fracture risk prediction. Although MRI bone imaging techniques are becoming well established at assessing bone geometry and structural properties at peripheral regions, such as the radius and calcaneus, there still remain challenges in image acquisition and standardization at the proximal femur **[19, 118, 124].** Bone imagining by MRI is also hindered by its expensive operational costs and limited access**.** Thus, despite MRI's technical potential, its clinical application remains unsubstantiated.

1.2.4.4 Hip Structural Analysis (HSA)

Hip structural analysis (HSA) is a technique used to estimate the geometric properties of bones at three locations of the proximal femur. This technique uses the two dimensional bone mass profiles derived from DXA to estimate the geometric properties of bone based on the principles described by Martin and Burr **[24]**. Martin and Burr indicated that a line of pixels across a bone axis is equivalent to a cut plane traversing the bone at that location **[24]**. According to this principle, the pixel mass profile can provide information on bone thickness, which in turn can be used to estimate geometric properties. Using the pixel mass profile, the HSA technique produces three, 5 mm thick cross sectional regions for analysis: 1) The Narrow Neck (NN) – the narrowest diameter of the femoral neck, 2) Intertrochanteric (IT)– along the bisector of the neck and shaft angle, and 3) the Shaft (S) – 2cm distal to the midpoint of the lesser trochanter **[25, 130]** (Figure1.7). From each region, a standard set of 10 outcome measures are

produced which include: aBMD, CSMI, CSA, Z, subperiosteal width (Wd), endocortical width (ED), cortical thickness (Ct.Th), profile centre distance, center of mass, and buckling ratio (BR) **[110, 130]**. By using DXA derived images, HSA maintains the inherent advantages associated with DXA (eg. quick, cost effective, safe within adult and pediatric populations). Additionally, the HSA program allows previous DXA scans to be reanalyzed in order to examine bone geometry at the proximal femur. The HSA program does have inherent limitations. Firstly, DXA design was not intended for geometric assessment. The HSA program provides simply an estimation of bone geometry based on several assumptions: 1) Bone shape based on simple cylindrical annuli; 2) Average tissue mineralization based on adult values; 3) Standardized cortical and trabecular distributions at the assessment sites (60/40 cortical to trabecular ratio at the NN, 70/30 at the IT, and 100% cortical at the S). These three assumptions result in potential underestimation in geometric estimation, specifically in pediatric populations. Despite these limitations, HSA remains a unique tool that is cost effective, relatively accurate, and provides an estimation of bone geometry and structural strength at a clinically significant fracture site.

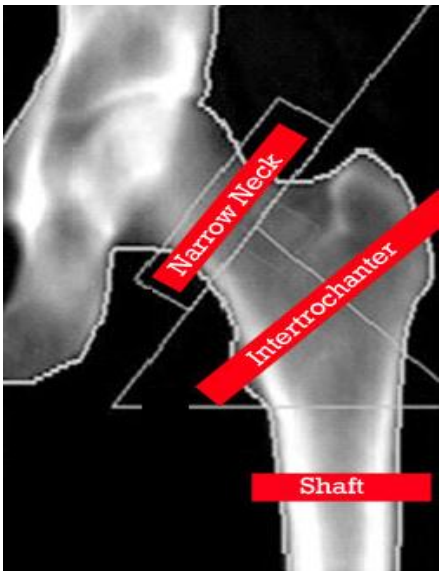


Figure 1.7: Depiction of the narrow neck, intertrochanter and femoral shaft regions as assessed by Hip Structural Analysis (HSA). Figure modified from Beck et al. **[130]**

1.2.5 Factors Influencing Bone Structural Strength

Bone structural strength is dependent on the delicate interaction between non-modifiable and modifiable factors. Although, the non-modifiable factors, such as genetics, have the largest influence bone structural strength development, modifiable factors may facilitate the achievement of an individual's full genetic potential. With CSA and Z established as valuable measures in determining bone structural strength, this section will concentrate on the discussing some of the factors that influence CSA and Z as components of bone structural strength at the clinical relevant proximal femur.

1.2.5.1 Age, Genetics, and Sex and Bone Structural Strength

The early work of Smith et al. (1973) provided seminal insights into the relationship between genetics and bone strength. Smith and colleagues observed a significant relationship between bone mass and first-degree family relationship, emphasizing that heritability and genetics were major determinants of bone structural strength. Similar to the work of Smith et al, most early genetic work has concentrated on measures of bone mass, providing compelling evidence that genetics and heritability account for approximately 40-70% of the variability of bone mass. Recent work has also supported a strong link between bone geometric measures and genetic inheritance. Shen et al. (2005) reported that the heritability of cross sectional geometric femoral neck parameters ranged from 0.37 to 0.62, emphasizing that the genetic linkage is dispersed across a variety of candidate genes and chromosomes. Xiong et al. (2006) detected a number of femoral neck geometry quantitative trait loci (QTL's), identifying region 20q12 to be significantly linked to multiple femoral neck geometric traits, such as buckling ratio, CSA, and Z; however, region 20q12 may contain candidate genes for parathyroid hormone and insulin growth factor proteins, which may also contribute to factors of both bone and muscular development. Given that muscle and bone cells derive from a common mesenchymal precursor (Karasik & Kiel, 2008), the shared genetic contribution between muscle and bone may be difficult to discriminate. Nevertheless,

there is evidence suggesting that bone structural strength is largely determined by genetics and heritable traits.

Similar to genetics, age is also considered a highly influential factor on bone structural strength. In fact, genetic and age-related factors are by far the greatest influences on bone structural strength **[131]**. All other factors in comparison modify bone strength to much smaller degrees **[131]**. The geometric properties of bone change drastically with age. During childhood and adolescence, modelling and remodelling alter bone size and shape by adding and removing bone from the periosteal and endosteal surfaces **[132]**. Prior to puberty, skeletal growth is similar between the sexes, where there is considerable apposition of bone to the periosteal surface, and expansion at the endosteal surface (Figure 1.8). These increases alter both CSA and Z. The addition of bone to the periosteal and endosteal surfaces enlarges CSA, while the addition of bone to periosteal surface shifts the cortical shell away from the long bone neutral axis. This outward neutral axis shift increases bone bending strength Z to a greater extent than a shift resulting from endosteal apposition. Thus, prior to puberty both sexes experience relatively similar structural benefits to CSA and Z. During puberty, however, skeletal disparities between the sexes become apparent. In males, periosteal apposition and endocortical resorption continue, while periosteal apposition diminishes and endocortical apposition increases in females **[133, 134]** (Figure 1.8). This contributes to noticeable structural strength differences. In males, the periosteal apposition increases both CSA and Z, while in females there is continued increase in CSA due to endocortical apposition, but this contributes to relatively minor advantages to Z. In adulthood, CSA is argued to follow a contemporaneous pattern to aBMD in both sexes, where aBMD and CSA continue to increase until a plateau, around 20-30 years of age, and an eventual decline occurs. Supporting this supposition Zhang et al. **[32]** recently observed, in a cross sectional study, that CSA at the proximal femur begins to decline as early as the second to third decade of life. This would suggest that bone compressive strength would plateau between 20-30 years of age and may even be compromised with old age (>60 years old).

Compensating for this decline, it is hypothesized that Z continues to increase with age to help maintain structural strength. Beck et al **[33]** investigated this theory, using HSA, in a group of 20-80 year olds. They observed that the age related decline in BMD at the proximal femur was associated with a reduced age related loss in Z due to a linear compensation in subperiosteal expansion **[33]**. Their results would suggest that although bone mass and CSA decline with old age structural strength may be maintained through geometric adaptations. Additionally, it would appear as though the development of Z lags behind bone mass and CSA as a potential compensatory mechanism. It has been previously documented that the timing of peak Z velocity development proceeds peak CSA development at the proximal femur during adolescence **[135]**. Whether this pattern occurs when bone mass and geometry are argued to be plateauing in early adulthood remains unsubstantiated. Regardless of this pattern continuing to occur in adulthood, it remains evident that there are age-dependent influences on the development of bone structural strength, and that these parameters may be sex-dependent.

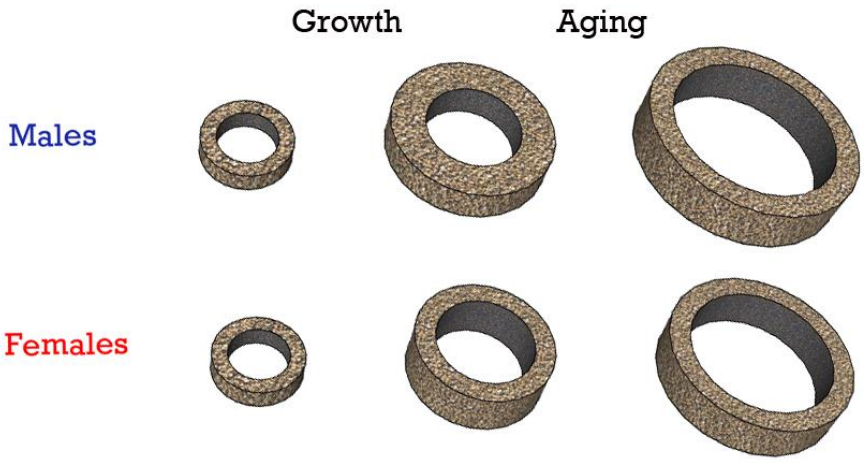


Figure 1.8: Depiction of the bone changes due to endosteal and periosteal apposition during growth and with aging. Skeletal growth prior to puberty is similar between sexes (far left circles), where there is considerable apposition of bone on the periosteal surface, and expansion at the endosteal surface. Once pubertal growth begins, sex discrepancies begin to become apparent (middle circles). In males, periosteal apposition and endocortical resorption continue, while periosteal apposition diminishes and endocortical apposition increases in females. With aging there is endosteal expansion that is seen in both males and females (far right circles).

1.2.5.2 Maturation and Bone Structural Strength

Alongside the non modifiable influences of genetics, age and sex, the role of puberty and maturation on bone structural strength has garnished recent attention. In particular, pubertal and maturational timing are documented as influential in determining the efficacy of mechanical loading on bone strength development and explaining the skeletal sex differences observed throughout life. Central to these explanations is the effects of estrogen on bone.

1.2.5.2.1 Estrogen and Bone Structural Strength

Estrogen has a diverse range of actions on the growth, differentiation and function of many target tissues within the body. With bone, it is well established that estrogen influences size, shape and density throughout life. As previously mentioned, during the pre-pubertal years skeletal growth is relatively similar between sexes, as are circulating estrogen levels **[9]**; however, once the onset of puberty begins skeletal disparities become apparent. Underpinning these observed skeletal dimorphisms are the increases in sex hormones in both sexes. Although it was previously hypothesized that the discernible skeletal disparities were primarily mediated by the of surge in sex specific hormones (eg. testosterone in males, estrogen in females), a significant body of evidence has now established that androgen-mediated bone growth in males is in part mediated through the aromatization of androgens to estrogens **[136, 137].** This acknowledges the fact that despite the common characterization of estrogen as an exclusive female hormone, circulating estrogen levels in males, resulting from the aromatization of testosterone, plays an equally pivotal role in bone growth and development **[34, 36, 38, 138]**. As circulating estrogen levels rise during puberty, linear bone growth is stimulated through activation of the estrogen receptors[2] on the bone surfaces and within bone cells **[36, 139]**. Estrogen receptor alpha (ERα) and beta (ERβ) are acknowledged as important estrogen-activated regulators of bone adaptation as they help

[2] Linear growth is stimulated through complex activation from various hormones receptors including androgens, growth hormones, and insulin like growth factors, and not exclusively from estrogen receptors.

retain estrogen within bone cells and help stimulate cell activity **[34, 38, 140, 141]**. Activation through ERα is associated with positive bone formation and osteoblastic stimulation **[142]**, while ERβ activation is associated with an inhibitory bone formation response **[34, 38]**. Thus, despite having similar estrogen affinities, ERα and ERβ may play antagonistic roles towards bone formation. Additionally, their distribution on bone surfaces may partially explain the skeletal disparities between sexes **[34, 38, 140, 143]**. The total distribution of estrogen receptors is similar between sexes, but their distribution on the periosteal and endosteal surfaces may differ. For instance, ERα is expressed on the both the periosteal and endosteal surfaces in both sexes, but males display a higher distribution on the periosteal surface, while females having increased numbers at the endosteal surface. In contrast, ERβ is found on both the periosteal and endosteal surfaces in both sexes with relatively similar distribution **[144, 145]**. This may partially explain why the surge in estrogen levels during puberty may mediate apposition of bone on the endosteal surface in females but at the periosteal surface in males through the activation of ERα. Furthermore, it would be reasonable to speculate then that as a result, estrogen activation would be influential in altering structural properties such as CSA and Z.

In addition to estrogen's direct effects on bone cells through activation of estrogen receptors, estrogen is purported to also modulate the response of bone to mechanical stimulation. The role of mechanical loading on bone formation is discussed in greater detail in proceeding sections (*Section 1.2.5.3.1*), but its relationship with estrogen will be briefly outlined here. According to the mechanostat theory, estrogen alters the mechanosensitivity of bone by lowering the stress strain set point **[52, 146].** This is purposed to supplement the already positive influences of mechanical loading on bone structural strength by allowing bone adaptation to be stimulated by a mechanical loading event that may not otherwise trigger an osteogenic response.

As a result of estrogen's positive influence on bone formation, bone apposition and its modulating effects on mechanical loading sensitivity, an earlier exposure to estrogen in both sexes would be

considered advantageous to an individual's bone structural strength. To investigate this notion, experiments exploring the relationship between pubertal timing, as a surrogate of timing of estrogen exposure, and bone strength measures have been conducted. Chevalley et al **[43]** investigated the influence of age of menarche on the bone mass, cortical, and trabecular architecture at the distal radius in young adult women. They observed that the age of menarche was inversely correlated with BMD and that individuals with an earlier menarche onset had significantly greater radial aBMD and cortical vBMD, along with greater cortical thickness. The authors concluded from these observations that a delayed age of menarche may be deleterious to bone strength at the distal radius in females **[43]**. Although the findings of Chevalley et al **[43]** are limited to females, similar observations have are also been reported in males. Kimblom et al. **[41]**, using a population of late adolescent (19 years of age) males investigated whether pubertal timing was associated with bone strength measures, derived from DXA and pQCT, and self reported fracture history. They observed that the age of PHV was an independent negative predictor of total body and radial cortical and trabecular vBMD in late adolescent males. Additionally, the age of PHV was a significant predictor of previous fractures, supporting the notion that an early pubertal onset may be advantageous not only to females but to male bone structural strength at the distal radius. Findings at the proximal femur have been limited to the assessment of bone mass, with conflicting results. Gilsanz et al. **[42]** recently reported that in both sexes the age of puberty significantly predicted BMC and aBMD measures at the proximal femur, with an earlier pubertal onset predicting greater proximal femur BMC and total body BMC. In contrast, Jackowski et al **[147]** observed no difference in BMC at the femoral neck between early, average and late maturers, in either sex; instead differences were seen in total body BMC, favoring early maturers. These conflicting results at the proximal femur may be the result of the age range of the participants assessed. Gilsanz et al followed individuals from 8-16 years of age, while Jackowski et al participants were 8-to nearly 30 years of age. These findings, taken as a whole though, would suggest that pubertal timing, may affect bone strength at the proximal femur

during adolescence, but these difference may no longer be apparent by adulthood. Whether pubertal timing influences bone structural strength variables, such as CSA and Z, at the proximal femur, remains unverified and an avenue of investigation.

1.2.5.3 Physical Activity and Bone Structural Strength

Physical activity is often cited as an important determinant for developing and maintaining strong, healthy bones. The mechanisms responsible for physical activity's associated benefits to bone are complex, but its foundation is based on its connection with mechanical loading. The next section will focus on characterising the foundation and theories that may help explain the positive association between physical activity and bone structural strength.

1.2.5.3.1 Mechanical Stimuli and Bone Adaptation

Bone adaptation is a result of a complex array of genotypic and environmental influences. Of the environmental factors, it is proposed that mechanical loading is essential. The notion of mechanical stimuli influencing bone dates back over a century with the early works of Jean Baptise Bourgery and Frederick Oldfield Ward who observed that tissue arrangements at the proximal femur are in line with the principle compressive and tensile forces acting at this bony region **[148]**. The seminal work of Wilhelm Roux and Julius Wolff expanded on these observations to suggest that the alignment of the bony tissue can be predicted from the mathematical calculations of the mechanical forces that act upon the body, and that altering these forces would result in predictable alterations in the tissue arrangement **[148]**. This concept of bone adaptation is often cited as *Wolff's Law*. Wolff's law would suggest that all mechanical loads would elicit some form of bone adaptation; however it has more recently been acknowledged that despite this prediction not all loads are osteogenic. Bone tissue is most responsive to dynamic loads rather than static loads **[149-152]**. Dynamic loads create hydrostatic pressure gradients within the bone's lacunar-canalicular network, generating fluid shear stresses which are experienced by the highly sensitive bone cells (e.g. osteocytes, bone lining cells, osteoblasts). This triggers a cascade of

events, including the release of intracellular calcium, hormone secretions, and bone matrix protein production. This process of transferring the mechanical loading stimuli into a complex reaction of chemical activity is termed mechanotransduction. This triggering of events would suggest that activities producing dynamic loads have a greater osteogenic potential than activities comprised of static loads and is supported by numerous animal **[152-154]** and human studies **[155-159]**. In a similar vein, Harold Frost theorized that integral to bone adaptation was the detection and transfer of mechanical strain. This seminal notion became to be the foundation of the mechanostat theory **[50, 52]**. According to the mechanostat theory, bone adaptation in load bearing bones is controlled by a mechanical mechanism that monitors the mechanical usage of bone and regulates the biological mechanisms, much like a household thermostat, that would turn on and off bone adaptation **[50, 52].** Central to the mechanostat theory is the production of strain and its detection. Strains above a genetically determined modelling minimum effective strain (MESm) stimulates bone apposition to strengthen the load bearing bone; whilst bone strains under the remodelling minimum effective strain (MESr) signal disuse remodelling, where whole bone strength is reduced. In addition to the MESm and MESr, a pathological minimum effective strain (MESp) also exists, where repeated strain causes microdamage **[52, 146]** (Figure 1.9). Above this threshold, microdamage accumulates, which may compromise the structural integrity of load bearing bones. Thus, according to the mechanostat theory, dynamic loads help to maintain bone strength when it produces strains that are above the MESr, but promotes bone formation when strains produced are above the MESm. It also purports that a static load, although it may produce strain, cannot be detected by the mechano-sensing cells, and thus unable to stimulate an adaptive response. This suggests that static loads can actually initiate bone loss. When strains, or lack of strain, are below the MESr, increased remodelling is initiated, which results in bone loss. This bone loss is speculated to continue until bone sufficiently adapts to state where the experienced stains are above the MESr, which would then trigger responses for bone adaptive maintenance.

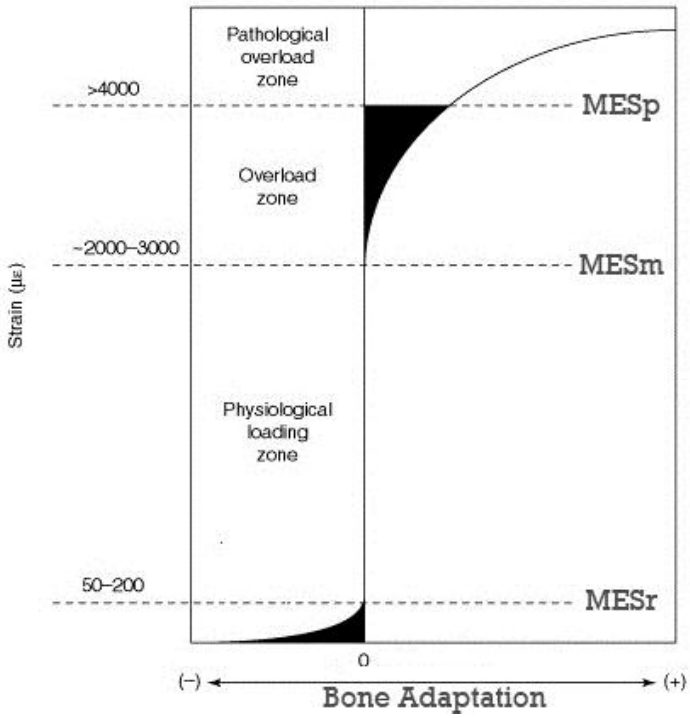


Figure 1.9: The effects of mechanical strain on bone adaptation following the postulates of the mechanostat theory. Strains below the resorptive minimum effective strain (MESr) result in negative bone adaptation or loss of bone. Strains levels above the modelling minimum effective strain (MESr) result in positive bone adaptations or the addition of bone. Strains above the pathological minimum effective strain (MESp) result in pathological bone adaptations (eg fractures) **[50-52]**. Modified from Bachrach **[160].**

In addition to the importance of strain, the mechanostat theory also acknowledges the importance of extrinsic factors and recruitment processes that may influence the detection of strain and its response triggering **[50-52, 146]**. For instance, the mechanostat identifies that precursor hormones, such as estrogen as mentioned previously, may shift the minimum effective strain thresholds, altering the sensitivity of mechano-sensing cells to a given strain **[52, 91, 146]**. These precursor hormones may also facilitate the triggering of response mechanisms, such as the stimulation of bone matrix protein synthesis. Although the mechanostat provides a foundation explaining the relationship between mechanical loading and bone adaptation, it is not without its limitations. The mechanostat provides a thorough explanation for the associated benefits attributed to the altered magnitude of loading, but it fails to incorporate the influence of load frequency. Load frequency deals with the rate at which the load is applied and it is documented to alter bone's adaptive response. Loads applied at a high frequency (20-50 hertz ,Hz) are documented to be more effective than low frequency loads (<10Hz) at stimulating

osteogenesis **[149, 150]**. Interestingly though, there appears to be a complex relationship between load magnitude and load frequency, as it has been reported that low magnitude loads can be osteogenic if applied at a high frequency **[161-163].** This would suggest that high magnitude and high frequency loading are beneficial in stimulating positive bone adaptation. Although there is literature to support the positive effects of high frequency and high magnitude loading on bone, like other mechano-sensing cells in the body, bone cells exhibit a desensitization phenomenon in the presence of extended mechanical loading sessions **[149]**. Thus, rest between loading bouts is also necessary to elicit positive bone adaptations. In summary, dynamic mechanical loading producing strains above a genetically determine mechano-sensing threshold at high frequencies appear to be ideal stimuli for eliciting positive bone adaptation.

1.2.5.3.2 Physical Activity and Bone Structural Strength Throughout Life

Activities that involve dynamic loads, which produce high magnitude strains at high frequencies may serve as the ideal stimulus for positive bone adaptations and thus, benefit overall bone structural strength. Not surprisingly then, physical activity is often used as a surrogate model of mechanical loading to investigate the influence of loading on bone structural strength in humans. The next section will discuss the role of physical activity on bone structural strength from childhood and adolescence into adulthood, with attention given to studies at the proximal femur.

1.2.4.3.3 Physical Activity and Bone Structural Strength in Childhood and Adolescence

The childhood and adolescent period is often referred to as a unique window of opportunity where the advantages to bone strength can be readily accrued. As a result, physical activity is purported to have its greatest influence on bone structural strength during this period. Early investigations with BMC have demonstrated that higher levels of physical activity in children, as early as 4-6 years in age, can confer 13% greater proximal femur BMC compared to children with lower activity levels **[59]**. Similarly, during adolescence, Bailey et al **[55]** have highlighted that physically active males and females have 9% and

17% greater total body BMC compared to their less active counterparts, supporting the notion that engaging in physical activity is important to the accrual of bone mass during adolescence. Similar to these findings in bone mass, there are data supporting the potential of physical activity to enhance geometric properties of bone structural strength too. Forwood et al. **[58]** investigated the relationship between daily physical activity and bone geometry at the femoral neck using HSA in 109 healthy males and 121 healthy females. These authors noted that physical activity significantly predicted CSA and Z in both sexes, and that the physically active individuals had approximately 4-5% greater CSA and Z than their less active counterparts. Janz et al. **[60]** reported comparable findings in young children aged 5-8 years. Using accelerometers, Janz and authors examined the longitudinal associations between physical activity and hip strength during childhood, concluding that engaging in 40 minutes of moderate-to-vigorous physical activity conferred a 3-5% advantage in proximal femur CSA and Z in both males and females throughout childhood **[60]**.

Exercise interventions also provide elegant evidence highlighting the importance of physical activity on childhood and adolescence bone structural strength. MacKelvie and colleagues **[164]** investigated whether a randomized control trial of a school based weight bearing, high impact circuit intervention resulted in structural bone strength benefits at the proximal femur in young males (~10 years of age) two years after the exercise intervention. These authors observed that even 2 years after the completion of the exercise intervention, participating males had significantly greater periosteal and endosteal diameters which resulted in a 7.5% greater Z than age and maturity matched controls. Similar results are reported by Weeks et al. **[165]** who report that an 8-month school based jumping intervention provided 3.8% greater femoral neck CSA and 18% greater femoral neck CSMI in young adolescent males (~14 years of age) compared to size and maturity matched adolescent male controls. These data suggest that physical activities, involving high impact loading are effective in eliciting bone structural strength benefits during childhood and adolescence.

Although physical activity throughout the childhood and adolescent period appear to provide favorable responses to bone structural strength, there is evidence to suggest that activities performed prior to puberty are further advantageous. Recently, Ducher et al **[166]** investigated whether involvement in competitive tennis in premenarcheal and postmenarcheal females resulted in exercise induced skeletal differences to bone structural strength at the humerus. They observed that the gains in total area (+10%) and cortical area (+16%) attributed simply to growth were three to fourfold greater in premenarcheal tennis players than the gains attributed to growth in postmenarcheal players **[166]**. Additionally, the repetitive loading in the playing arm resulted in an additional 3% to total bone area and 4% to cortical bone area in premenarcheal players, which was significantly greater than the exercise induced gains observed in the postmenarcheal players **[166]**. This would suggest that participating in load bearing physical activity during the adolescent period is favorable to induce positive skeletal adaptations that may benefit bone structural strength, but engaging in osteogenic physical activity prior to puberty may further supplement any bone structural strength advantages compared to the same activity post puberty.

In summary, the childhood and adolescent period may be an ideal time where the adaptation to bone structural strength associated with physical activity may be maximized. Additionally, it appears that activities engaged prior to puberty appear to be further advantageous.

1.2.4.3.3 Physical Activity and Bone Structural Strength in Adulthood

The adult period is characterised by a cessation in long bone growth in length through the closure of the epiphyseal growth plates. This cessation in bone length does not mean that skeletal adaptations fail to continue and bone structural strength remains static in adulthood. In fact, as mentioned in section 1.2.5.1, structural strength measures continue to change with age in adulthood, and, similar to the childhood and adolescent period, physical activity may influence the direction of these adaptations in adulthood too **[167, 168]**. Of notable difference though, is that the response to osteogenic stimuli may

be less vigorous in adult bones **[48, 169]**. Highlighting this point, Kontulainen et al **[48]** compared bone strength measures derived from DXA and pQCT at the radius between young female squash and tennis players who began training either at a young age (11 years of age, prior to menarche) or older age (26 years of age) observing that young starters had between 8-14% higher values for bone strength measures than older starters. Despite adult bone's having a reduced responsiveness to osteogenic stimuli, physical activity and exercise in adulthood has been documented to have positive skeletal effects, with emphasis placed on high impact type activities particularly at the proximal femur. Hind et al **[170]** compared hip structural geometry in young adult males participating in running, gymnastics and swimming. They noted that the young males engaging in running and gymnastic had significantly greater narrow neck CSA and CSMI than swimmers and controls. The authors suggested that the high ground reaction forces experienced at the hip associated with running and gymnasts serve as greater osteogenic stimuli than activities performed in weight support environments **[170]**. Further emphasising this point, premenopausal women participating in an 18-month high impact jumping and step aerobics randomized control trial were observed to have 3% greater CSA and Z at the proximal femur compared to normally active controls **[171]**. These authors also observed that these skeletal benefits to the proximal femur geometry were no longer apparent 3.5 years after the exercise intervention. This would suggest that in adulthood bone structural strength at the proximal femur adapts to current loading conditions and previous gains can be reversed if loading conditions diminish **[171]**. The premise of reversibility is particularly evident in immobilization studies in adults. Studies in patients with spinal cord injury have found that there is reduced cortical thickness, total bone area and section modulus at the weight bearing regions such as the tibia and femur **[172, 173]**. Similarly, simulated spaceflight using bed-rest models have suggested that there are marked reductions in cortical area, cortical thickness, and bone mass at the tibia even after short exposures to skeletal unloading **[174]**. These authors also indicated that recovery to baseline values took between 6 to 24 months. Irrespective of the mode of disuse,

pronounced bone loss is apparent, and the time to recover bone losses may be lengthy in adult bone **[175]**. In summary, physical activity appears to have positive effects on bone structural strength even in adulthood; however, these skeletal advantages can be quickly lost if physical activity patterns are reduced in adulthood.

1.2.4.3.5 Physical Activity and Bone Structural Strength From Childhood and Adolescence into Adulthood

With evidence to suggest that physical activity is beneficial to bone structural strength during childhood, adolescence and adulthood, and that the childhood and adolescence period may be the ideal opportunity to develop skeletal strength advantages, it seems reasonable to speculate that physical activity during the critical childhood and adolescent may confer lifelong skeletal advantages well into adulthood. Retired athlete models have been previously used to address this hypothesis with favourable results. Erlandson et al **[68]**, assessed whether the previously reported higher bone mass in a group of premenarcheal gymnasts was still apparent 10 years after the cessation of gymnastics participation. They observed that even when adjusted for size, the premenarcheal female gymnasts continued to display a 13-19% advantage in BMC measures, despite a 10 year cessation from gymnastics participation. Similar results are reported by Pollock et al **[69]** who observed that former adolescent female gymnasts continued to have 8-12% greater aBMD than age and size matched controls. Although these described studies focus on former female athletes, similar results have been observed in retired males involved in soccer **[72]**, and weightlifting **[176]**. The results of retired athlete studies are not limited to bone mass measures. Similar findings have been observed for bone structural strength measures. Ducher et al **[177]** compared bone strength measures using pQCT between former artistic gymnasts and non-gymnastic controls. It was observed that former gymnasts who had been retired for approximately 6 years continued to have greater total bone area, bone strength index, cortical density and stress strain index than their non-gymnastics counterparts. Theses authors also observed that there

were site specific differences within the forearm, concluding that long term skeletal benefits are bone and site specific **[177]**. Similarly, Eser et al **[178]** compared bone geometry and densitometry at the tibia, femur, radius, and humerus of former gymnasts and non gymnastic controls. They observed that retired gymnasts had 20-25% greater CSA, 13-25% greater cortical CSA, 35-38% greater stress strain index at the radius and humerus compared to the non gymnastic controls even after 6 years of retirement from the sport. Additionally, CSA, cortical CSA and stress strain index were significiantly greater at the mid femur and tibia than non gymnastic controls, but with a lower percent difference (3-13%) than those observed in the upper limbs. From these observations, the authors concluded that elite gymnastics training may confer positive skeletal benefits even after retirement, but these estimated bone strength benefits may be site specific with weight bearing regions showing relatively small advantages after the cessation of elite activity **[178].** These previous studies would suggest that the skeletal benefits to both bone mass and bone structural strength may be maintained from adolescent activity, but the advantages may depend on skeletal site and the load bearing nature of that region.

These retired athlete models may provide compelling evidence to support the notion of maintained skeletal benefits from adolescent activity, but data derived from athlete models may be predisposed to selection bias. Thus, the skeletal attributes of the individuals may influence the initial choice to participate in the sport as well as the ability to successfully continue within the activity without injury **[167]**. As a result, non-athlete specific populations are necessary to address the question of whether adolescent activity can confer skeletal advantages into adulthood. Baxter-Jones et al **[57]** assessed the long-term benefits of adolescent physical activity on BMC in a cohort of non-athlete specific males and females. They noted that individuals classified as active during adolescence maintained 8-10% greater BMC at the total body, lumbar spine and femoral neck than individuals classified as inactive during adolescence. These findings support the conjecture that adolescent physical activity may confer skeletal benefits into adulthood, but these conclusions are limited to bone mass assessments. Studies

investigating the long term effects of adolescent physical activity on adult bone structural strength measures are scarce. This is mainly due to the lack of technology that was previously available to assess bone structural strength measures using longitudinal design; however, there are a few studies limited to physical activity assessments determined retrospectively that assess bone structural strength **[63, 70]**. Daly et al **[63]**, classified males, 50-87 years of age, from retrospectively determined previous sports and leisure time activities into osteogenic weight bearing activities and assessed bone strength measures at the radius, spine, hip, mid femur, and heel using DXA (radius, spine hip), QCT (mid femur) and quantitative ultrasounds (QUS, heel). They reported that higher levels of osteogenic weight bearing sports and leisure time activity participation was associated with greater CSA, cortical area and polar moments of inertia at the mid femur and higher velocity of sound at the heel. These results suggest that greater lifetime leisure and sports activity is an important determinant of bone size and strength in men over 50 years of age **[63]**. This conjecture is further supported by observations from males involved in the Gothenburg Osteoporosis and Obesity Determinants (GOOD) study **[70]**. Here, bone strength measures derived from pQCT at the distal radius and distal tibia were assessed, and similar to the Daly et al **[63]**, previous sports and leisure activity participation was determined and an osteogenic value determined based on the load bearing nature of the activity. It was observed that individuals who participated in sport activities during adolescence had 3.2% greater periosteal circumference and 7% greater CSA at the distal tibia compared to those who were classified as inactive during adolescence. Additionally, these authors noted that individuals who were physically active during adolescence and maintained higher levels of activity into adulthood had significantly greater cortical width and CSA than individuals who were only highly active during adolescence **[70]**. These findings would suggest that not only is previous adolescent activity beneficial to adult bone structural strength at the weight bearing tibia, but that continued activity supplements these skeletal advantages into adulthood. Although these findings provide novel insight on the long term effects of adolescent physical activity on adult bone

structural strength, their conclusions are derived from retrospective data, limited to males, and provide only speculation about the effects at the clinically significant proximal femur.

In summary, it appears that adolescent activity may provide skeletal benefits to bone mass and potential bone strength measures at weight bearing regions in adulthood, but there continues to remain a paucity of information as to whether adult bone structural strength advantages at the proximal femur also result from previous adolescent activity levels.

1.2.6 Summary of Literature Review

The societal burden of osteoporosis related hip fractures is monumental. Understanding strategies for preventing these osteoporosis related fractures is of the upmost importance. Determining whether a bone may fracture is largely dependent on its structural strength. Geometric measures such as CSA and Z provide direct estimation of a bone's compressive and bending strength, providing valuable insight into whole bone strength. The childhood and adolescent period is identified as a unique time when bone structural strength undergoes drastic alterations. As a result, factors influencing bone structural strength during this period may have effects on development of bone strength throughout life. The mechanostat theory purports that bone strength is constantly altered based on the production and detection of strain by mechanosensing bone cells. The exposure to estrogen is documented to alter the sensitivity of the mechanosensing cells, allowing lower levels of strain to induce a positive osteogenic response. Previous cross sectional research has suggested that an earlier pubertal onset, associated with earlier estrogen exposure, may provide skeletal advantages to bone structural strength at the distal radius in both males and females; however a paucity of information remains regarding the role of pubertal timing and bone structural strength measures at the clinically relevant proximal femur. Additionally, the mechanostat theory predicts that dynamic strains, such as those found when engaging in physical activity and exercise, have positive effects on bone size, shape and strength. Previous literature supports this notion, identifying that physical activity is beneficial to bone structural strength

throughout life. Furthermore, retired athlete models have provided evidence to suggest that individuals engaged in higher levels of physical activity during the critical adolescent period may maintain bone strength benefits into adulthood. This notion, however, is mainly derived from retrospective and cross sectional data within athletic populations. It remains unsubstantiated whether adolescent physical activity may result in sustain structural strength advantages at the proximal femur in adulthood. Prospective longitudinal designs are necessary to address these questions, and in turn, provide insight into long term bone strength development at a clinically relevant fracture site.

1.3 Aims and Hypotheses

1.3.1 Study One

The primary aim of the first study is to describe the longitudinal development of cross sectional area and section modulus at the proximal femur from childhood, through adolescent and into early adulthood in a healthy population of males and females. Within this primary aim, there are two sub-goals: i) to identify when peak values for cross sectional area and section modulus occur at the proximal femur, ii) to determine the percent of adult peak CSA and Z that is attain during the critical adolescence growth period. The determination of these peak values in study will be integral to establishing how growth influence bone structural strength at the proximal femur, and will serve as a foundation for establishing the outcome variables for study 2 and 3.

It is hypothesized that geometric bone structural strength measures at the proximal femur will increase rapidly during childhood and adolescence as a result of growth, and continue to increase into early adulthood where they will peak after the plateau in bone mass is achieved.

1.3.2 Study Two

The primary aim of the second study is to examine the relationship between maturational timing and bone structural strength development at the proximal femur in a healthy population of males and

females. Using the peak values derived from study one, this second study will build on the effects of growth and help to examine the role of maturation on the development of the bone structural strength peaks.

It is hypothesized that cross sectional area and section modulus measures would be dependent on maturational onset, with early maturing individuals having an advantage in their estimated bone structural strength at the proximal femur.

1.3.3 Study Three

The aim of the third study is to investigate whether physical activity during adolescence confers bone structural strength advantages in adulthood at the proximal femur.

It is hypothesized that cross sectional area and section modulus adapt to current mechanical loading, and although adolescent physical activity will confer bone structural strength advantages, these advantages will disappear once current activity is accounted.

Chapter Two

Research Studies

2.1 Study One: The Timing of Bone Structural Strength Adaptations at the Proximal Femur from Childhood to Early Adulthood in Males and Females

2.1.1 Abstract

During adolescence, the peak velocity in bone mass accretion precedes the peak velocity of estimated geometry at the hip. Whether this pattern continues into adulthood when maximum values are achieved remains unknown. The purpose of this study was 1) to identify the ages at which peak values of aBMD, cross sectional area (CSA) and section modulus (Z) occur; 2) to determine the percent of adult peak attained during adolescence for each of these measures; and 3) to determine the relationship between body composition and the timing of the adult peak values.

One hundred sixty-five (92 females) individuals' aBMD, CSA and Z were serially assessed at the narrow-neck (NN), intertrochanter (IT) and shaft (S) using hip structural analysis (HSA). Peak bone values for BMD (aBMDp), CSA (CSAp) and Z (Zp) and the age of attainments for each were assessed using factorial MANOVA.

In males, aBMDp (NN, 19.4 ± 2.7y; IT, 20 ± 3.4y; S, 21.8 ± 2.8y) occurred significantly earlier than CSAp at all sites (NN, 21.6 ± 3.2y; IT, 21.1 ± 3.4y; S, 22.3 ± 3.1y) and earlier than Zp at the NN (22 ± 3.2y) and IT (21.3± 2.9y). In females, aBMDp (NN, 17.9 ± 2.7y; IT, 18.7 ± 3.5y; S, 19.7 ± 3.3y) occurred significantly earlier than CSAp at all sites (NN, 20.6 ± 3.6y; IT, 19.4 ± 3.9y; S, 21.0 ± 3.3y) and earlier than Zp at the NN (20.7 ± 3.4y) and S (20.6 ± 3.5y).

The changes in bone mass precede changes in geometric CSA and this timing may be integral for the development and maintenance of bone strength.

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2.1.2 Introduction

Bone is constantly repaired through the dynamic process of bone formation and resorption, altering the structural integrity and strength of the bone within limits of the applied loads. Disequilibrium between periosteal bone formation and endosteal bone resorption is proposed to be the underlying mechanism for osteoporosis **[179, 180]**. Recently it has been observed that the decrease in areal bone mineral density (aBMD) in adulthood is compensated by adaptations in bone geometry, in both men and women, to maintain structural integrity and strength **[181-183]**. Additionally, it has been noted that the rate of endosteal resorption is higher in women, proposing a sex differences in the geometric adaptation with aging **[184]**. Zhang and colleagues **[32]** recently observed in a cross sectional study that regardless of sex and ethnicity, there is a natural age related decline in cross sectional area (CSA) and cortical thickness at the proximal femur beginning around 20 years of age. This suggests that geometric properties may peak near the second decade of life. Similarly, Beck et al. **[33]**, using hip structural analysis, observed an age related decline in aBMD at the proximal femur in a group of 20-80 year olds, with a reduced age related loss in section modulus (Z) due to a linear compensation in subperiosteal expansion. This highlights that although bone mass is lost, mechanical strength may be maintained through geometric adaptation; however, these suppositions are derived from cross sectional data, which may be biased and lead to underestimation of the subsequent age related geometric adaptations **[184]**. Similarly, the majority of this literature has focused on adult populations, resulting in a paucity of information surrounding the adaptation of bone geometry during childhood into early adulthood. Although hip bone fragility and related fractures are largely experienced during adulthood, this disease may have pediatric antecedents **[7]**; thus it is important to understand potential childhood and adolescent mechanisms that may influence this disease. We have shown previously that during the adolescent growth spurt, peak height velocity (PHV) is followed by a peak in the velocity in total body bone mineral accrual **[54, 82, 185]** and the peak in CSA and Z velocities at the proximal femur **[135]**. It is

therefore purported that these timing patterns of peak tissue velocities observed during rapid growth in adolescence may be replicated when values reach their maximum or peak absolute values in young adulthood. Given that peak bone mass is achieved between the second and fourth decade of life **[27, 31, 186]** it remains imperative that geometric assessment also be determined longitudinally into this age range. Additionally, lean tissue mass (LTM) has been documented to influence bone mass and geometry development during adolescence **[29, 135, 185, 187-197]**. Frost's mechanostat theory postulates that the development of LTM increases mechanical strains that would be sufficient to elicit an adaptive bone response **[50]**. Similarly, fat mass (FM) has been also been acknowledged as a predictor of bone strength, although with more controversy **[196-198]**, with FM even suggested to stimulate bone changes during growth **[199]**; however, there remains a scarcity of information investigating whether LTM and FM influence the timing of aBMD and estimated bone geometry at the hip in adult males and females. Moreover, the process of growth and maturation is continuous through childhood and adolescence and may influence the associated sex differences in bone strength development **[11]**. Thus, an assessment of biological age is necessary to control for the confounding effects of maturation **[11]**. Therefore, the present study describes the longitudinal development of bone geometry at three sites of the clinically relevant proximal femur from childhood, through adolescence and into early adulthood in a healthy population of males and females aligned by biological age. The purpose was to identify the ages in which peak values of aBMD and bone geometry occurred; to determine the percentage of adult peak accrued during adolescence, and to determine the relationship between LTM and FM development and the timing of the adult peak values. It was hypothesized that there would be sex differences in the chronological ages of peak bone measures, but this would disappear once maturation was controlled, and that the age of peak aBMD would occur prior to age of peak geometric bone measures.

2.1.3 Methods

2.1.3.1 Participants

Participants were drawn from the University of Saskatchewan's Pediatric Bone Mineral Accrual Study (PBMAS). Details of the PBMAS participants have been described previously **[53, 55]**. In brief, in 1991, of the 375 eligible students attending two elementary schools in the city of Saskatoon (population 200,000), the parents of 228 students (113 boys and 115 girls) provided written consent for their children to be involved in this study and 220 had bone mass scans using dual energy x-ray absorptiometry (DXA). From 1992 to 1993 an additional 31 participants were recruited and scanned. After 7 years of data collection, 197 participants had been measured on two or more occasions (median 6 scans). Between 2002 and 2006, 169 participants returned and were measured on at least 1 occasion (ages range 18.2 to 27.5 years). To be included in the present study, participants had to have a valid assessment of peak height velocity (PHV), have geometric bone assessments during both adolescence and at least once in adulthood, and have no diseases known to affect growth or bone development. This resulted in the inclusion of 165 participants (73 males and 92 females) covering the age span of approximately 8-30 years of age. In the present sample, each individual had on average 8 measurement occasions with, on average, 3 of these data points between 18 and 28 years of age. Ninety eight percent of the participants were of Caucasian descent. Written consent was obtained from all participants. All procedures were approved the University of Saskatchewan's biomedical review committee.

2.1.3.2 Anthropometry

Height was assessed annually following the anthropometric standards outlined by Ross and Marfell-Jones **[200]**. Height was recorded without shoes to the nearest 0.1 cm using a wall mounted stadiometer (Holtain Limited, Crymych, UK).

2.1.3.3 Maturation

An important advantage of a longitudinal design is the ability to compare individuals on a common maturational landmark found in males and females, specifically the attainment of peak linear growth (peak height velocity, PHV). To determine the age at PHV, whole year height velocities were calculated for each participant. A cubic spline fitting procedure was applied to each individual's whole year velocity values and the age at the highest point was estimated (GraphPad Prism 5, GraphPad Software, San Diego, CA, USA). The cubic spline curve fitting provides a smooth velocity curved based on polynomial algorithms which provide an estimation of age and magnitude while maintaining the original integrity of the data. Biological age was then calculated by subtracting the age at PHV from the age at time of measurement for each individual (e.g. Age at time of measurement = 9.3 years, age of PHV = 13.4 years, biological age at measurement = 9.3-13.4 = -4.1 years).

2.1.3.4 Lean Tissue Mass and Fat Mass

Total body lean tissue mass (LTM) and fat mass (FM) were assessed annually using dual energy x-ray absorptiometry (DXA, Hologic QDR-2000, array mode) by a trained technician following the procedures outlined in the Hologic's operators manual and user guide. LTM and FM were analyzed using software version 5.67A. Scanner drift was assessed prior to each scan using the manufactures lumbar spine phantom. The inter-assay precision (CV%) in vivo for LTM and FM in our lab have been previously reported as 0.54% and 2.95%, respectively **[53, 56]**. To determine the peak value for LTM (LTMp) and FM (FMp), the annual absolute values for each tissue was independently plotted against chronological age and the peak value was determined as the maximal absolute value.

2.1.3.5 Bone Measures

Bone measures were derived using the hip structural analysis (HSA) program. The HSA program has been previously reported elsewhere in greater detail **[25]**. In brief, the HSA program estimates the structural geometry of the proximal femur from DXA derived images of the hip. Employing the principles

originally reported by Martin and Burr **[24]**, a line of pixels values traversing the bone axis in a bone mass image is a projection of the corresponding cross section and its dimensions. To determine these cross sections, the HSA algorithms divide the pixel mass values in g/cm^2 by the effective mineral density of fully mineralized adult cortical bone. Cross sections are evaluated by averaging geometry over 5 parallel lines spaced 1mm apart at: 1) The Narrow Neck (NN) – the narrowest diameter of the femoral neck; 2) Intertrochanteric (IT) – along the bisector of the neck and shaft angle; and 3) the Shaft (S) – a distance of 1.5 times the minimum neck width to the intersection of the neck and shaft axes **[130]**. The HSA program locates these regions on the DXA bone mineral image then derives the estimates of structural geometry. From each region, the HSA program produces ten output variables, of which three were assessed for this study: *Areal bone mineral density* (aBMD) – the estimated amount of bone mineral within the program-selected defined bone area; *Cross Sectional Area (CSA)*– the estimated amount of bone surface area in the cross section after excluding all the trabecular and soft tissue space; and, *Section Modulus (Z)* – an indicator of bending strength calculated as the CSMI/ the maximum distance from the center of mass to outer cortex [130]. The short-term precision for aBMD, CSA and Z derived from a hip scan using a Hologic QDR 2000 range from 2.2% to 2.3%, 2.3% to 2.8% and 2.8% to 3.4%, respectively **[26]**. All HSA analyses were completed by a single technician and derived from proximal femur scans using a Hologic QDR-2000 (Bedford, MA, USA).

2.1.3.6 Bone Strength Peak Assessment

The age at peak proximal femur aBMD, CSA and Z were determined for each participant. The annual absolute values for aBMD, CSA and Z were independently plotted against chronological age (years from birth) from which the peak values of aBMD (aBMDp), CSA (CSAp) and (Zp) were derived at the three locations of the proximal femur. The peak values were determined as the maximal absolute value for each bone measure at each site. Once the peak values were identified, the chronological ages at which

these peaks occurred was determined. These ages were then converted to a biological age (years away from PHV) as outlined above.

Additionally, a linear interpolation routine (Matlab 2006b, Mathworks, Natick, MA, USA) was used to determine the values for aBMD, CSA and Z at PHV (biological age zero). Since the age of PHV often falls in between two data points, an estimation of the bone measures between these data points was also required. The linear interpolation uses the raw bone measures from the two nearest data points surrounding the age of PHV and generates a line between these two points. The value for each bone measure is then derived as the point where the age of PHV intersects this line. This value at PHV was then compared to the respective peak adult value to determine the percent of peak achieved at PHV.

2.1.3.7 Statistical Analysis

From the peak bone strength assessments, the age at aBMDp, CSAp and Zp at the NN, IT, and S sites of the proximal femur were determined. These age values were used for the subsequent analyses. A 2x3 (sex by site) factorial MANOVA with repeated measures (ages of bone strength) was used to test for differences between the ages at aBMDp, CSAp and Zp in males and females at each site of the proximal femur. If significant sex by site by bone strength interactions were observed, subsequent analyses were sex segregated, and a single factor (site) MANOVA with repeated measure was conducted. If a significant multivariate main effect was found, a univariate ANOVA was conducted for each site. Site specific differences between the ages at peak bone strength measure were evaluated with post hoc paired t-test comparisons with Bonferroni adjustments. Data were checked for skewness, kurtosis and sphericity violations. Sphericity violations were assessed using Mauchley's test of sphericity, and where necessary, were adjusted using the Greenhouse-Giesser method. Percentage differences from peak aBMD, CSA and Z achieved at PHV for each site were also calculated. Sex differences in the percent of peak achieved for aBMD, CSA and Z at PHV at each site were assessed by MANOVA. If significant

multivariate main effect was found, a univariate ANOVA was conducted for each site. Additionally, p*ost hoc* multiple regression analyses were conducted to assess the influence of LTMp and FMp on the age of peak bone strength measures at each site. To control for the influence of size differences, adult height and the absolute peak value for each individual bone strength measure (aBMD, CSA, Z) at each site (NN, IT and Z) were also included in the regressions models; thus final models included, adult height, absolute peak bone strength values, LTMp and FMp entered as predictor variables. An alpha of $p<0.05$ was considered significant. All analyses were performed using SPSS 18.0 for Windows (SPSS, Chicago, IL, USA).

2.1.4 Results

2.1.4.1 Participants

Participant age of peak proximal femur aBMD, CSA and Z measures at the NN, IT and S sites are presented in Table 2.1. There were significant sex differences in the chronological ages at which all peak measurement occurred ($p<0.05$) excluding NNCSAp and SZp ($p>0.05$). On average, the age at peak for most measures occurred 1-2 years earlier in females; however, when aligned by biological age (years from age of PHV, instead of chronological age), only the biological age at ITZp continued to be significantly different between sexes ($p<0.05$), with females age at ITZp occurring 1.3 years earlier than males (Table 2.1). As a result of the similarity in the timing of peak measures by biological age, all subsequent results were analyzed and presented aligned by biological age. Additionally, 10 males and 14 females had only a single adult measurement between 18 and 28 years of age. Removing these individuals did not significantly alter the present findings, thus these individuals were included for all analyses.

2.1.4.2 Bone Strength Timing

There was a significant sex by site by bone strength interaction ($p< 0.05$), thus subsequent analyses were sex separated. When separated by sex, there was a significant site by bone strength interaction ($p<0.05$)

for both sexes. Site specific univariate analyses indicated there were significant differences in the age at peak bone strength at all sites of the proximal femur(NN, IT, and S sites) for both sexes (p<0.05).

Table 2.1: The chronological and maturation ages for peak bone strength and height measurements in males and females (Means ± SD).

	Males (n=73)		Females (n=92)	
Variable	**Chronological age**	**Biological Age**	**Chronological age**	**Biological Age**
PHV	13.4 ± 1.1*	0	11.9 ± 0.9	0
NN CSAp	21.6 ± 3.3	8.1 ± 3.2	20.6 ± 3.6	8.8 ± 3.6
NN aBMDp	19.4 ± 2.7 *	6.0 ± 2.7	17.9 ± 2.9	6.0 ± 2.7
NN Zp	22.0 ± 3.2 *	8.7 ± 3.2	20.7 ± 3.4	8.8 ± 3.5
IT CSAp	21.1 ± 3.4 *	7.7 ± 3.4	19.4 ± 3.9	7.5 ± 3.5
IT aBMDp	20.0 ± 3.4 *	6.6 ± 3.4	18.7 ± 3.5	6.8 ± 3.5
IT Zp	21.3 ± 3.4 *	7.9 ± 3.4 **	18.5 ± 3.9	6.6 ± 4.0
S CSAp	22.3 ± 2.9 *	8.9 ± 3.1	21.0 ± 3.3	9.2 ± 3.3
S aBMDp	21.8 ± 2.8 *	8.4 ± 2.8	19.7 ± 3.3	7.8 ± 3.3
S Zp	21.2 ± 2.8	7.8 ± 2.8	20.6 ± 3.5	8.7 ± 3.5

PHV = Peak Height Velocity; NN = Narrow neck site; IT= Intertrochanter site; S= Femoral shaft site. CSAp = peak cross sectional area, aBMDp = peak areal bone mineral density; Zp = Peak section modulus

* indicates a significant difference in chronological age between sexes

** indicates a significant difference in biological age between sexes

Figure 2.1 displays the timing of bone strength measures at the NN site for males (A) and females (B). At the NN, male's age at aBMDp occurred significantly earlier than both the ages at CSAp and Zp (Table 2.1; p<0.05), while the age at Zp occurred significantly later than the age at CSAp (p<0.05, Figure 2.1A). In females, the age at aBMDp also occurred significantly earlier than both the ages at CSAp and Zp (Table 2.1; p<0.05), but there was no significant difference between the age at CSAp and Zp (Figure 2.1B).

Figure 2.1C and D displays the timing of bone strength measures at the IT site for both sexes. In males, the age at aBMDp occurred approximately one year prior to the age at CSAp and Zp (Table 2.1; p<0.05), with no significant difference observed between the age at CSAp and Zp (Figure 2.1C). In females, there was a significant difference in the age at CSAp and both the age of aBMDp and Zp (Table 2.1; p<0.05),

with the age at CSAp occurring approximately one year after aBMDp and Zp (Figure 2.1D). There was no significant difference between the age at aBMDp and Zp (p>0.05).

Figure 2.1E and F display the timing of bone strength measures at the S site for both males and females. In males, there was significant difference in the timing of bone strength measures (p<0.05), with the age at CSAp occurring significantly later than both the ages at aBMDp and Zp (Table 2.1; Figure 2.1E); however, there was no significant difference between the age at aBMDp and Zp. In females, the age at CSAp occurred over one year after the age at aBMDp (Table 2.1; p<0.05). Similarly, the age at Zp also occurred significantly later than the age at BMDp (Table 2.1; p<0.05). There were no significant differences between the age at CSAp and Zp in females at the S site (Figure 2.1F).

2.1.4.3 Percent of Peak Achieved at Peak Height Velocity

Figure 2.2 displays the percent of adult peak bone strength achieved at PHV at each site of the proximal femur in both males and females. At the NN site, males and females achieved approximately 67% of their adult peak in CSA and 76-78% of their adult peak in aBMD by age at PHV (Figure 2.2A). In contrast, there was a significant sex difference in the percent of peak achieved for Z at the NN. Females attained 4% more of their adult peak (61%) than their male counterparts (57 %) at PHV.

Similar to the NN, there was no significant sex difference in the percent of peak achieved at PHV for CSA and aBMD, with males and females attaining 70% of adult CSAp and 80% of adult aBMDp (p>0.05); however, there was a significant sex difference in the percent of peak achieved for Z (p<0.05). Males attained only 61% of their adult peak, while females achieved 68% (Figure 2.2B).

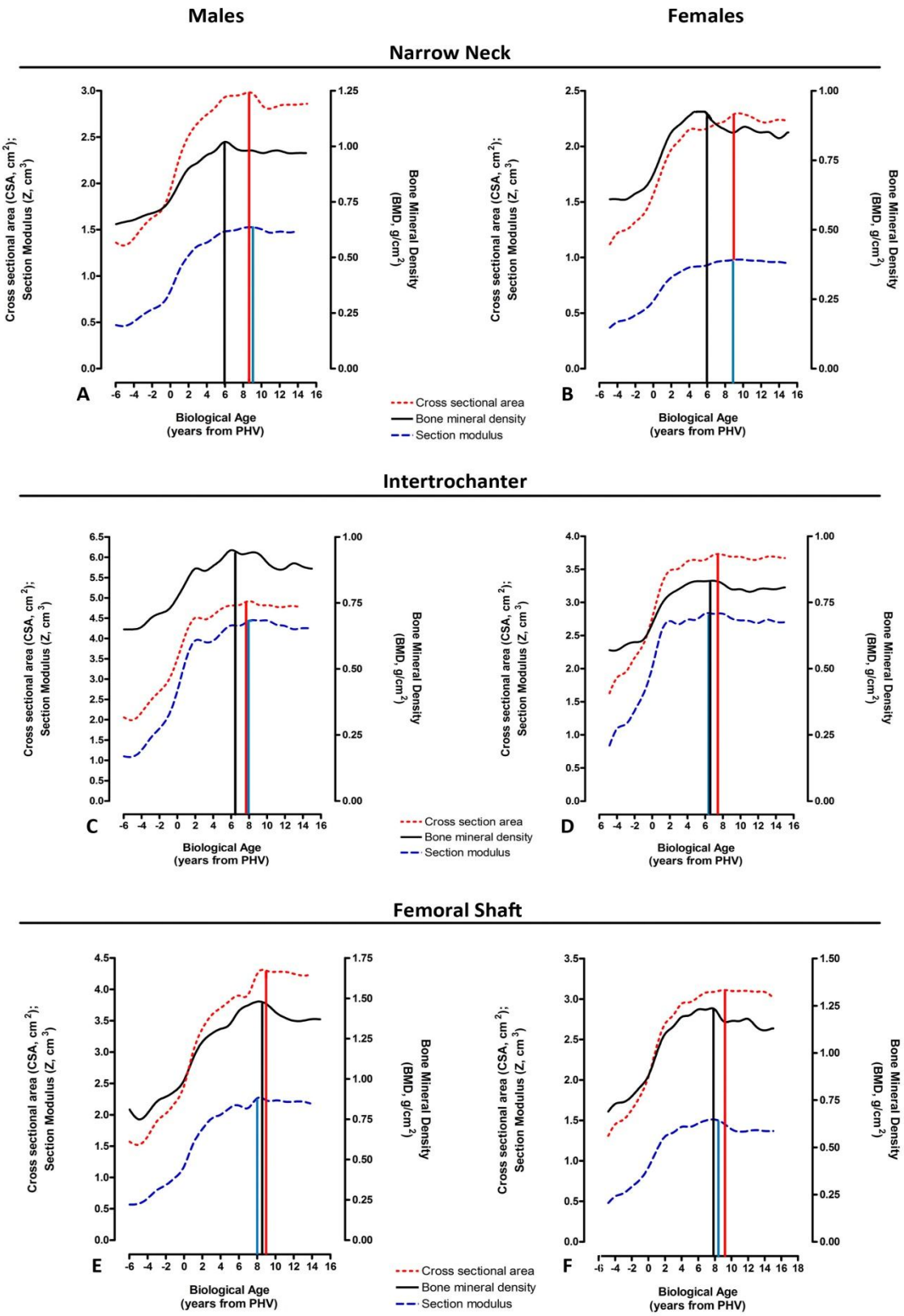

Figure 2.1: The average growth curves for cross sectional area, bone mineral density and section modulus at the narrow neck (A, B), intertrochanter (C, D) and femoral shaft (E, F) sites of the proximal femur in males (A, C, E) and females (B, D, F) aligned by biological age. The solid drop down lines identify the biological ages at which the peak bone strength measures occurred.

At the femoral shaft the same trend continued. There was no significant sex difference in the percent of peak achieved for CSA and aBMD, but there were significant sex differences in Z (p<0.05). Males and females attained 62-65 % of their adult CSAp and approximately 70% of their aBMDp at PHV. Males continued to attained significantly less of their adult Zp at the S site at PHV, achieving only 55% of their adult Zp, while females attained approximately 6% more, with 61% of their adult Zp (Figure 2.2C)

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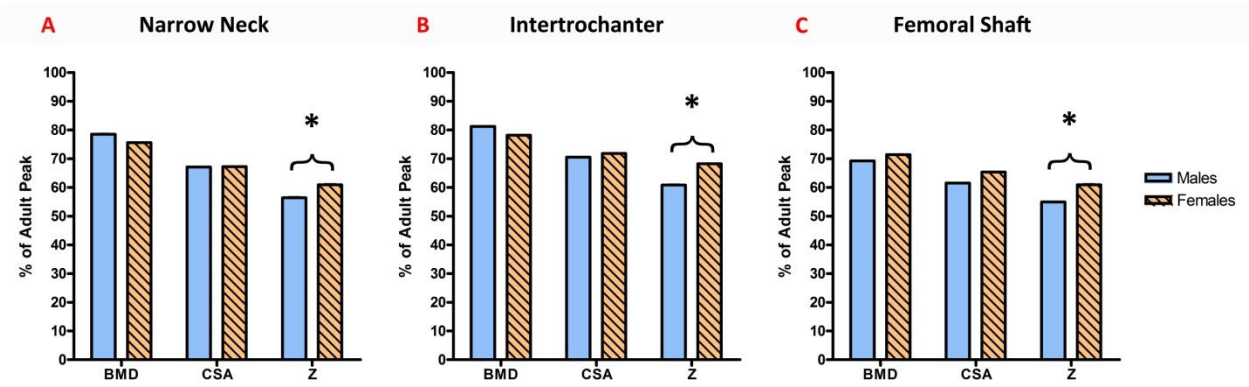

Figure 2.2: The percentage of adult peak achieved at peak height velocity (PHV) for cross sectional area (CSA), areal bone mineral density (BMD) and section modulus (Z) at the narrow neck (NN; A), intertrochanter (IT; B) and femoral shaft (S; C) sites of the proximal femur in males and females.
* indicates a significant sex difference (p<0.05)

2.1.4.4 Lean Tissue Mass and Fat Mass Multiple Regression Models

The results of the multiple regression analyses and the contribution of each predictor variable on the age of peak bone strength measures at each site are displayed in Table 2.2. At the NN site, LTMp was a significant independent predictor of the age of aBMDp in males. FMp was only a significant predictor of the age of NNCSAp in females. Neither LTMp nor FMp were a significant predictor of the age of NN Zp in both sexes (p>0.05).

At the IT site, LTMp significantly predicted the ages of aBMDp and CSAp but not the age of Zp in males. In females, the peak in LTM significantly predicted the age of CSAp and Zp, while FMp was a significant predictor of only IT CSAp; however LTMp and FMp did not significantly predict the age of aBMDp at the IT site in females (p>0.05).

At the femoral shaft, LTMp and FMp did not significantly predict the ages of aBMDp, CSAp and Zp in males (p>0.05). In females, LTMp was only a significant predictor of the age of S Zp, while FMp significantly predicted the age of both S CSAp and S Zp (p<0.05).

2.1.5 Discussion

It was found in both males and females, the peak in BMD occurred significantly earlier than estimated geometric CSA at all sites of the proximal femur. Additionally, a significant sex difference was observed in the percent of peak achieved by age at PHV, with females possessing a significantly greater percentage of their adult peak section modulus than their male counterparts. This is the first study, to our knowledge, to longitudinally assess the development of proximal femur bone geometry from late childhood into early adulthood and observe the timing of peak bone geometry measures in a normal healthy population of males and females.

2.1.5.1 Timing of Bone Strength Measures

Males and females have been previously described to display sex differences in the timing and tempo of maturation **[11],** as well as geometric bone properties during childhood and adolescence **[187]**. On average, female bone mass and geometric peaks occurred 1-2 years prior to male peak values. This corresponds to the average difference in maturational timing, as indicated by age of PHV (Table 2.1), between sexes **[11].** It also explains why, once aligned by maturational age, the significant age differences disappeared for all bone mass and geometric measures, except intertrochanter section modulus. Although there were no sex differences in the age at peak for the majority of the bone strength measures when aligned by maturational age, significant sex by site by bone strength interactions were observed, suggesting there were differences in the ages of peak bone strength measures amongst the three sites between sexes. At the IT and S sites, marked sex differences in the sequential timing of aBMDp, CSAp and Zp were observed. In males, the age at aBMDp occurred nearly 1

Table 2.2: Multiple regression models with height, peak bone strength measures, lean tissue mass peak and fat mass peak inputted as predictor variables.

	Age at NN aBMDp		Age at IT aBMDp		Age at S aBMDp	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
Model adjusted R squared	0.06	-0.01	0.04	0.10*	0.01	0.11*
Predictors	**Standardize Beta**		**Standardize Beta**		**Standardize Beta**	
Height	-0.38#	-0.18	-0.27	-0.17	-0.14	-0.13
aBMDp	-0.04	0.11	-0.02	-0.16	0.11	0.13
LTMp	0.41#	0.14	0.36#	0.11	0.06	0.11
FMp	-0.05	0.02	0.03	0.23	0.08	0.13
	Age at NN CSAp		**Age at IT CSAp**		**Age at S CSAp**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
Model adjusted R squared	0.02	0.08*	0.10*	0.15*	0.12*	0.12*
Predictors	**Standardize Beta**		**Standardize Beta**		**Standardize Beta**	
Height	-0.16	-.0.134	-0.17	-0.28#	0.11	-0.22
CSAp	0.01	0.19	0.05	0.07	0.39#	0.08
LTMp	0.26	-0.06	0.43#	0.24#	-0.03	0..14
FMp	-0.06	0.28#	-0.02	0.26#	0.09	0.27#
	Age at NN Zp		**Age at IT Zp**		**Age at S Zp**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
Model adjusted R squared	0.02	0.04	0.07	0.12*	0.01	0.17*
Predictors	**Standardize Beta**		**Standardize Beta**		**Standardize Beta**	
Height	-0.24	-0.10	-0.17	-0.29#	0.05	-0.24#
Zp	0.07	0.17	0.18	-0.20	0.23	-0.12
LTMp	0.18	-0.08	0.32	0.62#	-0.18	0.38#
FMp	-0.09	0.24	-0.08	0.02	0.21	0.29#

NN = Narrow neck site; IT= Intertrochanter site; S= Femoral shaft site

CSAp = peak cross sectional area, aBMDp = peak areal bone mineral density; Zp = Peak section modulus; LTMp = peak lean tissue mass; FMp = peak fat mass

* indicates the model is significant ($p<0.05$)

# indicates the predictor coefficient is significant in the model ($p<0.05$)

year before the ages of CSAp and Zp at the IT site. In contrast, the ages of both aBMDp and Zp occurred approximately 1 year prior to the age of CSAp in females. At the femoral shaft, the ages of both aBMDp and Zp occurred earlier than CSAp in males and in female the ages of both CSAp and Zp occurred later than aBMDp. Although it remains difficult to determine the mechanisms responsible for this difference based on observational data, it is reasonable to speculate that body compositional differences and the inherent limitations of HSA may have had an influence. First, body composition has been described to influence bone mass and bone geometry development during childhood and adolescence **[29, 187-189, 191-194]** and in adulthood **[195-197]** in both males and females **[29, 187-189, 191-197]**. It has also been documented previously that the peak velocity in LTM precedes and influences the peak velocity of total body bone mineral content **[185]** and estimated bone geometry at the hip **[135]** during adolescence. In the present study it was observed that the peak in LTM continues to influence the age of bone strength measures in adulthood (Table 2.2); however, this influence was relatively minor, with prediction models that included height and LTM only accounting for approximately 7-13% of the variance in the ages of aBMDp, CSAp and Zp (data not shown). The addition of FM (Table 2.2) did not alter the contribution of LTM on the prediction of the age of peak bone measures in either sex, but FM significantly contributed to the prediction of the age peak bone strength measures in females, particularly the age of CSAp. Given that females tend to accrue more adipose tissue during adolescence than males, the addition of FM may have directly altered the mechanical loads (compressive forces) experienced at the hip which may in turn influence the developmental timing of CSA; however, the inclusion of FM, along with height and LTM, in the model still only predicts less than 15% of the variance in the age of peak bone measures at the proximal femur. These findings suggest that the total amount of LTM and FM contributes relatively little to the timing of bone strength development in adulthood and that their influence may be site and sex specific.

Similarly, because there are known body compositional difference between sexes, the distribution of body mass may alter the loads experienced at the proximal femur. This may have resulted in alterations in geometric properties along an axis that is not assessed in the current study due to the inherent limitations of the HSA procedure. The HSA procedure is only able to estimate geometric properties along the scanner's plane of assessment **[25, 130]**. Thus, the observed geometric adaptations are limited to the frontal plane, but geometric adaptation may be better reflected by assessments along other planes of motion. These limitations suggest further prospective research using three-dimensional imagery is necessary to elaborate on these HSA findings along the multiple planes in which geometric adaptations may occur.

Despite these sex differences, males and females followed a similar pattern in the timing of aBMDp and CSAp at all sites of the proximal femur. The age at aBMDp occurred approximately one year prior to the occurrence of CSAp at all sites of the proximal femur. This pattern parallels observations during adolescence **[54, 82, 135, 185]** as well as that proposed for later changes in architecture associated with aging. These observations suggest that during development, optimization of bone strength continues after the attainment of peak bone density and is similar between sexes. During adolescence bone mass and geometry rapidly increase, but by early adulthood when bone mass begins to plateau, and net bone mass is unchanged, it is possible that geometric adjustments are made to fine tune overall bone strength. Conversely, the earlier onset of aBMDp observed may be an artifact of the planar projection of DXA derived aBMD. If volumetric density remains constant while size increases one can anticipate a potential drop in aBMD **[201]**. Thus, if geometry and volumetric density change contemporaneously, this may result in an earlier peak in aBMD. To more precisely decipher the sequential timing between bone mass and geometry, longitudinal studies with the high resolution three dimensional modalities are required to confirm the present observations.

The peak in aBMD and geometric properties observed in the current study coincides with previous literature suggesting that bone strength peaks between 20-40 years of age **[27, 31, 32]**. In the present study it was observed that BMD peaked at approximately 20 years of age (6 years post-PHV), while peak CSA and Z occurred between 20-22 years of age (6-9 years post-PHV). Zhang et al **[32],** in using a cross sectional design, speculated that geometric properties may plateau around 20 years of age. Our data supports this supposition, but we are limited in suggesting whether these geometric properties plateau in our data.

2.1.5.2 Percent of Peak Achieved at Peak Height Velocity

The present study also assessed the percent of adult peak achieved at PHV for aBMD, CSA and Z at the three sites of the proximal femur. Males and females attained approximately 60-70% of their adult peak in CSA and 75-80% of their adult peak in aBMD by the age of PHV, but females achieved 4-7% more of their adult peak in Z than their male counterparts. The aBMD findings parallel the observations from previous studies suggesting that a substantial amount of adult bone mass is accrued during this adolescent period **[54, 80, 202]**. These findings suggest that similar to bone mineral accrual, the adolescence period is vital to the development of adult CSA and Z. Additionally, the data indicates there is asynchrony between the gain in bone mass and geometry, with individuals achieving a greater percentage of adult bone mass during adolescence than adult bone geometry. This phenomenon may explain why fracture rates increase during adolescence **[203-205],** supporting the supposition that during the adolescent growth spurt there is a transient period of skeletal weakness, where a significant amount of bone mass is accrued, but the skeleton may not be geometrically optimized. Similarly, males are documented to have more adolescent fractures than females **[204]** and the present study results suggests that this sex discrepancy may be a result of weakened bending strength, as indexed by Z, in males. Although hip fractures are scarce during adolescence, long bone adaptations at this site may be

contemporaneous to those at the distal radius; however, whether bending strength is compromised in males during adolescence at the distal radius is an avenue for future research.

The results of this study are unique in that we used longitudinal measurements, assessed the timing of bone mass and geometric development and concentrated on the clinically significant proximal femur; however, the conclusions are limited by several factors. First, this study was observational in nature. Observation studies are limited due to inherent susceptibility of observational associations related to uncontrolled variables, selection bias, and reverse causality **[206]**. This may limit the ability to generalize the findings to other populations. Further longitudinal research in other populations is required to supplement these observations. Next, the HSA program also has inherent limitations. DXA images are often noisy and blurred resulting in the difficulty of locating precise edge margins **[25, 207]**. In addition, the positioning of femur is important as small changes in femur rotation have a large effect on the geometric dimensions **[207]**. All DXA scans were performed by qualified radiologists familiar with proper positioning of the proximal femur to ensure hip scans were performed with care to limit these potential errors; nevertheless, it is difficult to position the hip consistently in repeated measures over time.

In conclusion, there were observed sex differences in the developmental timing of geometric bone measures at the proximal femur, but despite these differences, in both males and females, the peak in BMD occurred significantly earlier than CSA at all assessment sites using HSA. Additionally, significant sex differences were observed in the percent of peak achieved by age at PHV, with females possessing a significantly greater percentage of their adult peak section modulus than their male counterparts. These findings suggest that changes in bone mass precede the changes in bone geometry and this timing of events may be integral for the development and maintenance of bone strength; however, further research on geometric adaptations using three dimensional imagining is necessary.

2.2 Study Two: Maturational Timing and Bone Structural Strength at the Proximal Femur

2.2.1 Abstract

Late maturational timing is documented to be detrimental to bone strength primarily at the distal radius. Studies at the proximal femur have focused on bone mass and the results remain controversial. The purpose of this study was to examine the long term relationship between the onset of maturation and the development of cross sectional area (CSA) and section modulus (Z) at the proximal femur using a longitudinal design.

Two hundred and twenty six individuals (108 males and 118 females) from the Saskatchewan Pediatric Bone Mineral Accrual Study (PBMAS) were classified into maturity groups based on age of attainment of peak height velocity. CSA and Z were serially assessed at the narrow neck (NN), intertrochanter (IT) and proximal shaft (S) sites using hip structural analysis (HSA). Multilevel models were constructed to examine the development of CSA and Z by maturity group.

Cross sectional observations indicated that during adolescence, early maturing males had significantly greater CSA and Z than late maturing males at all sites of the proximal femur, while early maturing females had greater Z at the NN and S, and greater CSA at the NN, IT and S sites compared to late maturing females. In contrast, longitudinal observations indicated that when age, body size, body composition, physical activity and dietary intake were controlled no significant effects of maturational timing were found in the development of CSA and Z at the NN, IT or S regions ($p>0.05$) in either males or females.

In this population of healthy individuals there appears to be no effect of the onset of maturation on estimated CSA and Z development at the proximal femur in both males and females. This may be a result of the proximal femur's loading environment. Future research is required to determine the role of loading on the relationship between maturational timing and bone structure and strength development at the proximal femur.

2.2.2 Introduction

Loss of bone mass is an inherent part of the aging process in both men and women. The attainment of peak bone mass is considered an important aspect in the prevention of osteoporosis and the debilitating fractures that coincide with this disease **[134]**. The adolescent period is crucial for bone mass development with approximately 40% of peak bone mass laid down during the 5 years surrounding the pubertal growth spurt **[208]**. It is purported that the timing of maturational onset may influence the development of peak bone mass; however this relationship between maturational timing and bone mass accrual remains controversial. The few studies that have investigated this maturation-bone relationship have focused primarily on females **[43-45, 209]** leaving a paucity of information in males. We have previously shown, in a cohort of boys and girls followed continuously for 15 years, there appears to be a deleterious effect of a naturally delayed maturation on total body bone mineral content (BMC) development in females but not males, with late maturing females developing less bone mass throughout adolescence and into adulthood than their early and late maturing peers **[147]**. These finding suggest a potential sex-dependent effect of maturational timing on bone mass development and perhaps sex difference in the development of bone structure and strength at the hip. Further supporting this supposition, Forwood et al. **[58]** observed apparent sex differences in cross sectional area (CSA) and section modulus (Z) at the proximal femur during adolescence, even after maturational difference were controlled; however, Forwood and colleagues **[58]** did not identify whether the timing of maturation (ie. being an early, average, or late maturer) influenced adult proximal femur bone strength and whether these differences were sex dependent.

In general, clinicians do not use BMC as a clinical assessment tool, rather areal bone mineral density (aBMD) is used as a measure to diagnose osteoporosis and predict fracture risk **[210]**; however, aBMD is only a single component of whole bone strength. Although it remains difficult to assess bone strength *in vivo*, it can be estimated from knowledge of geometry, material composition and the loading

configuration **[19, 211].** There is a growing body of literature highlighting the importance of geometric measures, such as CSA and Z, which may serve to provide further unique insights into bone strength and fracture risk **[13, 20-22, 125]**.

Previous literature suggests that there is a deleterious effect of late maturational timing on bone geometry and architecture **[41-45, 147]**. It has been documented that a natural delay in maturation increases fracture prevalence in males **[41]**, reduces bone mineral mass development during both adolescence**[42]** and in adulthood **[43-45, 147]** and decreases cortical volumetric BMD (vBMD) and cortical thickness at the distal radius in adult females **[43]**; however, there is currently a paucity of research investigating the relationship between maturational timing and bone strength at the clinically relevant proximal femur. Hip structural analysis (HSA) has proven to be a promising technique for estimating geometric properties at this unique fracture site **[125],** with literature supporting that HSA geometric measures add to the prediction of hip fracture risk beyond that of aBMD **[13, 20, 22]**. Therefore, the purpose of this study was to determine whether the timing of maturation influences the development of HSA estimated geometric properties of bone strength at the proximal femur in a healthy population of males and females followed continuously from childhood through adolescence and into young adulthood. It was hypothesized that HSA estimated CSA and Z development would be significantly different between early and late maturational groups in both males and females, with early maturing individuals having an advantage in their estimated geometric bone strength at the proximal femur.

2.2.3 Methods

2.2.3.1 Participants

Participants were drawn from the University of Saskatchewan's Pediatric Bone Mineral Accrual Study (PBMAS). The details of the PBMAS participants and their recruitment have been described previously **[29, 53, 57]**. In brief, between 1991-2006, two hundred and fifty-nine individuals provided written

consent and/or assent (in childhood) to participate in the PBMAS and 251 were initially DXA scanned (1991-1993). Table 1 shows the number of subjects tested in each year thereafter. To be included in the present analysis, participants required the following: 1) A measured age of attainment of peak height velocity (PHV); 2) HSA derived bone measures from at least two testing occasions, and 3) Have no diseases known to affect growth or bone development. This resulted in the inclusion of 226 participants (108 males and 118 females) covering the age span of approximately 8-30 years of age. Ninety eight percent of the participants were of Caucasian descent. Written consent was obtained from all participants. All procedures were approved the University of Saskatchewan's biomedical ethical review committee.

2.2.3.2 Anthropometry

Height and weight were assessed annually following the anthropometric standards outlined by Ross and Marfell-Jones **[200]**. Height was recorded without shoes to the nearest 0.1cm using a wall mounted stadiometer (Holtain Limited, Crymych, UK). Weight was measured on a calibrated electronic scale to the nearest 0.1kg (Toledo Scale Company, Ontario, Canada).

2.2.3.3 Bone Measures

Bone measures were derived using the hip structural analysis (HSA) program. The HSA program has been previously reported in greater detail **[25]**. In brief, the HSA program estimates the structural geometry of the proximal femur from DXA derived images of the hip. Employing the principles originally reported by Martin and Burr **[24]**, a line of pixels values traversing the bone axis in a bone mass image is a projection of the corresponding cross section and its dimensions. To determine these cross sections, the HSA algorithms divide the pixel mass values in g/cm^2 by the effective mineral density of fully mineralized adult cortical bone. Cross sections are evaluated by averaging geometry over 5 parallel lines spaced 1mm apart at three sites of the proximal femur: 1) The Narrow Neck (NN); 2) Intertrochanteric

(IT); and 3) Femoral Shaft (S). The HSA program locates these regions on the DXA bone mineral image and derives the geometric estimates. From each region, the HSA program produces ten output variables, of which two were assessed for this study: *Cross Sectional Area (CSA)*– an estimate of axial compressive strength, which is defined as the estimated amount of bone surface area in the cross section after excluding all the trabecular and soft tissue space; and, *Section Modulus (Z)* – an indicator of bending strength calculated as the cross sectional moment of inertia (CSMI) divided by the maximum distance from the center of mass to outer cortex **[130]**. The short-term precision for CSA and Z derived from a hip scan using a Hologic QDR 2000 range from 2.2% to 2.3%, 2.3% to 2.8% and 2.8% to 3.4% for the NN, IT and S, respectively **[26]**. All HSA analyses were completed by a single technician and derived from proximal femur scans using a Hologic QDR-2000 (Bedford, MA, USA).

2.2.3.4 Lean Tissue Mass and Fat Mass

Lean and fat tissue mass were assessed annually using dual energy x-ray absorptiometry (DXA, Hologic QDR-2000, array mode) by a trained technician following the procedures outlined in the Hologic's operators manual and user guide. Total body lean tissue mass (LTM) and fat mass (FM) were analyzed using software version 5.67A. Scanner drift was assessed prior to each scan using the manufactures lumbar spine phantom. The inter-assay precision (CV%) in vivo in our lab have been previously reported as 0.54% and 2.95%, LTM and FM, respectively **[53, 56, 57].**

2.2.3.5 Calcium and Vitamin D Intake

Nutritional intake was assessed via annual 24 hour food recalls. Dietary intake from the 24 hour recall was entered and analyzed using the nutritional assessment software package (NUTS Nutritional Assessment System, version 3.7 Quilchena Consulthing Ltd., Victoria, BC, Canada) which used the 1988 Canadian Nutrient File information. The same individual coded, checked, and analyzed all dietary intakes

according to procedures described elsewhere **[212]**. On the basis of these recalls, average daily intake of calcium and vitamin D was determined for each individual each measurement year.

2.2.3.6 Physical Activity

Physical activity (PA) was assessed by serially administered self reported questionnaires. The details of the physical activity questionnaires as used in the PBMAS have been reported elsewhere **[56, 57, 213]**. In brief, during childhood and adolescence physical activity was assessed by the Physical Activity Questionnaire for Children (PAQ-C) and the Physical Activity Questionnaire for Adolescence (PAQ-A). The PAQ-C/A are designed to assesses general physical activity levels over the previous seven days, scoring nine items on a five point Likert-type scale. PA scores range from one to five, with higher scores indicating higher levels of physical activity. During adulthood the Physical Activity Questionnaire for Adults (PAQ-AD), a 7 item version of the PAQ-C/A was used, again, scoring individuals on a five point scale. The PAQ-C/A/AD have been previously reported to be a valid and reliable measure of physical activity levels in children, adolescents and adults **[214-216]**.

2.2.3.7 Maturation

Maturation was determined using the common maturational landmark age of peak linear growth, as indexed by peak height velocity (PHV). PHV is the most commonly employed indicator of maturity in longitudinal studies **[217]** and provides an accurate benchmark to reflect the timing of maturation. To establish the age of PHV for each child, whole year height velocities were calculated for each participant by dividing the difference between the annual height increments by the age increment (the mean age increment was 0.998 ± 0.048 years). A cubic spline fit was then applied to the whole year velocity values and the age at the highest velocity point interpolated to identify the age at PHV. The cubic spline curve fitting provides a smooth velocity curved based on polynomial algorithms which provide an estimation of age and magnitude while maintain the original integrity of the data. Participants were separated into

early, average, and late maturational categories based on their age of PHV. Participants whose age of PHV preceded the average age of PHV by one year or more were categorized as early maturers; individuals with a PHV greater than one year after the average age of PHV were categorized as late maturers. All remaining participants were categorized as average maturers. This resulted in the current population to be separated into 38 early (24 males, 14 females), 160 average (73 males, 87 females), and 28 late (11 males, 17 females) maturers.

2.2.3.8 Statistical Analyses

All variables were assessed for normality and violations were adjusted using logarithmic transformations. Differences between maturation groups were assessed cross sectionally, at three distinct growth periods [Childhood (8-12 years of age), Adolescence (13-19 years of age) and Adulthood (20+ years of age)], by averaging each individuals' data during that specific time period. All cross sectional analyses were assessed by analysis of variance (ANOVA) using SPSS version 18.0 (Statisical Package for Social Sciences, Chicago, IL). All values are presented as means ± standard deviation unless otherwise specified. For the longitudinal analyses, multilevel (hierarchical) random effects models were constructed using a multilevel modeling approach (MlwiN version 2.22, Multilevel Models Project; Institute of Education, University of London, UK **[218]**). A detailed description of the multilevel modeling procedure as applied to the PBMAS has been previously reported **[56, 57]** and complete details of this approach are presented elsewhere **[206]**. In brief, bone parameters (CSA and Z) were measured repeatedly in individuals (level 1 of the hierarchy) and between individuals (level 2 of the hierarchy). Analysis models that contain variables measured at different levels of a hierarchy are known as multilevel regression models. Specifically, the following additive random effects multilevel regression models were adopted to describe the developmental changes in bone parameters with chronological age following the format of equation 2.1 below.

$$y_{ij} = \alpha + \beta_j x_{ij} + k_1 z_{ij} + \ldots k_n z_{ij} + \mu_j + e_{ij} \qquad (2.1)$$

In equation 2.1, y is the bone parameter (CSA or Z) on measurement occasion *i* in the *j*-th individual; α is a constant; $\beta_j x_{ij}$ is the slope of the bone parameter over time (in this model chronological age is centered around 16 years, the average age of the sample; this is required because the minimum value of chronological age in the sample is 8 years, if the origin was not shifted (centered) from 0 to 16 years the model would be estimating the variance of the intercepts at a bone parameter that never occurred in the sample and thus reduce the variance between individuals [level 2]) for the *j*-th individual; and k_1 to k_n are the coefficients of various explanatory variables (ie. height, LTM, FM, physical activity, calcium intake, vitamin intake and maturity group) at assessment occasion *i* in the *j*-the individual. These are the fixed parameters in the model. Both μ_j and ε_{ij} are random quantities, whose means are equal to zero; they form the random parameters in the model. They are assumed to be uncorrelated and follow a normal distribution and thus their variances can be estimated; μ_j is the level-2 (between subjects variance) and ε_{ij} the level-1 residual (within individual variance) for the *i*-th assessment of the bone parameter in the *j*-th individual. Models were built in a stepwise procedure, i.e. predictor variables (κ - fixed effects) were added one at a time, and the log likelihood ratio statistics was used to judge the effects of including further variables. Predictor variables (κ) were accepted as significant if the estimated mean coefficient was greater than twice the standard error of the estimate (SEE) i.e. $p < 0.05$. If the retention criteria were not met the predictor variable was discarded. To allow for the non-linearity of growth, age centered power functions were introduced into the linear models (e.g. age centered^2 and age centered^3). These variables are introduced to shape the development curve. The predictor variable coefficients were used to predict CSA and Z development with chronological age, height, LTM, FM, physical activity levels, and calcium and vitamin D intake controlled in the prediction equations using population averages at chronological age category for each maturity category. A total of six independent multilevel (hierarchical) random effects models were constructed for each bone parameter (CSA and Z) at each assessment site of the proximal femur (NN, IT and S).

2.2.4 Results

2.2.4.1 Cross Sectional Analyses

Table 2.3 displays the participant characteristics for early, average and late maturing males during childhood, adolescence and adulthood. Early maturing males' PHV occurred at 12.1 years of age (Range 11.3-12.7y); average maturing males at 13.6 years (Range 13.1 – 14.3y); and late maturing males at 15.1 years of age (Range: 14.6 – 15.9y). During childhood, late maturing males were significantly shorter than their early maturing peers (p<0.05), but there were no significant differences in CSA or Z between maturational groups at any of the three sites of the proximal femur (p>0.05). In adolescence, late maturing males continued to be shorter than their average and early maturing peers and possessed less CSA, Z and LTM, but greater FM, compared to early and average maturing males (p<0.05). Additionally, average maturing males were significantly shorter and had less CSA and Z compared to early maturing males at all sites of the proximal femur (p<0.05). In adulthood, there was no significant difference in height, estimated bone geometry measures and body composition between early and late maturing males (p>0.05). Instead, average maturing males had significantly greater NN CSA, NN Z, S CSA, LTM and FM than they early maturing counterparts (p<0.05), but only significantly greater LTM and FM compared to their late maturing peers (p<0.05).

Table 2.4 displays the age and estimated bone geometry characteristics for early, average and late maturing females during childhood, adolescence and adulthood. Early maturing females' PHV occurred at 10.1 years of age (Range: 9.2 – 11.0y); average maturing females at 11.8 years (Range: 11.2 – 12.6y); and late maturing females at 13.1 years of age (Range: 12.8 – 13.6y). During childhood early maturing females were significantly taller, had more LTM, and had greater CSA and Z at all sites compared to their average and late maturing peers (p<0.05). Average maturers also were significantly taller, had greater LTM and greater estimated bone geometry at each site compared to late maturing females during childhood (p<0.05). During adolescence, late maturing females had significantly less LTM, FM and

estimated CSA and Z at the NN and S sites than both early and average maturing females (p<0.05). Late maturing females were also significantly shorter and had less IT CSA then average maturing females (p<0.05). In adulthood, average maturing females were significantly older and had greater estimated CSA and Z at the NN and IT sites than both early and late maturing females (p<0.05). Additionally, average maturing females had significantly greater LTM than their late maturing peers (p<0.05).

2.2.3.2 Longitudinal Multilevel Analyses

2.2.3.2.1 Narrow Neck Site

Table 2.5 summarizes the results for the multilevel models for the development of CSA and Z at the NN, site in males and females. In males, at the NN, age centered and LTM significantly contributed to the prediction of CSA and Z (p<0.05, Table 2.5 Model A and B). Once age and body mass adjustments were made, no independent maturity effects were observed between early, average and late developing males on NN CSA and NN Z. In females, age centered, height, LTM and FM all significantly contributed to the prediction of both NN CSA and NN Z (p<0.05, Table 2.5 Model C and D); however, similar to their male peers, once adjusted for age, size and body composition, there were no significant independent maturity effects on the development of NN CSA and NN Z.

2.2.3.2.2 Intertrochanteric Site

Table 2.6 presents the results for the multilevel models for the development of CSA and Z at the IT site for males and females. In males, height, LTM and PA significantly contributed to the prediction of both ITCSA and IT Z (p<0.05, Table 2.6 Model E and F). In females, height and LTM significantly predicted the IT CSA, while age centered, age centered squared, height, LTM and FM contributed to the prediction of IT Z (p<0.05, Table 2.6 Model G and H). Once age, body mass, and PA adjustments were made, no independent maturity effects were observed between maturing groups in either sex for IT CSA and IT Z(p>0.05, Table 2.6).

Table 2.3. Characteristics of early, average and later maturing males during childhood (8-12 yrs), adolescence (13-19 yrs), adulthood (20 + yrs). Presented as means ± standard deviations.

Males

	Childhood			Adolescence			Adulthood		
Variables	Early N=21	Average N=67	Late N=10	Early N=23	Average N=73	Late N=11	Early N=23	Average N=72	Late N=9
Age of PHV (y)	12.1 ± 0.5	13.5± 0.5 [a]	15.1 ± 0.3 [a,b]	12.2 ± 0.5	13.6 ± 0.6 [a]	15.2 ± 0.4 [a,b]	12.1 ± 0.5	13.7 ± 0.6 [a]	15.0 ± 0.1 [a,b]
Age (y)	10.6 ± 1.3	11.0 ± 1.1	11.1 ± 1.1	15.5 ± 1.9	15.2 ± 1.8	15.2 ± 1.6	23.5 ± 2.3	23.6 ± 2.1	23.7 ± 2.5
Height (m)	1.50 ± 0.1	1.47 ± 0.8	1.44 ± 0.8[a]	1.74 ± 0.9	1.72 ± 0.1 [a]	1.68 ± 0.1 [a,b]	1.76 ± 0.8	1.80 ± 0.7	1.80 ± 0.6
NN CSA (cm^2)	1.5 ±0.2	1.6 ± 0.2	1.5 ± 0.3	2.54 ± 0.4	2.35 ± 0.5 [a]	2.12 ± 0.4 [a,b]	2.79 ± 0.3	2.93 ± 0.4[a]	2.75 ± 0.6
NN Z (cm^3)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	1.22 ± 0.3	1.12 ± 0.3 [a]	0.94 ± 0.4 [a,b]	1.43 ± 0.3	1.53 ± 0.3 [a]	1.41 ± 0.4
IT CSA (cm^2)	2.6 ± 0.5	2.6 ± 0.5	2.6 ± 0.6	4.34 ± 0.6	4.11 ± 0.9 [a]	3.88 ± 0.8 [a]	4.67 ± 0.6	4.90 ± 0.8	4.86 ± 1.0
IT Z (cm^3)	1.7 ± 0.6	1.6 ± 0.4	1.5 ± 0.5	3.68 ± 0.7	3.47 ± 1.0 [a]	3.08 ± 0.9 [a,b]	4.16 ± 0.6	4.39 ± 0.9	4.86 ± 0.8
S CSA (cm^2)	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.4	3.42 ± 0.6	3.05 ± 0.7 [a]	2.72 ± 0.6 [a,b]	3.96 ± 0.4	4.17 ± 0.6 [a]	4.06 ± 0.7
S Z (cm^3)	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	1.78 ± 0.4	1.57 ± 0.5 [a]	1.33 ± 0.4 [a,b]	2.12 ± 0.3	2.25 ± 0.5	2.16 ± 0.4
LTM (kg)	30.0 ± 5.9	28.5 ± 4.3	26.2 ± 3.6	52.0 ± 7.2	48.4 ± 10.1 [a]	42.1 ± 9.4 [a,b]	59.6 ± 6.6	62.2 ± 8.0 [a]	58.3 ± 3.7 [b]
FM (kg)	8.7 ± 5.3	9.0 ± 5.5	9.3 ± 6.8	11.2 ± 7.0	11.4 ± 6.5	14.6 ± 1.0 [a,b]	16.2 ± 7.0	19.5 ± 9.2 [a]	16.1 ± 13.1 [b]

NN = Narrow Neck; IT = Intertrochanter; S = Femoral Shaft; LTM = Lean tissue mass; FM = Fat mass; CSA = Cross sectional area; Z = Section modulus

[a] indicates a significant difference from early maturers, [b] indicates a significant difference from average matures

Table 2.4: Characteristics of early, average and later maturing females during childhood (8-12 yrs), adolescence (13-19 yrs), adulthood (20 + yrs). Presented as means ± standard deviations.

Females

	Childhood			Adolescence			Adulthood		
Variables	**Early N=13**	**Average N=72**	**Late N=16**	**Early N=14**	**Average N=84**	**Late N=17**	**Early N=12**	**Average N=81**	**Late N=16**
Age of PHV (y)	10.0 ± 1.0	11.8 ± 0.6 [a]	13.1 ± 0.2 [a, b]	10.2 ± 0.4	11.8 ± 0.5 [a]	13.1 ± 0.2 [a, b]	10.0 ± 0.5	11.8 ± 0.5 [a]	13.1 ± 0.2 [a, b]
Age (y)	10.4 ± 1.4	10.8± 1.2	10.5 ± 1.3	14.7 ± 2.0	15.4 ± 1.8 [a]	15.1 ± 1.8	21.8 ± 1.7	23.3 ± 2.1 [a]	22.8 ± 2.2
Height (m)	1.51 ± 0.1	1.48 ± 0.1 [a]	1.39 ± 0.8 [a, b]	1.63 ± 0.6	1.64 ± 0.6	1.61 ± 0.7 [b]	1.64 ± 0.7	1.66 ± 0.6	1.65 ± 0.5
NN CSA (cm^2)	1.61 ±0.4	1.43 ± 0.3 [a]	1.28 ± 0.2 [a, b]	2.04 ± 0.4	2.05 ± 0.3	1.90 ± 0.4 [a, b]	2.00 ± 0.5	2.25 ± 0.4 [a]	2.16 ± 0.4
NN Z (cm^3)	0.63 ± 0.2	0.55 ± 0.2 [a]	0.45 ± 0.1 [a, b]	0.84 ± 0.2	0.87 ± 0.2	0.80 ± 0.2 [a, b]	0.83 ± 0.3	0.98 ± 0.2 [a]	0.94 ± 0.2
IT CSA (cm^2)	2.79 ± 0.9	2.40 ± 0.6 [a]	2.09 ± 0.4 [a, b]	3.49 ± 0.8	3.52 ± 0.5	3.35 ± 0.7 [b]	3.13 ± 0.7	3.71 ± 0.6 [a]	3.48± 0.6
IT Z (cm^3)	2.0 ± 0.7	1.65 ± 0.6 [a]	1.31 ± 0.4 [a, b]	2.61 ± 0.7	2.70 ± 0.6	2.55 ± 0.7	2.25 ± 0.6	2.78 ± 0.6 [a]	2.60 ± 0.5
S CSA (cm^2)	2.03 ± 0.6	1.83 ± 0.4 [a]	1.58 ± 0.3 [a, b]	2.79 ± 0.4	2.82 ± 0.4	2.58 ± 0.5 [a, b]	2.89 ± 0.5	3.13 ± 0.5	2.95 ± 0.4
S Z (cm^3)	0.91 ± 0.3	0.80 ± 0.2 [a]	0.64 ± 0.2 [a, b]	1.35 ± 0.3	1.36 ± 0.3	1.21± 0.3 [a, b]	1.39 ± 0.3	1.52 ± 0.3	1.39 ± 0.3
LTM (kg)	29.1 ± 7.4	26.6 ± 5.2 [a]	23.0 ± 3.9 [a, b]	36.5 ± 4.7	37.6 ± 4.9	34.2 ± 5.5 [a, b]	39.2 ± 6.2	40.1 ± 5.8	37.4 ± 4.7 [b]
FM (kg)	10.6 ± 6.4	11.2 ± 5.2	8.6 ± 4.4 [b]	19.3 ± 9.6	18.4 ± 8.2	16.0 ± 7.9 [a, b]	23.7 ± 10.7	24.4 ± 11.2	25.5 ± 7.6

NN = Narrow Neck; IT = Intertrochanter; S = Femoral Shaft; LTM = Lean tissue mass; FM = Fat mass; CSA = Cross sectional area; Z = Section modulus

[a] indicates a significant difference from early maturers, [b] indicates a significant difference from average matures

2.2.3.2.3 Femoral Shaft Site

Table 2.7 displays the results for the multilevel models for the development of CSA and Z at the S site in males and females. In males, age centered, LTM and PA significantly contributed to the prediction of SCSA, while only age centered and LTM significantly contributed to the prediction of S Z (p<0.05, Table 2.7 Model I and J). Comparable to the findings at the NN and IT sites, once adjusted for age, height, body composition, physical activity and dietary calcium and vitamin D, no independent maturity effects were observed in the development of S CSA and S Z between early, average and late developing males. In females, age centered, height, LTM and FM all significantly contributed to the prediction of S Z (p<0.05), but only age centered, height and LTM significantly contributed to the prediction of SCSA (p<0.05, Table 2.7 Model K and L); however, comparable to observations at the other site, once adjusted for age, size and body composition, there were no significant independent maturity effects on the development of S CSA and S Z.

2.2.5 Discussion

The purpose of this study was to investigate the effects of maturational timing on HSA derived estimated CSA and Z at three sites of the proximal femur. When assessed cross sectionally, there were significant differences in the raw absolute estimated bone geometry observed during childhood, adolescence and adulthood in both males and females. During adolescence, late maturing males and females were observed to possess less CSA and Z compared to their early maturing peers. In adulthood, however, these differences in CSA and Z between early and late maturing individuals were no longer apparent. In the longitudinal models, once age, size, body composition, physical activity, calcium and vitamin D intake were adjusted, estimated CSA and Z development at all sites of proximal femur did not differ significantly between maturational groups in either sex. To our knowledge, this is the first study to use a longitudinal design to investigate the independent effects of maturational timing on the development of adult estimated CSA and Z at the clinically relevant proximal femur in both sexes.

Table 2.5: Multilevel regression models for cross sectional area (CSA) and section modulus (Z) for males and females at the narrow neck site.

	Males (n= 108)				Females (n=118)			
Variable	Model A Narrow Neck CSA (cm^2)		Model B Narrow Neck Z (cm^3)		Model C Narrow Neck CSA (cm^2)		Model D Narrow Neck Z (cm^3)	
Fixed Effects								
Constant	532.90 ± 64.0 #		-89.60 ± 39.1 #		NS		-583.1 ± 123.9 #	
Age C	22.00 ± 5.2 #		15.10 ± 3.2 #		20.8 ± 3.1 #		11.8 ± 1.6 #	
Age C^2	-0.26 ± 0.37 #		0.30 ± 0.2 #		NS		NS	
Age C^3	0.21 ± 0.04 #		-0.20 ± 0.03 #		-0.2 ± 0.05 #		-0.2 ± 0.02 #	
Height	NS		NS		5.7 ± 1.9 #		4.2 ± 0.9 #	
Lean	0.04 ± 0.001 #		0.03 ± 0.007 #		0.04 ± 0.003 #		0.02 ± 0.001 #	
Fat	NS		NS		0.005 ± 0.001 #		0.003 ± 0.001 #	
Physical Activity	NS		NS		NS		NS	
Calcium	NS		NS		NS		NS	
Vitamin D	NS		NS		NS		NS	
Average vs Early	NS		NS		NS		NS	
Average vs Late	NS		NS		NS		NS	
Random Effects								
Level 1								
Constant	10.40 ± 0.5 #		3.9 ± 0.2 #		9.1 ± 0.5 #		2.6 ± 0.1 #	
Level 2	Constant	Age Centered	Constant	Age Centered	Constant	Age Centered	Constant	Age Centered
Constant	48.8 ± 7.1 #	4.5 ± 0.8 #	15.1 ± 2.2 #	1.5 ± 0.3 #	34.7 ± 4.8 #	2.2 ± 0.3 #	8.1 ± 1.1 #	0.7 ± 0.1 #
Age Centered	4.5 ± 0.8 #	0.6 ± 0.1 #	1.5 ± 0.3 #	0.3 ± 0.05 #	2.2 ± 0.3 #	0.2 ± 0.04 #	0.7 ± 0.1 #	0.07 ± 0.01 #

CSA = Cross sectional area; Z = Section modulus; AgeC = Age centered; NS = Not significant

Fixed effect values are Estimated Mean Coefficients ± SEE (Standard Error Estimate) of CSA (cm^2) and Z (cm^3)

Random effects values Estimated Mean Variance ± SEE [CSA (cm^2) and Z (cm^3)]

Age Centered (AgeC) is age in years centred around 16 year of age (yrs).

Height (cm); Total Body Lean Mass (g); Total Body Fat Mass (g). Physical Activity (Score from 1-5)

ł Maturity - average compared to early (Average vs Early) and average compared to late (Average vs Late).

indicate numerical values are multiplied by 10^{-3}

Numerical values are all significant, $p < 0.05$ (mean > 2*SEE). Non significant variables removed from the final model.

Table 2.6: Multilevel regression models for cross sectional area (CSA) and section modulus (Z) for males and females at the intertrochanter site.

	Males (n= 108)		Females (n=118)	
Variable	Model E Intertrochanter CSA (cm^2)	Model F Intertrochanter Z (cm^3)	Model G Intertrochanter CSA (cm^2)	Model H Intertrochanter Z (cm^3)
Fixed Effects				
Constant	-2.70 ± 0.50	-4.20 ± 0.60	-3.20 ± 0.50	-5.4 ± 0.50
Age C	NS	NS	NS	-32 ± 5.2 #
Age C^2	NS	NS	NS	2.1 ± 0.9 #
Age C^3	NS	NS	NS	NS
Height	24.90 ± 4.0 #	25.90 ± 4.2 #	28.30 ± 3.5 #	36.7 ± 3.4 #
Lean	0.05 ± 0.004 #	0.06 ± 0.005 #	0.06 ± 0.004 #	0.06 ± 0.004 #
Fat	NS	NS	NS	-0.004 ± 0.002 #
Physical Activity	39.20 ± 12.70	47.30 ± 16.30	NS	NS
Calcium	NS	NS	NS	NS
Vitamin D	NS	NS	NS	NS
Average vs Early	NS	NS	NS	NS
Average vs Late	NS	NS	NS	NS
Random Effects				
Level 1				
Constant	30.40 ± 2.0 #	50.8 ± 3.4 #	22.3 ± 1.2 #	24.9 ± 1.4 #
Level 2	Constant / Age Centered	Constant / Age Centered	Constant / Age Centered	Constant / Age Centered
Constant	238.2 ± 34.1 # / 19.4 ± 3.4 #	160.2 ± 24.2 # / 16.4 ± 3.0 #	164.9 ± 22.2 # / 7.2 ± 1.2 #	119.7 ± 16.3 # / 4.8 ± 1.0 #
Age Centered	19.4 ± 3.4 # / 2.1 ± 0.4 #	16.4 ± 3.0 # / 2.1 ± 0.4 #	7.2 ± 1.2 # / 0.6 ± 0.1 #	4.8 ± 1.0 # / 0.4 ± 0.1 #

CSA = Cross sectional area; Z = Section modulus; AgeC = Age centered; NS = Not significant
Fixed effect values are Estimated Mean Coefficients ± SEE (Standard Error Estimate) of CSA (cm^2) and Z (cm^3)
Random effects values Estimated Mean Variance ± SEE [CSA (cm^2) and Z (cm^3)]
Age Centered (AgeC) is age in years centred around 16 year of age (yrs).
Height (cm); Total Body Lean Mass (g); Total Body Fat Mass (g). Physical Activity (Score from 1-5)
ǂ Maturity - average compared to early (Average vs Early) and average compared to late (Average vs Late).
indicate numerical values are multiplied by 10^{-3}
Numerical values are all significant, $p < 0.05$ (mean > 2*SEE). Non significant variables removed from the final model.

Table 2.7: Multilevel regression models for cross sectional area (CSA) and section modulus (Z) for males and females at the femoral shaft site.

	Males (n= 108)		Females (n=118)	
Variable	Model I Femoral Shaft CSA (cm^2)	Model J Femoral Shaft Z (cm^3)	Model K Femoral Shaft CSA (cm^2)	Model L Femoral Shaft Z (cm^3)
Fixed Effects				
Constant	0.60 ± 0.10	NS	-0.8 ± 0.40	-1.3 ± 0.20
Age C	73.60 ± 8.3 #	34.40 ± 5.2 #	42.1 ± 3.9 #	15.9 ± 2.5 #
Age C^2	-1.30 ± 0.6 #	-1.60 ± 0.4 #	-2.3 ± 0.7 #	NS
Age C^3	-0.40 ± 0.09 #	-0.20 ± 0.05 #	-0.2 ± 0.06 #	-0.1 ± 0.03 #
Height	NS	NS	10.7 ± 2.7 #	9.2 ± 1.6 #
Lean	0.05 ± 0.002 #	0.04 ± 0.001 #	0.05 ± 0.003 #	0.03 ± 0.002 #
Fat	NS	NS	NS	0.002 ± 0.001 #
Physical Activity	20.20 ± 9.50	NS	NS	NS
Calcium	NS	NS	NS	NS
Vitamin D	NS	NS	NS	NS
Average vs Early	NS	NS	NS	NS
Average vs Late	NS	NS	NS	NS
Random Effects				
Level 1				
Constant	14.60 ± 1.1 #	11.1 ± 0.7 #	14.9 ± 0.8 #	5.4 ± 0.3 #
Level 2	Constant / Age Centered	Constant / Age Centered	Constant / Age Centered	Constant / Age Centered
Constant	82.8 ± 12.2 # / 6.3 ± 0.9 #	26.4 ± 4.0 # / 2.2 ± 0.5 #	58.0 ± 8.0 # / 2.9 ± 0.6 #	18.6 ± 2.6 # / 0.9 ± 0.2 #
Age Centered	6.3 ± 0.9 # / 0.9 ± 0.2 #	2.2 ± 0.5 # / 0.4 ± 0.07 #	2.9 ± 0.6 # / 0.4 ± 0.07 #	0.9 ± 0.2 # / 0.1 ± 0.03 #

CSA = Cross sectional area; Z = Section modulus; AgeC = Age centered; NS = Not significant
Fixed effect values are Estimated Mean Coefficients ± SEE (Standard Error Estimate) of CSA (cm^2) and Z (cm^3)
Random effects values Estimated Mean Variance ± SEE [CSA (cm^2) and Z (cm^3)]
Age Centered (AgeC) is age in years centred around 16 year of age (yrs).
Height (cm); Total Body Lean Mass (g); Total Body Fat Mass (g). Physical Activity (Score from 1-5)
ǂ Maturity - average compared to early (Average vs Early) and average compared to late (Average vs Late).
# indicates numerical values are multiplied by 10^{-3}
Numerical values are all significant, $p < 0.05$ (mean > 2*SEE). Non significant variables removed from the final model.

Late maturational timing is documented to have a deleterious effect on bone mass, bone geometry and bone architecture **[41-45, 147]**, with a natural delay in maturation increasing fracture prevalence in males **[41]**, reducing bone mineral mass development during adolescence and adulthood **[41-45, 147]**, and decreasing bone microstructure in adult females **[44]**. The negative associations of late maturational timing on bone structure and strength have largely been observed at the distal radius, with only two recent studies focusing on the proximal femur but using bone mineral mass as their bone strength measure **[42, 147]**. Using a prospective design, Gilsanz et al. **[42]** documented a negative association between maturational timing and areal bone mineral density (aBMD) at the proximal femur in both sexes at 16 years of age. The observations for estimated CSA and Z in the current cross sectional analyses parallels the findings of Gilsanz et al. **[42],** with late maturing males and females having less CSA and Z then their early maturing peers at 15 years of age; however, to draw conclusions based on these cross sectional observations ignores differences in body size and other determinants of bone mass and structure (such as LTM and vitamin D intake) as well as the independent linear trajectories of growth associated with maturation which may influence bone development. When estimated CSA and Z were assessed longitudinally, and the independent effects of growth, size, body composition, dietary intake and physical activity were accounted, there was no significant difference viewed between maturity groups in CSA and Z development at any site of the proximal in both sexes. Instead, LTM significantly predicted the development of CSA and Z in both sexes, with FM significantly contributing to CSA at the NN and Z at the NN, IT, and S in females. PA also significantly contributed to IT CSA, IT Z and SCSA for males only. Normally, it would be expected that PA would have a greater predictive contribution to CSA and Z development in both sexes; however, given the strong documented interaction between PA and LTM **[56],** when both LTM and PA are included in the models, the predictive contribution of LTM masks that of PA. These findings are comparable to our earlier BMC observations, where no significant differences were noted in the development of BMC between maturational groups

at the proximal femur **[147]**. It is not surprising that LTM and FM contributed to the prediction of estimated geometry, as their influence on bone mass and bone geometry have been published extensively **[60, 103, 135, 185, 188, 192, 195, 219]**. What is novel is that when the influence of LTM and FM are accounted for, maturational timing does not influence estimated bone geometry at the proximal femur in both males and female. These current longitudinal findings, alongside those previously published in the same cohort **[147],** would suggest that maturation timing does not compromise bone geometry or mass at the proximal femur. This contradicts previous cross sectional data at the hip **[42],** which noted a 3-5% decrease in aBMD at the femoral neck in late maturing males and females compared to their earlier maturing counterparts. Because the rate of bone mass in adulthood declines at approximately 1-2% a year, Gilsanz and colleagues proposed this 3-5% advantage in aBMD seen in early maturers may correspond to 3-5 years of protection against normal age related bone loss and skeletal fragility. Although fracture incidence was not assessed in the current study, the current findings imply that maturational timing does not influence fracture risk in adulthood. In contrast, Chevalley et al **[43-45]** and Kindblom et al. **[41]** make a compelling alternative argument at the distal radius, suggesting that individuals with a natural delayed maturation may be at a greater risk of skeletal fragility and fracture. The discrepancy between the current findings at the proximal femur and those at the distal radius may be the result of mechanical loading disparities at each site. Given that the proximal femur is constantly loaded through muscular contractions and gravitational forces, these factors may supersede the potential effects of maturation at this site; however, at sites not regularly exposed to intense muscular contractions or body weight loading (eg. distal radius), the potential influence of maturational timing may be more pronounced. Future research investigating the effects of maturational timing on potential site specific differences in geometric properties is required to confirm these speculations.

The results of this study are unique in that longitudinal measurements in both males and females were employed, maturational timing was prospectively determined, and the independent effects of growth,

body composition and dietary intake were controlled, but the conclusions are limited by several factors. First, this study was observational in nature. Observation studies are limited due to inherent susceptibility of observational associations related to uncontrolled variables, selection bias, and reverse causality **[10, 206]**. This may limit the ability to generalize the findings to other populations. Further longitudinal research in other populations is required to supplement these observations. Next, the HSA program also has inherent limitations. DXA images are often noisy and blurred resulting in the difficulty of locating precise edge margins **[110, 207].** In addition, the position of femur is important as small changes in femur rotation have a large effect on the geometric dimensions **[207].** All DXA scans were performed by qualified radiologists familiar with proper positioning of the proximal femur to ensure hip scans were performed with care to limit these potential errors; nevertheless, it is difficult to position the hip consistently in repeated measures over time. Despite these limitations, the current study provides novel information surrounding the relationship between maturational timing and bone strength development at the proximal in males and females.

In conclusion, cross sectional unadjusted data may suggest there is a negative effect of maturational timing on bone strength, but longitudinal assessments that account for the variability in the timing and tempo of growth, and the independent effects of size, body composition, physical activity and nutritional intake, suggest that there is no effect of maturational timing on estimated CSA and Z development at the proximal femur in both males and females. Future research is required to investigate potential site specific differences that may alter the influence of maturational timing on geometric bone properties.

2.3 Study Three: Adolescent Physical Activity and Adult Bone Structural Strength at the Proximal Femur

2.3.1 Abstract

Physical activity enhances bone structural strength at the proximal femur in adolescence, but whether these benefits are maintained into early adulthood remains unknown. The purpose of this study was to investigate whether males and females, described as active, average and inactive during adolescence, display differences in structural strength at the proximal femur in early adulthood (20-30 years of age).

One hundred and four participants (55 males, 49 females) from the Pediatric Bone Mineral Accrual Study (PBMAS) were separated into four adolescent physical activity groupings using the Physical Activity Questionnaire for Adolescents (PAQ-A). Cross sectional area (CSA) and section modulus (Z) at the narrow neck (NN), intertrochanter (IT) and femoral shaft (S) sites of the proximal femur were assessed using hip structural analysis (HSA) in young adulthood from femoral neck DXA scans. Group differences were assessed using analyses of covariance (ANCOVA), controlling for adult height (Ht), weight (Wt), adolescent bone geometry, percentage adult total body lean tissue (LTM%) and adult physical activity levels (PA).

When adjusted for Ht, Wt and adolescent bone geometry, adolescent participants classified as inactive had significantly lower adult bone geometric measures at the proximal femur than adult participants who were active during adolescence ($p<0.05$); however, once LTM% and PA were included as covariates no significant differences in adult geometric bone measures were observed at any site of the proximal femur in either sex ($p>0.05$).

Although physical activity may confer skeletal advantages during adolescence, maintaining these adolescent benefits into young adulthood may be dependent on a preserved active lifestyle

2.3.2 Introduction

Osteoporosis is a disease characterized by deterioration of the bone structure leading to subsequent bone fragility **[77]**. Hip fractures are arguably the most costly consequence of osteoporosis, resulting in increased mortality, compromised functional capacity and an amplified economic burden on the public health care systems **[3, 5, 6, 220]**. Although the early determinants of osteoporosis and fracture risk are still poorly understood, it has been proposed that optimizing and maintaining bone structural strength, particularly during adolescence, can potentially reduce the risk of osteoporosis later in life **[221]**.

According to the mechanostat theory, dynamic loads are essential to elicit bone adaptation **[50-52]**. Physical activity via muscular actions places such loads on the skeleton and is documented to provide osteogenic benefits to bone structural strength during childhood, adolescence and adulthood **[48, 62, 222, 223]**. Engaging in physical activity during childhood and adolescence is highlighted as a unique opportunity where the benefits of mechanical loading on bone structural strength can be maximized **[53, 55, 58-60, 64, 208, 217, 224-227]**. It has been observed that individuals who participate in higher levels of physical activity during adolescence have greater bone mass between 20 and 30 years of age **[57, 63, 67-71]**, the time when peak bone mass is achieved **[27-31]**. Additionally, the mechanostat suggests that the removal of these dynamic loads, or the reduction in physical activity, would result in negative bone adaptation with declines to both bone mass and bone structural strength **[50-52]**. Supporting this supposition, immobilizations studies have documented quick and efficient declines to bone mass and structural parameters with the removal of dynamic loads **[174, 175, 228, 229]**. This would imply that in order to maintain any benefits to structural strength from childhood and adolescence into adult, physical activity levels must also be prolonged.

Although the Saskatchewan Pediatric Bone Mineral Accrual Study (PBMAS) provided evidence to support the supposition that skeletal benefits resulting from early life habitual physical activity persist into young adulthood **[208]**, these results focused primarily on bone mineral content (BMC) accrual and

areal bone mineral density (aBMD) development. While strength generally trends in the same direction as aBMD and BMC, this is not always the case. In addition these parameters are not themselves properties that govern strength. Bone strength is determined by structural dimensions (eg. bone geometry) and material strength **[18]**. It has been acknowledged that geometric measures may improve fracture prediction beyond that provided by aBMD alone, as cross sectional area (CSA) and section modulus (Z) measures are better able to differentiate osteoporotic and non-osteoporotic individuals **[20-22, 125]**. Retired athlete models have been commonly used to investigate the association between early life physical activity and adult geometric bone strength, with retired athletes reported to have significantly greater total bone area, CSA, and bending strength at cross sections of the radius and humerus **[48, 177, 230]**. These data suggest that childhood and adolescent physical activity is not only associated with improved bone mass, but also improved bone geometric properties; however, such conclusions derived from retired athletes are confounded by the possibility of genetic predispositions. Muscular strength is a strong predictor of bone structural strength **[135, 185, 231-233]**. Physical activity and lean tissue mass development **[56]**, a surrogate of muscular strength, are also strongly linked. Given these relationships the independent role of physical activity on bone structural strength development may be confounded or masked by the effects of physical activity on lean tissue mass development. It is therefore suggested that investigations in healthy non-athletic populations which consider the confounding effects of lean tissue mass development would be more applicable to the general public and provide further evidence to support the investment of physical activity in childhood and adolescence **[69]**.

Previously, in this cohort of healthy children participating in the PBMAS, it has been shown that higher levels of habitual physical activity are positively associated with the development of improved geometric bone measures at the hip during adolescence **[58].** Furthermore, geometric bone measures have been shown to peak and/or plateau between the second and third decade of life **[234].** What remains

unknown is whether (i) these benefits of physical activity, observed during adolescence, are maintained into young adulthood and (ii) whether the current adult physical activity influences maintenance of the bone benefits observed in adolescence. The longitudinal nature of the PBMAS provides a unique dataset to address these questions. Therefore, the purpose of this present study is to investigate whether adolescent physical activity is related to geometric bone strength estimated at the proximal femur in young adulthood. It is hypothesized that once the confounders of height, weight, lean tissue mass and adult physical activity levels are accounted, adults identified as active in adolescence will have greater CSA and Z at the proximal femur than adults classified as average and/or inactive in adolescence.

2.3.3 Methods

2.3.3.1 Participants

Participants were drawn from the University of Saskatchewan's Pediatric Bone Mineral Accrual Study (PBMAS). Details of the PBMAS participants and the recruitment process have been described previously **[29, 53, 55]**. In brief, in 1991, 375 eligible students, aged 8-15 years, were recruited from two elementary schools in the city of Saskatoon, of which the parents of 228 students (113 boys and 115 girls) provided written consent for their children to be involved in this study. Two hundred and twenty of these individuals underwent dual energy x-ray absorptiometry (DXA) scans. From 1992 to 1993 an additional 31 participants were recruited and scanned. After 7 years of annual data collection 230 participants (109 males and 121 females) had been measured on two or more occasions (median 6 occasions) and comprised the adolescent longitudinal dataset. Between 2002 and 2007, 169 participants returned and were measured on at least 1 occasion (ages range 17 to 30 years). To be included in the present study, participants had to have: 1) a valid assessment of peak height velocity (PHV); 2) an assessment of peak geometric bone measures (peak CSA and/or peak Z, as determined in Study 1) in adulthood **[234]**; 3) physical activity scores at PHV and in adulthood; and 4) no diseases known to affect growth or bone development. This resulted in the inclusion of 104 participants (55 males and 49

females). Ninety eight percent of the participants were of Caucasian descent. Written consent was obtained from all participants. All procedures were approved the University of Saskatchewan's biomedical ethics review committee.

2.3.3.2 Anthropometry

Height and weight were assessed annually following the anthropometric standards outlined by Ross and Marfell-Jones **[200]**. Height (Ht) was recorded without shoes to the nearest 0.1 cm using a wall mounted stadiometer (Holtain Limited, Crymych, UK). Weight (Wt) was measured without shoes on a calibrated scale to the nearest 0.1kg (Toledo, Columbus, USA).

2.3.3.3 Peak Height Velocity

The attainment of peak height velocity (PHV), a measure of maximum linear growth during adolescence, is a commonly used maturational landmark in longitudinal studies **[9, 10]**. Given that maturity influences adolescent physical activity levels in both sexes in a contemporaneous manner **[235]**, it is imperative that when assessing physical activity during adolescence individuals been aligned at a comparable maturity milestone **[9, 11]**. To determine the age at PHV, whole year height velocities were calculated for each participant from serial measures of stature. A cubic spline fitting procedure was applied to each individual's whole year velocity values and the age at the highest point was estimated (GraphPad Prism 5, GraphPad Software, San Diego, CA, USA). The cubic spline curve fitting procedure provides a smooth velocity curved based on polynomial algorithms that maintain the original integrity of each individual's data. From these curves an estimation of attainment of peak statural growth are identified.

2.3.3.4 Physical Activity and Physical Activity Groupings

Physical activity (PA) was serially assessed using self-report questionnaires. Details of the physical activity questionnaires used have been reported elsewhere **[56, 213]**. In brief, during childhood and adolescence physical activity was assessed using the Physical Activity Questionnaire for Children (PAQ-C)

and the Physical Activity Questionnaire for Adolescence (PAQ-A). The PAQ-C/A were designed to assess general PA levels over the previous seven days, scoring nine items on a five point Likert-type scale. Final PA scores range from one to five, with higher scores indicating higher levels of PA. In adulthood the Physical Activity Questionnaire for Adults (PAQ-AD), a 7 item version of the PAQ-C/A was used; again, individuals PA was scored on a five point scale. The PAQ-C/A/AD have been previously reported to be a valid and reliable measure of PA levels in children, adolescents and adults **[214, 215, 236].**

Adolescent activity groups were formed based on the PAQ-C/A scores using procedures described in detail elsewhere **[57]**. Briefly, for each individual an age and sex specific Z score was determined for each test administration. These Z scores were based on the mean and SD for the entire sample at the same chronological age. All childhood and adolescent Z score, from each measurement occasion, were then summed and averaged; the median number of annual visits being 6 (minimum 3, maximum 7). Individuals were then ranked into quartiles according to their average adolescent activity Z score. Those whose Z-score fell in the highest quartile were classified as active, those in the middle two quartiles were classified as average active, and those whose score was in the lowest quartile were classified as inactive.

To control for current adult physical activity, a physical activity score occurring when peak geometric bone measures were reached was ascertained using a linear interpolation routine (MatLab 2006b, Mathworks, Natick, MA, USA) for each participant. This value (1 low to 5 high) was then used as the adult PA score specific to the geometric bone outcome measure (e.g. CSA or Z).

2.3.3.5 Lean Tissue Mass

Total body lean tissue mass (LTM) was assessed annually using dual energy x-ray absorptiometry (DXA, Hologic QDR-2000, array mode) by a trained technician following the procedures outlined in the operators manual and user guide. Adult LTM was determined as the adult values at the age at which the peak in geometric bone measures occurred for each individual. Total body lean tissue mass (LTM) was

analyzed using software version 5.67A. The inter-assay precision (CV%) in vivo in our lab have been previously reported as 0.5% for LTM **[56]**. LTM percentage (LTM%) was determined as the ratio of LTM (kg) to total body Wt (kg)

2.3.3.6 Bone Measures

At each measurement occasion participants underwent a DXA scan of the total body, lumbar spine and proximal femur following the procedures outlined in Hologic operator's manual. For the current study, only proximal femur DXA scans were used and all bone measures were derived using the hip structural analysis (HSA) program. The HSA program has been previously reported elsewhere in greater detail **[25]**. In brief, the HSA program estimates the structural geometry of the proximal femur from DXA derived images of the hip. Employing the principles originally reported by Martin and Burr **[24]**, a line of pixels values traversing the bone axis in a bone mass image is a projection of the corresponding cross section and its dimensions. To determine these cross sections, the HSA algorithms divide the pixel mass values in g/cm^2 by the effective mineral density of fully mineralized adult cortical bone. Cross sections are evaluated by averaging geometry over 5 parallel lines spaced 1mm apart at: 1) the Narrow Neck (NN) – the narrowest diameter of the femoral neck; 2) the Intertrochanteric (IT) – along the bisector of the neck and shaft angle; and 3) the Shaft (S) – a distance of 1.5 times the minimum neck width to the intersection of the neck and shaft axes **[130]**. The HSA program locates these regions on the DXA bone mineral image and then derives the estimates of structural geometry. From each region, the HSA program produces ten output variables, of which two were assessed for this study: *Cross Sectional Area (CSA)*– the estimated amount of bone surface area in the cross section after excluding all the trabecular and soft tissue space; and, *Section Modulus (Z)* – an indicator of bending strength calculated as the cross sectional moment of inertia/ the maximum distance from the center of mass to outer cortex **[110, 130, 207]**. The short-term precision for CSA and Z derived using a Hologic QDR 2000 hip scan ranges from

2.3% to 2.8% and 2.8% to 3.4%, respectively **[26]**. All HSA analyses were completed by a single technician and derived from proximal femur scans using a Hologic QDR-2000 (Bedford, MA, USA).

Following the procedures previously outlined by Jackowski et al. **[234]**, the ages and absolute values for peak proximal femur CSA and Z were determined for each participant. The peak values were determined as the maximal absolute value for each bone measure at each site, resulting in peak values for CSA (CSAp) and Z (Zp) at each site (eg NN, IT, S). The ages at peak were used to determine the time point for selecting adult covariates (eg Ht, Wt, LTM %, and PA scores).

2.3.3.7 Statistical Analysis

Differences in adolescent CSA and Z, between adolescent PA groups, were first assessed using sex separated analysis of variance (ANOVA). This was performed to confirm previous observation in this population **[57, 58]** that differences in geometric bone properties existed during adolescence between adolescent PA groups. To test for potential differences in adult peak bone geometric measures between adolescence PA groups, three progressive sex separated ANCOVAs were performed. The first sex separated ANCOVA (Adult Model 1) included adult Ht, adult Wt, and geometric bone measurements at PHV as covariates to assess differences after controlling for size and adolescent bone geometry. The second sex-separated ANCOVAs (Adult Model 2) included adult Ht, adult Wt, geometric bone measurements at PHV and adult LTM% as covariates to assess differences once relative muscular strength was also accounted. Finally, the third sex-separated ANCOVA (Adult Model 3) included adult Ht, adult Wt, geometric bone measurements at PHV, adult LTM% and adult PA as covariates to determine the effects of relative muscular strength and current physical activity levels on adult bone geometry. If significant group differences were observed in either model, post-hoc pairwise comparisons with Bonferroni adjustments were used to ascertain individual group differences. All adult covariates were the adult values corresponding to the age at peak bone measure being assessed. For example, in one participant, the age at peak NN CSA occurred at 7 years post PHV (21 years of age), while NN Z occurred

at 8 years post PHV (22 years of age). Therefore, the adult values for Ht, Wt, LTM%, and PA score at 7 years post PHV were used as covariates for NN CSA analyses, while values at 8 years post PHV were used for NN CSA analyses. Data were checked for normality using skewness and kurtosis. Any violations were adjusted using logarithmic transformations. An alpha of $p<0.05$ was considered significant. All analyses were performed using Statistical Package for the Social Sciences 19.0 for Windows (SPSS, Chicago, IL, USA).

2.3.4 Results

2.3.4.1 Adolescent Measures

Table 2.8 provides a summary of the participants' anthropometric, body composition and geometric bone measures in adolescence and adulthood by sex. There were no significant differences observed in adolescent Ht and Wt between adolescent physical activity groups in either males or females at PHV ($p>0.05$), but there were significant differences in LTM % ($p<0.05$). Inactive adolescent males and females had significantly lower LTM % that their active classified peers at PHV ($p<0.05$). Significant differences were also observed between groups in absolute proximal femur bone geometric measurements. Inactive males had significantly lower NN CSA, NN Z, IT Z, and S CSA than both their average and active classified counterparts; inactive males also had significantly less S Z than active males at PHV ($p<0.05$, Table 2.8). No significant differences were observed in absolute adolescent bone geometric measures between average and active males ($p>0.05$, Table 2.8). In females, the inactive group had significantly lower absolute NN CSA, NN Z, and IT Z than the active group at PHV ($p<0.05$, Table 2.8). No significant differences at PHV in absolute bone measures were observed between the inactive and average groups ($p>0.05$), and, similar to the males, no significant differences were observed between the average and active females at PHV ($p>0.05$, Table 2.8).

2.3.4.2 Adult Measures

Comparisons in adulthood (at the time of achievement of peak geometric bone CSA and Z as derived from Jackowski et al **[234]**) found there were no significant differences in adult anthropometrics or body composition between adults classified by adolescent physical activity (p>0.05, Table 2.8), despite active adolescent males and females maintaining significantly higher levels of self-reported physical activity in adulthood (p<0.05, Table 2.8).

2.3.4.3 Adult Model 1 (adjustments for height, weight, and bone geometry at PHV)

Figure 2.3 displays the results for adult adjusted CSA and Z at the NN, IT and S sites for males and females, when adjusted for Model 1 covariates (Tables 2.9 and 2.10). Inactive males had significantly lower adult adjusted CSA and Z at all sites of the proximal femur than males classified as average active and active in adolescence (p<0.05, Figure 2.3); means adjusted for adult ht, wt and bone geometry at PHV. For all adult geometric bone measures in males, wt and bone geometry significant contributed to the model, with the Model 1 explaining 28-52% of the variance in adult bone geometric measures (Table 2.9).

In females, individuals who were classified as inactive during adolescence had significantly lower adjusted adult NN Z, IT CSA and IT Z than females classified as active active in adolescence (p<0.05, Figure 2.3); means adjusted for adult height, adult LTM, adult FM and bone geometry at PHV. Additionally, these inactive classified females had significantly lower adjusted adult S CSA and S Z than females classified as average in adolescence (p<0.05). Similar to males, wt and bone geometry significantly contributed to models for the majority of adult geometric measures, with ht also being a significant predictor for NN CSA and S Z (Table 2.10).

Table 2.8: Anthropometric, body composition and absolute geometric bone measures in male and female adolescent activity groups at peak height velocity and adulthood. Means ± standard deviations

		Males			Females		
		Inactive (n= 17)	Average (n= 32)	Active (n= 12)	Inactive (n= 14)	Average (n=33)	Active (n= 14)
Values at PHV	Age of PHV (y)	13.3 ± 1.1	13.4 ± 1.1	13.5 ± 0.9	12.2 ± 0.8	11.9 ± 0.8	11.6 ± 1.0
	Height (cm)	164.8 ± 6.3	163.9 ± 7.1	164.9 ± 3.4	151.5 ± 5.8	152.4 ± 6.4	156.5 ± 4.0
	Weight (kg)	48.5 ± 6.7	50.7 ± 8.3	52.6 ± 9.8	39.8 ± 6.6	39.7 ± 6.7	41.1 ± 5.8
	Percent LTM	72.0 ± 7.6	79.4 ± 8.6	81.2 ± 6.8[a]	65.7 ± 8.9	67.4 ± 9.4	74.4 ± 9.2[a]
	PA Score	2.33 ± 0.47	3.11 ± 0.36[a]	3.7 ± 0.33[a,b]	2.32 ± 0.44	2.80 ± 0.29[a]	3.31 ± 0.40[a,b]
	NN CSA (cm^2)	1.82 ± 0.19	1.96 ± 0.25[a]	2.05 ± 0.31[a]	1.49 ± 0.19	1.53 ± 0.23	1.62 ± 0.18[a]
	NN Z (cm^3)	0.79 ± 0.13	0.86 ± 0.15[a]	0.90 ± 0.15[a]	0.56 ± 0.11	0.58 ± 0.11	0.64 ± 0.10[a]
	IT CSA (cm^2)	3.23 ± 0.40	3.52 ± 0.44[a]	3.77 ± 0.76	2.61 ± 0.21	2.66 ± 0.38	2.85 ± 0.48
	IT Z (cm^3)	2.50 ± 0.52	2.77 ± 0.48[a]	2.91 ± 0.61[a]	1.83 ± 0.28	1.91 ± 0.39	2.11 ± 0.38[a]
	S CSA (cm^2)	2.34 ± 0.24	2.50 ± 0.33[a]	2.66 ± 0.52[a]	2.00 ± 0.19	2.00 ± 0.33	2.10 ± 0.20
	S Z (cm^3)	1.11 ± 0.17	1.21 ± 0.21	1.27 ± 0.27[a]	0.85 ± 0.12	0.92 ± 0.24	0.93 ± 0.10
		Males			**Females**		
		Inactive (n= 17)	Average (n= 32)	Active (n= 12)	Inactive (n= 14)	Average (n=33)	Active (n= 14)
Adult Values	Age	21.0 ± 0.8	22.3 ± 0.6	22.6 ± 0.9	22.5 ± 0.9	21.7 ± 0.6	21.0 ± 0.9
	Height (cm)	180.7 ± 7.4	180.0 ± 7.7	178.3 ± 7.1	167.2 ± 6.11	166.2 ± 6.0	166.9 ± 5.8
	Weight(kg)	82.9 ± 16.2	79.3 ± 11.6	86.0 ± 15.0	71.7 ± 17.7	65.6 ± 15.2	65.0 ± 10.0
	LTM Percentage	74.2 ± 8.1	78.1 ± 7.5	74.7 ± 6.3	57.8 ± 8.1	61.9 ± 7.0	65.3 ± 6.9[a]
	PA Score	2.07 ± 0.49	2.53 ± 0.54[a]	2.74 ± 0.47[a]	1.71 ± 0.61	2.07 ± 0.43	2.72 ± 0.41[a,b]
	PA Change	-0.18 ± 0.73	-0.64 ± 0.55	-1.18 ± 0.75	-0.66 ± 0.71	-0.74 ± 0.54	-0.63 ± 0.54
	NN CSA (cm^2)	2.73 ± 0.41	2.97 ± 0.40	3.17 ± 0.53[a]	2.33 ± 0.37	2.27 ± 0.31	2.33 ± 0.32
	NN Z (cm^3)	1.39 ± 0.59	1.55 ± 0.28	1.67 ± 0.33[a]	1.00 ± 0.20	0.98 ± 0.18	1.03 ± 0.15
	IT CSA (cm^2)	4.61 ± 0.60	5.01 ± 0.59	5.44 ± 1.01[a]	3.82 ± 0.56	3.82 ± 0.63	3.96 ± 0.60
	IT Z (cm^3)	4.14 ± 0.59	4.57 ± 0.62	4.97 ± 1.22[a]	2.92 ± 0.54	2.93 ± 0.68	3.07 ± 0.54
	S CSA (cm^2)	3.86 ± 0.67	4.15 ± 0.44	4.50 ± 0.84[a]	3.17 ± 0.50	3.23 ± 0.56	3.20 ± 0.39
	S Z (cm^3)	2.06 ± 0.44	2.24 ± 0.29	2.46 ± 0.60[a]	1.55 ± 0.34	1.61 ± 0.35	1.54 ± 0.24

NN= Narrow Neck,; IT = Intertrochanter; S = Femoral Shaft; LTM = Lean Tissue Mass; FM = Fat Mass; PA = Physical Activity; CSA = Cross sectional area; Z = Section modulus. [a] indicates a significant difference from the inactive adolescent physical activity group ($p<0.05$)

[b] indicates a significant difference from the average adolescent physical activity group ($p<0.05$)

Table 2.9: Beta coefficients and model variance for the analyses of covariance (ANCOVA) in males. Beta coefficients ± standard error.

		Adjusted R^2	Height	Weight	Adolescent Geometry	Lean Tissue Mass %	Adult Physical Activity
Model 1[a]	**NNCSA**	0.42	0.008 ± 0.007	0.016 ± 0.004*	1.068 ± 0.168*	Not included in model	
	NNZ	0.42	0.009 ± 0.004	0.011 ± 0.002*	1.100 ± 0.206*		
	ITCSA	0.28	0.011 ± 0.012	0.019 ± 0.006*	1.011 ± 0.112*		
	ITZ	0.52	0.040 ± 0.012	0.018 ± 0.007*	0.867 ± 0.134*		
	SCSA	0.42	0.006 ± 0.009	0.026 ± 0.005*	0.890 ± 0.612*		
	SZ	0.5	0.016 ± 0.006	0.016 ± 0.003*	0.813 ± 0.146*		
Model 2[b]	**NNCSA**	0.65	Not included in model	0.016 ± 0.005*	1.088 ± 0.188*	1.800 ± 0.835*	Not included in model
	NNZ	0.63		0.014 ± 0.003*	1.112 ± 0.226*	1.705 ± 0.560*	
	ITCSA	0.71		0.022 ± 0.007*	1.021 ± 0.121*	2.809 ± 1.180*	
	ITZ	0.63		0.020 ± 0.009*	0.876 ± 0.153*	2.361 ± 1.452*	
	SCSA	0.70		0.033 ± 0.006*	0.909 ± 0.156*	3.762 ± 1.010*	
	SZ	0.73		0.021 ± 0.004*	0.822 ± 0.164*	2.445 ± 0.586*	
Model 3[c]	**NNCSA**	0.71	Not included in model	0.015 ± 0.005*	1.286 ± 0.190*	2.120 ± 0.775*	0.066 ± 0.070*
	NNZ	0.66		0.013 ± 0.003*	1.038 ± 0.231*	1.676 ± 0.560*	0.068 ± 0.053*
	ITCSA	0.73		0.022 ± 0.007*	0.995 ± 0.123*	2.898 ± 1.180*	0.144 ± 0.107*
	ITZ	0.66		0.019 ± 0.009*	0.886 ± 0.152*	1.987 ± 1.460*	0.161 ± 0.122*
	SCSA	0.72		0.033 ± 0.006*	0.898 ± 0.160*	3.698 ± 1.031*	0.107 ± 0.091*
	SZ	0.75		0.021 ± 0.004*	0.851 ± 0.170*	2.540 ± 0.604*	0.010 ± 0.051*

NN= Narrow Neck; IT = Intertrochanter; S = Femoral Shaft; CSA = Cross sectional area; Z = Section modulus

[a] model included height, weight and bone geometry at PHV as covariates

[b] model included weight, bone geometry at PHV, and total body lean tissue mass percentage as covariates

[c] model included weight, bone geometry at PHV, total body lean tissue mass percentage, and adult physical activity as covariates

* indicates covariate is significant ($p<0.05$)

Table 2.10: Beta coefficients and model variance for the analyses of covariance (ANCOVA) in females. Beta coefficients ± standard error.

		Adjusted R^2	Height	Weight	Adolescent Geometry	Lean Tissue Mass %	Adult Physical Activity
Model 1[a]	**NNCSA**	0.31	0.017 ± 0.006*	0.009 ± 0.002*	0.825 ± 0.210*	Not included in model	
	NNZ	0.48	0.011 ± 0.003	0.006 ± 0.001	0.903 ± 0.206*		
	ITCSA	0.34	0.030 ± 0.011	0.019 ± 0.004*	0.814 ± 0.194*		
	ITZ	0.50	0.044 ± 0.010	0.020 ± 0.004*	0.597 ± 0.174*		
	SCSA	0.53	0.027 ± 0.008	0.021 ± 0.003*	0.142 ± 0.164*		
	SZ	0.63	0.027 ± 0.004*	0.012 ± 0.002*	0.577 ± 0.140*		
Model 2[b]	**NNCSA**	0.49	Not included in model	0.007 ± 0.006	0.857 ± 0.213*	0.478 ± 0.973	Not included in model
	NNZ	0.63		0.006 ± 0.002	0.920 ± 0.209*	0.273 ± 0.443	
	ITCSA	0.60		0.019 ± 0.009	0.847 ± 0.197*	1.387 ± 1.597*	
	ITZ	0.70		0.025 ± 0.008*	0.610 ± 0.176*	1.879 ± 1.346*	
	SCSA	0.70		0.027 ± 0.006*	0.625 ± 0.165*	2.558 ± 1.02*	
	SZ	0.74		0.015 ± 0.005*	0.590 ± 0.144*	1.419 ± 0.581*	
Model 3[c]	**NNCSA**	0.59	Not included in model	0.008 ± 0.006*	0.835 ± 0.215*	0.223 ± 0.773*	0.060 ± 0.066*
	NNZ	0.66		0.006 ± 0.003	0.900 ± 0.221*	0.286 ± 0.553	0.010 ± 0.029
	ITCSA	0.61		0.019 ± 0.009	0.850 ± 0.202*	1.369 ± 1.18*	0.019 ± 0.100
	ITZ	0.73		0.025 ± 0.008*	0.625 ± 0.178*	1.962 ± 1.459*	0.070 ± 0.088*
	SCSA	0.73		0.027 ± 0.006*	0.620 ± 0.167*	2.546 ± 1.028*	0.036 ± 0.079*
	SZ	0.77		0.015 ± 0.003*	0.586 ± 0.146*	1.464 ± 0.604*	0.020 ± 0.033*

NN= Narrow Neck; IT = Intertrochanter; S = Femoral Shaft; CSA = Cross sectional area; Z = Section modulus

[a] model included height, weight and bone geometry at PHV as covariates

[b] model included weight, bone geometry at PHV, and total body lean tissue mass percentage as covariates

[c] model included weight, bone geometry at PHV, total body lean tissue mass percentage, and adult physical activity as covariates

* indicates covariate is significant ($p<0.05$)

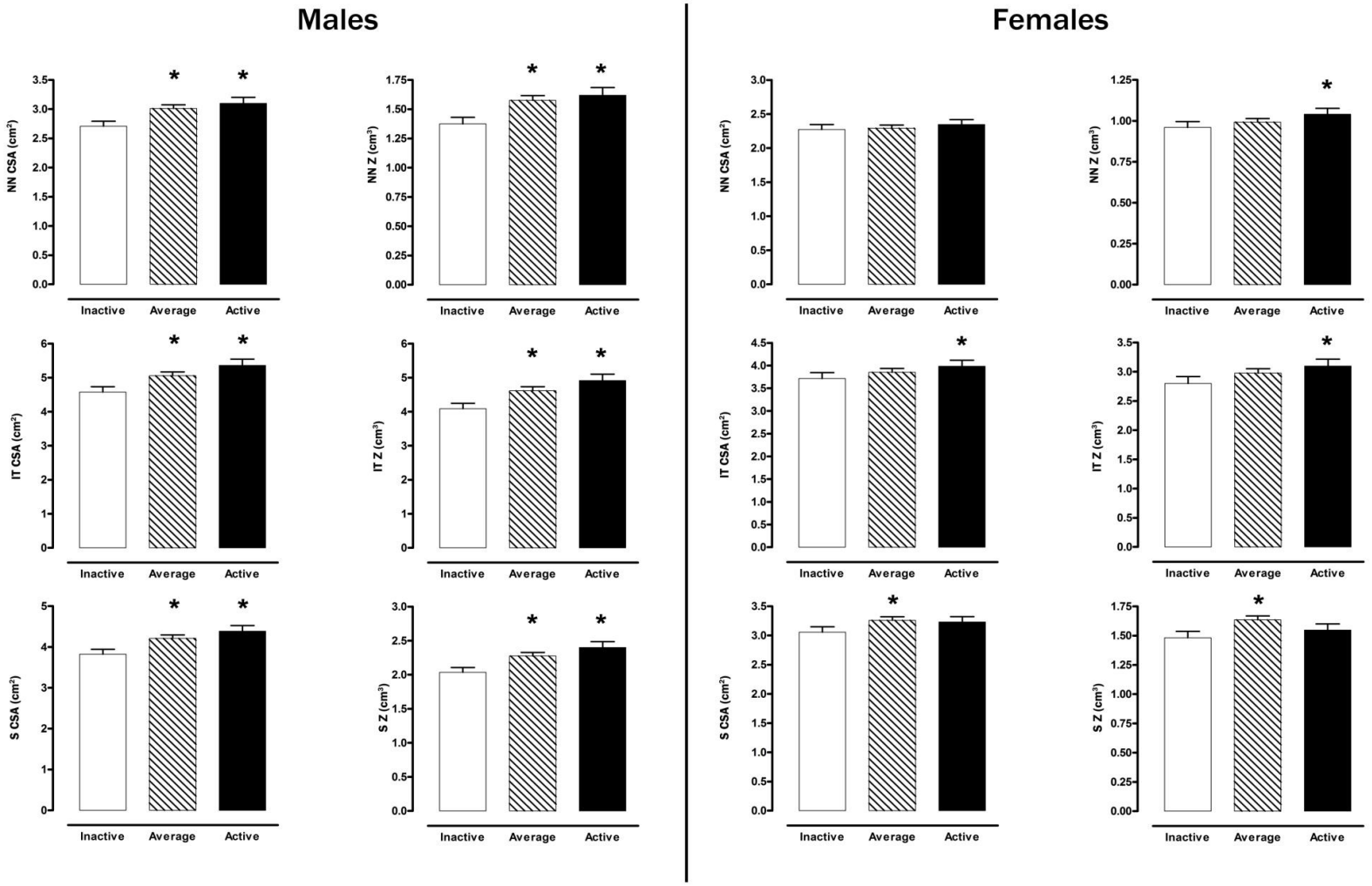

Figure 2.3: Model 1 adjusted adult geometric bone measures for males and females at the narrow neck (NN), intertrochanter (IT) and femoral shaft (S) site of the proximal femur grouped by adolescent physical activity. Means adjusted for height, weight and adolescent bone geometry. Adjusted means ± standard error.
CSA = Cross sectional area; Z = Section modulus
* indicates a significant difference from the inactive physical activity group ($p<0.05$)

2.3.4.4 Adult Model 2 (adjustments for height, weight, geometric measure at PHV, total body lean tissue mass percentage)

Figure 2.4 displays the results for adult adjusted CSA and Z at the NN, IT and S sites for males and females, when adjusted for Model 2 covariates. Similar to model 1, inactive males had significantly lower adult adjusted CSA and Z at the NN and IT sites, but only significantly lower Z at the S site of the proximal femur than males classified as active in adolescence (p<0.05, Figure 2.4); means adjusted for adult ht, wt, bone geometry at PHV and LTM %. No significant difference were observed in adult adjusted bone measure between males classified as inactive and average in adolescence once model 2 covariates were accounted. For all adult geometric bone measures in males, wt, adolescent bone geometry and LTM % significantly contributed to the models, with the Model 2 explaining 63-73%% of the variance in adult bone geometric measures (Table 2.9).

In females, individuals who were classified as active during adolescence had significantly higher adjusted adult ITCSA, but significantly lower S Z than females classified as average in adolescence (p<0.05, Figure 2); means adjusted for adult ht, wt, bone geometry at PHV and LTM %. Model 2 covariates accounted for 51-75% of the variance in adult bone geometric measures in females, with adolescent bone geometry being a significant contributor at all sites (p<0.05; Table 2.10). LTM % was also a significant model contributor for CSA and Z at the IT and S sites, while wt was significant for NNCSA, ITZ, S CSA and S Z (p<0.05; Table 2.10). Height remained a significant predictor only for NNCSA and S Z. (p<0.05; Table 2.10).

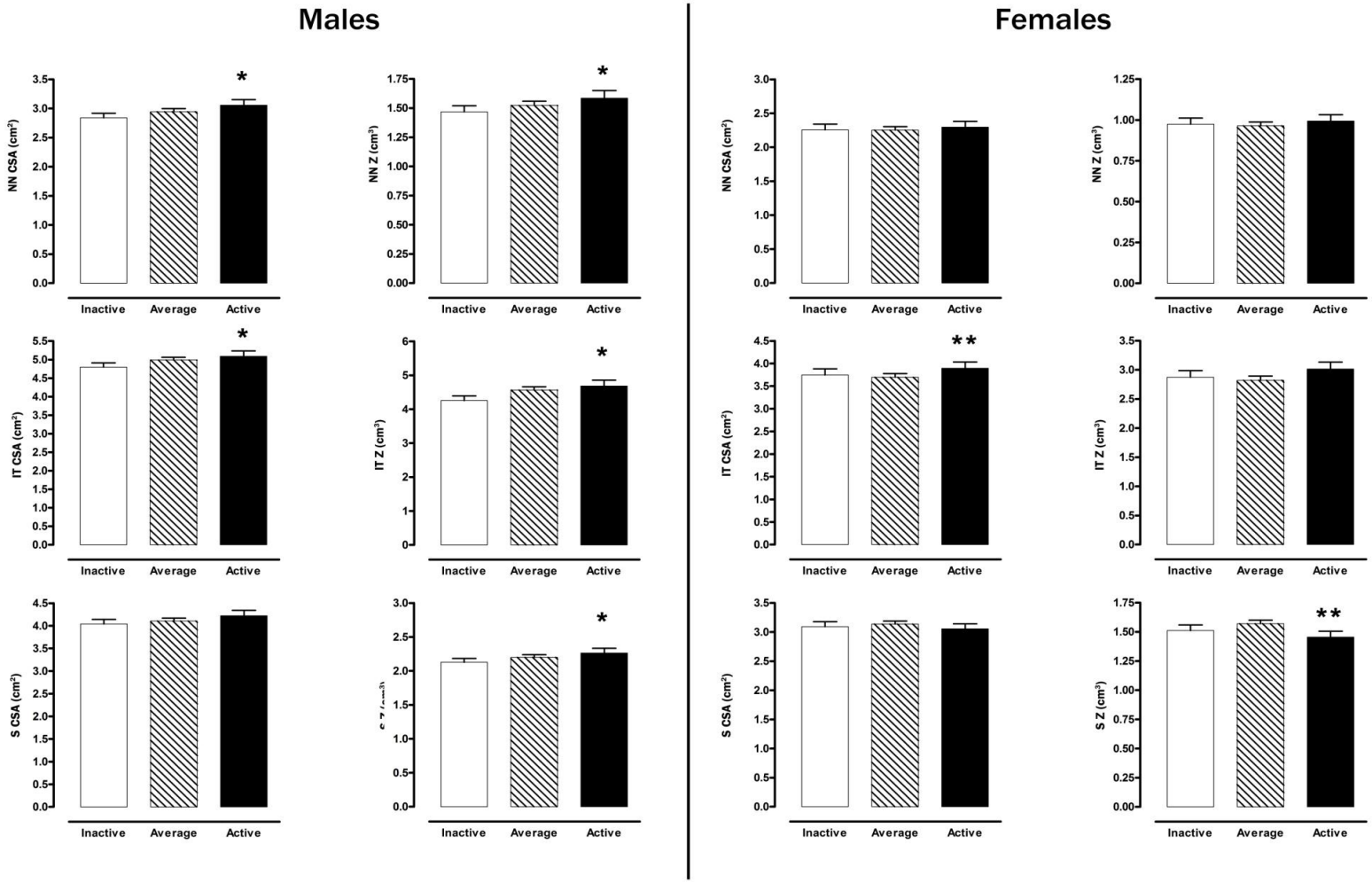

Figure 2.4: Model 2 adjusted adult geometric bone measures for males and females at the narrow neck (NN), intertrochanter (IT) and femoral shaft (S) site of the proximal femur grouped by adolescent physical activity. Means adjusted for weight, adolescent bone geometry, and total body lean tissue mass percentage. Adjusted means ± standard error. CSA = Cross sectional area; Z = Section modulus
* indicates a significant difference from the inactive physical activity group (p<0.05)
** indicates a significant differences from the average physical activity group (p<0.05)

2.3.4.5 Adult Model 3 (adjustments for height, weight, geometric measure at PHV, total body lean tissue mass percentage and adult physical activity)

No differences were found in adjusted CSA and Z at the NN, IT and S sites between adult males grouped by their adolescent physical activity once adult PA was included into the model (p>0.05, Figure 2.5); means adjusted for adult ht, wt, bone geometry at PHV, LTM % and adult PA. Model 3 covariates accounted for 60-78% of the variance in adult male bone geometric measures, with wt, adolescent geometry, LTM % and adult PA all observed to be significant predictors for all adult geometric measures (p<0.05; Table 2.9).

Similarly, during adulthood, no differences were observed in adjusted CSA and Z at any site of the proximal femur once adult PA was included between female adolescent physical activity groups (p>0.05, Figure 2.5); means adjusted for adult ht, wt, bone geometry at PHV, LTM % and adult PA. In females, the model 3 covariates accounted for 60-78% of the variance in adult bone geometric measures (Table 2.10). Adult PA was a significant predictor for adult CSA and Z in females at all sites except NNZ and IT CSA (Table 2.10).

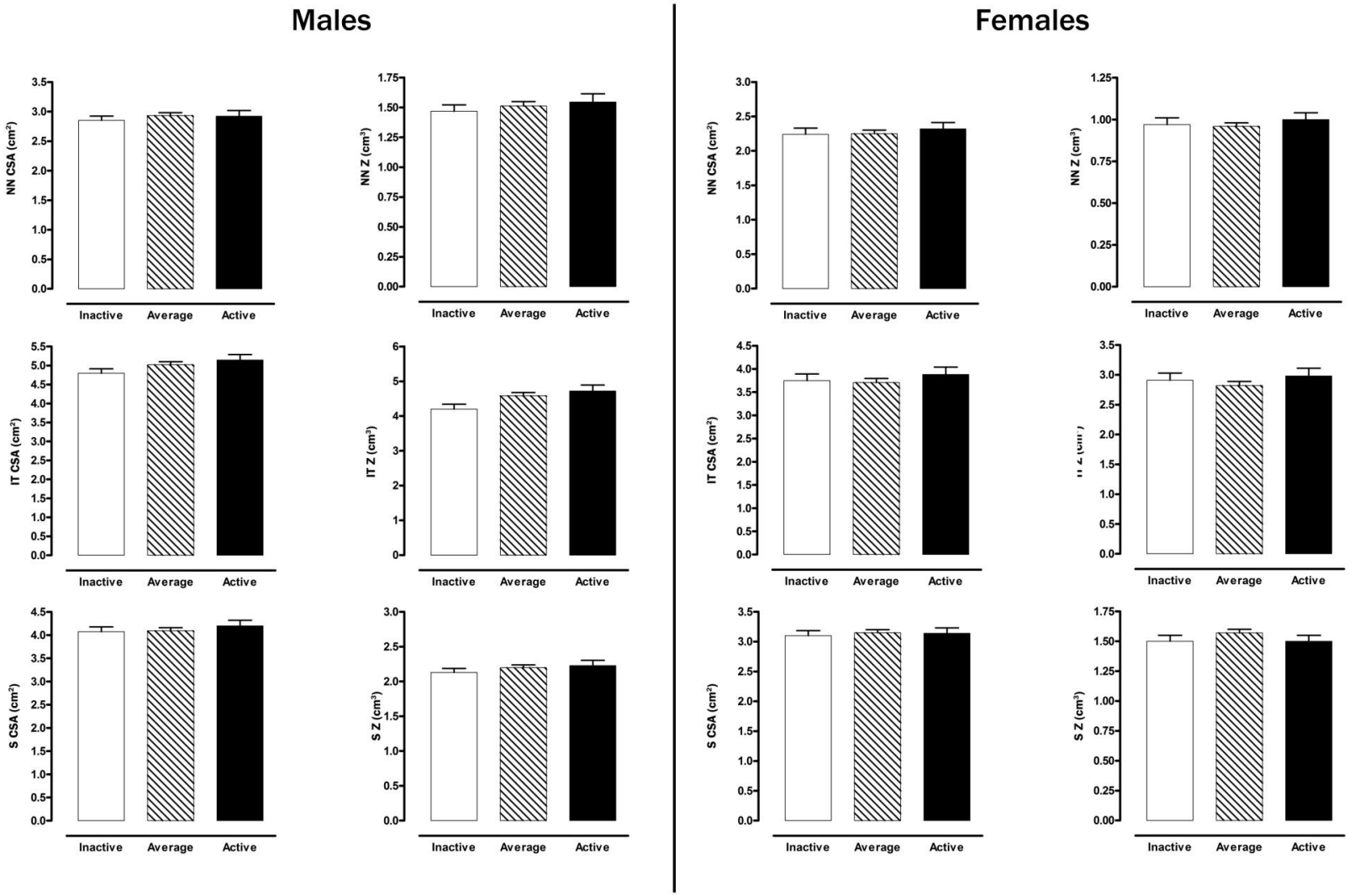

Figure 2.5: Model 3 adjusted adult geometric bone measures for males and females at the narrow neck (NN), intertrochanter (IT) and femoral shaft (S) site of the proximal femur grouped by adolescent physical activity. Means adjusted for weight, adolescent bone geometry, total body lean tissue mass percentage and adult physical activity. Adjusted means ± standard error.
CSA = Cross sectional area; Z = Section modulus

2.3.5 Discussion

The aim of the present study was to investigate whether the positive effects of adolescent physical activity on bone strength were associated with enhanced geometric bone strength measures in young adulthood. It was observed that physical activity during adolescence was positively related with estimated bone cross sectional area and section modulus at the proximal femur but that these benefits did not persist into early adulthood once current physical activity was accounted. This is the first study, to our knowledge, to assess the relationship between adolescent physical activity and adult geometric bone properties in a healthy non-athlete specific cohort at the clinically relevant proximal femur using a longitudinal dataset.

According to the mechanostat theory **[50, 52]**, physical activity provides novel dynamic loads that can elicit adaptations to bone mass, geometry and architecture. Supporting this supposition, investigations in adolescents and adult populations have documented the positive effects of physical activity on various parameters of bone strength **[48, 53, 55, 58, 60, 62, 65, 222, 223, 225, 226, 237, 238]**. In the present study, it was observed that at PHV active adolescents had 8-12% greater CSA and 12-14% greater Z than their inactive peers at the proximal femur. These adolescent findings parallel those previously reported for adolescent BMC and bone geometry in the PBMAS cohort **[57, 58]**, and further support the conjecture that childhood and adolescence physical activity is positively associated with adolescent CSA and Z at the proximal femur in both sexes.

Although the current adolescent findings reinforce the positive relationship between physical activity and adolescent bone strength, the purpose of this current study was to investigate whether these adolescent benefits were sustained into young adulthood, when the peak in proximal femur CSA and Z have been identified to occur **[234]**. When adult CSA and Z was adjusted for height, weight and adolescent bone geometry, males and females identified as active during adolescence continued to maintain a 7-14% benefit in CSA and 8-17% benefit in Z in adulthood compared to their inactive

adolescent counterparts. These findings would suggest that the skeletal benefits of adolescent physical activity on adolescent CSA and Z at the proximal femur are maintained into adulthood, supporting conclusions drawn from other estimates of bone strength **[57, 63, 67-70, 217, 226]**. This conclusion, however, ignores the potential role of muscular strength on bone structural strength. It has been documented that physical activity has a positive influence on lean tissue mass development **[56]**, a surrogate of muscular strength. And given that lean tissue mass also has positive effects on bone structural strength **[135, 185, 231-233]**, ignoring this connection may result in spurious conclusions. To address this concern, relative lean tissue mass was included as a covariate in Model 2, alongside height, weight and adolescent bone geometry. It was observed that when relative lean tissue mass was accounted, active males continued to maintain a 4-7% benefit in CSA and a 6-9% advantage in Z over their inactive peers. In contrast, any benefits previously observed for active females over their inactive counterparts were no longer apparent. These findings would suggest that adult CSA and Z in females is appropriately adapted to their current relative lean tissue mass, while in males adolescent activity may confer skeletal advantages to CSA and Z into adulthood.

PA is also documented to have, positive effects, which are independent of lean tissue mass, on bone geometry throughout life **[58, 155, 239, 240]**. When adult PA was included as an additional covariate in Model 3 (Table 2.9 and Table 2.10, Figure 2.5) any previously observed adolescent benefits were no longer evident in adult CSA and Z at any site of the proximal femur in either sex. These findings would suggest that current adult activity is integral to current adult CSA and Z more so than that of previous adolescence activity. These findings contrast those published in recent athlete models which have documented continued skeletal advantages from high levels of early life physical activity on adult bone mass, geometry and architecture **[63, 67-71, 155, 177, 230]**. The discrepancy between findings may result from differences in outcome measures as well as assessment sites. When examining the studies at the proximal femur, these investigations have concentrated primarily on bone mass measures,

documenting greater maintained total body and femoral neck BMC and aBMD in former elite gymnasts **[68, 69, 155]** as well as non-athletic active adolescents **[57, 63]**. Although bone mass and bone geometry are strongly correlated **[19, 25, 110],** the alterations to geometric properties may not be contemporaneous to adaptations in bone mass observed at the proximal femur. Additionally, the type of activity may play an important role on the transfer of skeletal gains from adolescence to adulthood **[241]**. High impact and odd impact loading activities (i.e. gymnastics, soccer, hockey) are associated with higher aBMD and enhanced bone geometry at regions specific to the loading pattern, while low impact/non-impact activities (i.e. swimming, cycling) are associated with greater areal BMD but reduced hip geometric measures **[241]**. Literature documenting the skeletal advantages to geometric bone properties with increased physical activity has focused on athletes involved in intense high and odd impact weight bearing activities. These high impact weight bearing activities are ideal for bone adaptation because they produce novel and dynamic strains on the bony tissue **[50, 52]**; however, engaging in higher levels of habitual physical activity, does not necessarily equate to engaging in activities that are also osteogenic. Since the PBMAS cohort consists of healthy, mostly non-athlete specific males and females, and the PAQ only provides information on habitual physical activity levels, being classified as active merely identifies an individual as participating in activities resulting in greater energy expenditure, but it does not guarantee that these activities are stimuli for geometric bone adaptations. Thus, the type of activity exposed to during adolescence, rather than the level of activity, may better reflect whether adolescence skeletal advantages are maintained into adulthood. Recently, Breban and colleges **[155]** investigated the relationship between high-intensity long-term weight bearing exercise on hip geometric bone measures, observing that individuals involved in high intensity type of exercise prior to puberty and practiced for at least 10 years maintained greater CSA and Z at the proximal femur in young adulthood; however, alongside these higher adult geometric bone measures, Breban et al **[155]** also reported that these former adolescent athletes retained higher levels of general

physical activity in adulthood. Thus, the observed benefits to adult CSA and Z could be attributed to current activity levels as well as those sustained from early adolescent activity **[155]**. Therefore, it remains unsubstantiated whether it is adolescent activity and/or current adult activity that is/are influencing adult geometric bone properties. According to the present finding, current adult activity levels is a significant predictor of estimated CSA and Z at the proximal femur; however, once adult PA was controlled, along with height and body composition, the previously reported group differences in CSA and Z in adulthood were no longer apparent. Given that there is low to moderate tracking between childhood, adolescence and adult physical activity levels **[242-244]**, the geometric bone adaptations may be better reflected by current activity levels rather than physical activity history. Future research that discriminates between the type, amount, and maintenance of activity are necessary to identify their independent roles on the development and transfer of geometric bone properties from adolescence to adulthood.

Despite the unique longitudinal data, in both males and females, the prospectively determined physical activity levels, and careful control of potential confounding variables, the conclusions of the present study are limited by several factors. First, this is an observational study, susceptible to observational associations that may be related to uncontrolled factors such as selection bias and reverse causality. Also, since the PBMAS is drawn from a small cohort of regionally selected Caucasian adolescents, the present observations and conclusions may have limited application to other cohorts. Further longitudinal research in other populations is required to supplement these observations. Next, physical activity was assessed using a subjective questionnaire. Although the PAQ is a reliable and valid method for assessing physical activity in children, adolescents and adults **[214, 215, 236]**, it provides little information on discriminating the nature of the activity. Studies utilizing objective measures of physical activity, with greater sensitivity to categorizing activity type would supplement the present study findings. Finally, the geometric bone measures were derived using HSA. While HSA geometric measures

have been validated against other three dimensional assessment techniques **[125, 245]**, the HSA geometric measures are derived using noisy two-dimensional DXA images which may hinder the detection of precise edge margins **[110, 130].** In addition, the position of the femur is important as small changes in femur rotation have a large effect on the geometric dimensions **[110, 130]**. All DXA scans were performed by qualified radiologists familiar with proper positioning of the proximal femur to ensure hip scans were performed with care to limit these potential errors; nevertheless, it is difficult to position the hip consistently in repeated measures over time. Regardless of the HSA's inherent limitations, it remains one of the few modalities that are safe, easy and cost effective in assessing the clinically relevant region of the proximal femur. Despite these limitations, the current study provides novel information surrounding the relationship between adolescent physical activity and adult bone geometric properties at the proximal femur in males and females.

In conclusion, there is increasing evidence to suggest that increased activity around the attainment of PHV has positive effects on bone structure and strength; however whether these adolescent benefits transfer to long term skeletal health remains unclear. Although the results of the present study are not definitive, the results do suggest that early life physical activity confers skeletal advantages to adolescent bone geometry at the proximal femur in both males and female, but whether these gains are maintained into adulthood may be dependent on multiple variables that include, but are not limited to, the type of activity engaged in as well as a continued physically active lifestyle.

Chapter Three

Discussion and Conclusions

3.1 Discussion

The overall aim of this dissertation was to investigate the effects of growth, pubertal timing and early life physical activity on adult bone strength measures at the clinically relevant fracture site of the proximal femur. To achieve this goal three studies were conducted. The purpose of the first study was to examine the longitudinal development of bone structural strength measures at the proximal femur from childhood, through adolescence and into early adulthood, with the aim to identify whether a peak/plateau in bone geometry occurs and to examine the contribution of the adolescent period towards the development of adult bone structural strength. In *Study 1*, it was observed there was a temporal pattern of bone structural strength development that was similar in both sexes, where the peak in aBMD occurred significantly earlier than geometric CSA at all site of the proximal femur. It was also observed that age at which aBMD, CSA and Z may peak and/or begin to plateau coincides with previous cross sectional literature **[27, 31-33, 186]**, suggesting that peak bone structural strength at the proximal femur may occur between 20-30 years of age. These observations also suggest that pursuing interventions prior to this period may be most advantageous for developing mechanically competent bone strength in adulthood. In addition, *Study 1* assessed the percent of adult peak achieved at PHV for CSA and Z at the three sites of the proximal femur. Males and females attained approximately 60-70% of their adult peak in CSA and 55-68% of their adult peak in Z by the age of PHV, with females achieving 4-7% more of their adult peak in Z than their male counterparts. These findings emphasize that the adolescent period is critical to the development of CSA and Z at the proximal femur, which parallels conclusions derived from bone mass measures **[54, 80, 202]**. Given that the period around PHV is hypothesized as an optimal time for bone strength development, observations from *Study 1* further support this supposition and provide further justification for the promotion of osteogenic activities as a potential avenue for developing bone structural strength.

With *Study 1* identifying the occurrence of peak CSA and Z values at the proximal femur at 20-30 years of age in both males and females, the purpose of *Study 2* was to examine the influence of pubertal timing on the development of these geometric bone strength peaks. When participants were grouped into early, average, and late maturers, derived from the age of PHV, and size, body composition, physical activity and nutritional calcium and vitamin D intake were accounted, it was observed that there was no significant difference in the development of CSA and Z between maturational group at any of the proximal femur sites in either male of female participants. These findings suggest that the onset of pubertal timing does not influence the development of CSA and Z at the proximal femur in both males and females. This conjecture is surprising given the disproportionate, though limited, literature which supports the deleterious effects of late pubertal timing on bone strength measures **[42-44]**. Estrogen exposure is proposed as the mechanism responsible for the deleterious bone structural strength differences previously cited between late and early maturers because of its documented effects on bone formation, bone apposition and modulating effects on mechanical loading sensitivity **[34, 36, 38, 39, 52, 91, 137, 140, 141, 146]**. Given that the majority of findings that suggest a deleterious effect of late pubertal development are observed in bone measures at the radius **[41, 43, 44]**, with only one at the hip **[42]**, the effects of estrogen may be mediated by habitual loading differences experienced at the radius and hip. Since bone becomes quickly desensitized to constant strains, the enhanced bone formation and modulating bone sensitivity associated with estrogen exposure may further stimulate bone formation at locations with habitual weight bearing loads, especially when LTM, a surrogate of muscle strength, and physical activity levels are also accounted. The effects of estrogen exposure may enhance osteogenic responses at non-weight bearing regions, where novel loads are less likely to be desensitized and heightened mechanical sensitivity may be beneficial. The mechanisms behind these observations are not well understood, and in fact may be due to effects completely unrelated to estrogen exposure all together.

Regardless of the underlying mechanisms, previous literature suggests that a late pubertal onset may be detrimental toward bone strength; however the observations from *Study 2* cannot support these sentiments. In fact, Study 2's findings suggest that the once growth, body composition, physical activity and nutrition are accounted, that a late maturational onset is not detrimental toward adult bone structural strength development at the proximal femur.

Building on the conclusions from Study 1, where the adolescent period was identified to be vital towards the development of adult bone structural strength, with 55-70% of adult CSA and Z developed by the time an individual achieves peak linear growth, the purpose of Study 3 was to determine the effects of physical activity during this critical period on the adult bone structural strength. Previous literature has documented that early life physical activity may provide sustained benefits to bone mass, bone architecture and geometric bone strength into adulthood **[57, 63, 68-71]**. These sustained benefits are believed to be the result of 'banked profits' from the adolescent period due to its osteogenically favorable environment which is unparalleled by any other period during the human life cycle; however according to the mechanostat theory bone adapts primarily to maintain strains within tolerable limits to prevent fracturing and alters bone strength according to habitual strain tolerances **[50-52, 146]**. Thus, the notion of bone 'banking' is not supported by the mechanostat theory as bone strength would be positively altered in environments of increased strain, but negatively altered if the strain stimulus was removed. As such, these sustained skeletal benefits in adulthood from early life physical activity may simply be the result of continued osteogenically favorable strain stimuli into adulthood. In Study 3, it was observed that when participants were separated into inactive, average, and active physical activity groupings during adolescence, participants in the active group has significantly greater adult CSA and Z compared to their inactive classified peers, when the model was adjusted for adult height, weight and adolescent bone geometry. In contrast, when the models also included body composition and current physical activity levels, any differences previously observed in adult CSA and Z between adolescent

physical activity groups were no longer apparent. This would suggest that adolescent physical activity may confer skeletal advantages into adulthood, but these benefits are appropriately adapted for the physiological strains imposed by current adult muscular strength, as estimated by LTM, and current physical activity levels. This would imply that adult bone structural strength is influenced by current physiological strains, rather than those previously experienced. Furthermore, it supports the postulates of the mechanostat theory that bone constantly adapts with dynamic efficiency to maintain bone strength within tolerable limits depending on the mechanical usage surrounding the bony tissues **[50-52, 91]**.

3.2 Conclusions

At the proximal femur, there appears to be a temporal pattern in structural strength development that is similar between sexes. These bone structural strength measures also appear to peak or begin to plateau around 20-30 years of age, with 55-70% of this accrued by the time of peak linear growth. Pubertal onset may not influence the development of these adult peaks in bone structural strength, but it may affect bone structural strength assessed during adolescence. Finally, adolescent physical activity may have positive effects on adolescent bone structural strength at the hip, but whether these skeletal benefits are maintained into adulthood may be dependent on current physical activity levels and muscular strength. Thus, the adolescent period may be vital to the growth and development of bone structural strength, but once there is a cessation in growth, bone structural strength may adapt appropriately to current loading levels and physiological strains.

3.3 Future Directions

This dissertation provides novel insight on the topics of growth, maturation and physical activity on the development of adult bone structural strength, but it also precipitates questions that may be the stimulus for future research. The studies included in this dissertation have focused strictly on bone

structural strength at the proximal femur. Given the unique loading environment the human proximal femur is exposed to as upright bipedal animals, it remains unsubstantiated whether the conclusions drawn from this research parallel adaptations occurring at other weight bearing sites (e.g. the tibia) or non weight bearing regions (e.g. radius). The timing of bone structural strength measures at regions such as the radius, ulna, tibia and fibula have yet to be researched and whether these sites undergo contemporaneous skeletal changes remains pure speculation. Furthermore, there remains a paucity of information around the influence of the timing of these events on adult bone structural strength and fracture incidence. These investigations may serve to provide valuable insight into bone health development and fracture risk assessment.

The imaging technology for assessing bone structural strength has also advanced rapidly and its incorporation into current research studies show promise for identifying novel indicators/measures of bone strength. How these new bone strength measures change over time, with growth and development, will require further longitudinal assessments. How these measures compare to the observations from the current studies and their contribution to our understanding of bone strength development and fracture risks remains largely unknown as well. In addition to the novel imaging technologies, highly sensitive objective measures of physical activity are also becoming more readily available. These devices can help better differentiate the types of physical activity, identifying the influence of type of load, load frequency, load intensity, and load duration on the development of bone structural strength. Studies incorporating these new technologies may also provide valuable insight on the long term effects of different types of physical activity on bone structural strength throughout life and help further expand upon the conclusions from this dissertation.

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Appendix A

Copy of Ethics Approval

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB)

Certificate of Approval
Study Amendment

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio #
Adam Baxter-Jones	Kinesiology	88-102

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
College of Kinesiology
87 Campus Drive
Saskatoon SK S7N 5B2

SPONSORING AGENCIES
CANADIAN INSTITUTES OF HEALTH RESEARCH (CIHR)

TITLE
Protocol NHRDP #6608-126-OS:: Relationship of Growth and Lifestyle to Peak Bone Mass

APPROVAL OF	APPROVED ON	CURRENT EXPIRY DATE
PBMAS DXA Upgrade Consent Form (rec'd 25-Nov-2010)	29-Nov-2010	20-Oct-2011

Delegated Review: ☒ Full Board Meeting: ☐

CERTIFICATION
The study is acceptable on scientific and ethical grounds. The Bio-REB considered the requirements of section 29 under the Health Information Protection Act (HIPA) and is satisfied that this study meets the privacy considerations outlined therein. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research study, and for ensuring that the authorized research is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved protocol or consent process.

FIRST TIME REVIEW AND CONTINUING APPROVAL
The University of Saskatchewan Biomedical Research Ethics Board reviews above minimal studies at a full-board (face-to-face) meeting. Any research classified as minimal risk is reviewed through the delegated (subcommittee) review process. The initial Certificate of Approval includes the approval period the REB has assigned to a study. The Status Report form must be submitted within one month prior to the assigned expiry date. The researcher shall indicate to the REB any specific requirements of the sponsoring organizations (e.g. requirement for full-board review and approval) for the continuing review process deemed necessary for that project. For more information visit http://www.usask.ca/research/ethics_review/.

REB ATTESTATION
In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

Michel Desautels, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board

Appendix B

Calculating Peak Height Velocity: A Working Example

The following is a working example illustrating how the age of peak height velocity is ascertained using serial height measurements during childhood and adolescence. Table B.1. provides a summary of the measurements and calculations that are necessary to determine the age of peak height velocity (APHV) and will be used to supplement the following procedural descriptions.

Table B.1. A working example of the measurements and calculations necessary for ascertaining the peak height velocity.

	A	B	C	D	E	F
	Age at Test	Height	Age Increment	Age Center	Simple Height Increment	Whole Year Velocity
1	8.209	132.8				
2	9.064	137.5	A2 - A1 = 0.855	(A2 + A1)/2 =8.6365	B2 - B1 = 4.7	E2/ C2 = 5.497
3	10.066	142.2	A3-A2 = 1.002	(A3 + A2)/2 = 9.565	B3 - B2 = 4.7	E3/C3 = 4.691
4	11.043	149.9	0.977	10.555	7.7	7.881
5	12.011	154.1	0.968	11.527	4.2	4.339
6	13.004	159.8	0.993	12.508	5.7	5.740
7	14.019	163	1.015	13.512	3.2	3.153

First, in order to determine age of peak height velocity, it is necessary to have serial measurements of height and the age when the measurement was assessed. Column A in Table B.1. displays the age at test of a female participant, while column B is her height measurement ascertained at that measurement occasion. Next, it is necessary to calculate the time that has elapsed between measurement occasions (age increment, column C). This is calculated by subtracting the age at test of the previous measurement occasion from the age of test at the present measurement occasion. Next, the midpoint age between the previous and present measurement occasions must be calculated. This calculation is known as the age center and is computed as the summation of the age of test from the previous and present measurement occasions and then dividing by two (column D). The gain in height between the two testing measurement occasions is calculated next and recorded as the simple height increment (Column E). Finally, the whole year velocity is calculated by adjusting the simple height increment by the time

elapsed between measurement occasions (Column F). With the whole year velocity values calculated, the APHV can be determined as the age center when the highest value for whole year velocity occurred. In the working example, the highest whole year velocity value was 7.881 cm/year (Column F), which corresponds with age center 10.555 years (Column D). Thus, this female's APHV is 10.56 years.

Given that the peak whole year velocity occurred somewhere between two measurement occasions (between 10.066 and 11.043 in the female participant's example data), a truer APHV is adjusted for this fact. This is done through proportional allotment. Equation B.1. summarizes the variables necessary to use proportional allotment for determination of APHV and Eq. B.2. provides a sample calculation for the working example. Thus, when the APHV in the example is adjusted using proportional allotment the female is estimated to reach PHV at 10.53 years of age.

(Eq B.1.) $APHV = A + [\frac{VA - VA_{-1}}{(VA - VA_{-1}) + (VA - VA_{+1})}] - 0.5$, where

A = age centered at peak whole year velocity
VA = whole year velocity value at peak
VA_{-1} = whole year velocity value before peak
VA_{+1}= whole year velocity value after peak

(Eq B.2.) $APHV = A + [\frac{VA - VA_{-1}}{(VA - VA_{-1}) + (VA - VA_{+1})}] - 0.5$

$$APHV = 10.555 + \frac{7.881 - 4.691}{(7.881 - 4.691) + (7.881 - 4.339)} - 0.5$$

$$APHV = 10.555 + \frac{3.19}{6.732} - 0.5$$

$$APHV = 10.555 + 0.4739 - 0.5$$

$$APHV = 10.53$$

Appendix C

The Timing of BMD and Geometric Adaptation at the Proximal Femur From Childhood to Early Adulthood in Males and Females: A Longitudinal Study. As published in the Journal of Bone and Mineral Research.

ORIGINAL ARTICLE

JBMR

The Timing of BMD and Geometric Adaptation at the Proximal Femur From Childhood to Early Adulthood in Males and Females: A Longitudinal Study

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ABSTRACT

During adolescence, the peak velocity in bone mass accretion preceded the peak velocity of estimated geometry at the hip. Whether this pattern continues into adulthood when maximum values are achieved remains unknown. The purpose of this study was (1) to identify the ages at which peak values of areal BMD (aBMD), cross-sectional area (CSA), and section modulus (Z) occur, (2) to determine the percent of adult peak attained during adolescence, and (3) to determine the relationship between body composition and the timing of the adult peak values.

One-hundred and sixty-five (92 females) individuals' aBMD, CSA, and Z values were assessed serially at the narrow neck (NN), intertrochanter (IT), and shaft (S) using hip structural analysis (HSA). Peak bone values and the ages of attainment were assessed using factorial MANOVA.

In males, aBMDp (NN 19.4 ± 2.7 years, IT 20 ± 3.4 years, and S 21.8 ± 2.8 years) occurred significantly earlier than CSAp at all sites (NN 21.6 ± 3.2 years, IT 21.1 ± 3.4 years, and S 22.3 ± 3.1 years) and earlier than Zp at the NN (22 ± 3.2 years) and IT (21.3 ± 2.9 years). In females, aBMDp (NN 17.9 ± 2.7 years, IT 18.7 ± 3.5 years, and S 19.7 ± 3.3 years) occurred significantly earlier than CSAp at all sites (NN 20.6 ± 3.6 years, IT 19.4 ± 3.9 years, and S 21.0 ± 3.3 years) and earlier than Zp at the NN (20.7 ± 3.4 years) and S (20.6 ± 3.5 years). The changes in bone mass precede changes in geometric CSA, and this timing may be integral for the development and maintenance of bone strength. © 2011 American Society for Bone and Mineral Research.

KEY WORDS: BONE GEOMETRY; PEAK HEIGHT VELOCITY; GROWTH CURVES; LONGITUDINAL; SEX DIFFERENCES

Introduction

Bone is constantly renovated through the dynamic process of bone formation and resorption, altering the structural integrity and strength of the bone within the limits of the applied loads. Disequilibrium between periosteal bone formation and endosteal bone resorption is proposed to be the underlying mechanism for osteoporosis.[1,2] Recently, it has been observed that the decrease in BMD in adulthood is compensated by adaptations in bone geometry in both men and women to maintain structural integrity and strength.[3–5] Additionally, it has been noted that the rate of endosteal resorption is higher in women, suggesting a sex differences in the geometric adaptation with aging.[6] Zhang and colleagues[7] recently observed in a cross-sectional study that regardless of sex and ethnicity, there is a natural age-related decline in cross-sectional area (CSA) and cortical thickness at the proximal femur beginning around 20 years of age. This suggests that geometric properties may peak near the second decade of life. Similarly, Beck and colleagues,[8] using hip structural analysis (HSA), observed an age-related decline in BMD at the proximal femur in a group of 20- to 80-year-old subjects, with a reduced age-related loss in section modulus (Z) owing to a linear compensation in subperiosteal expansion. This highlights the idea that although bone mass is lost, mechanical strength may be maintained through geometric adaptation. However, these suppositions are derived from cross-sectional data that may be biased and lead to underestimation of the subsequent age-related geometric adaptations.[9] Similarly, the majority of this literature has focused on adult populations, resulting in a paucity of information surrounding the adaptation of bone geometry during childhood into early adulthood. Although hip bone

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Appendix D

Multilevel Models Output for Study Two.

MLwiN Models for HSA variables between male maturation groups
Controlling for age center, height , lean tissue mass, fat mass, calcium, vitamin D, physical activity and interactions.

Males

NN Site in Males

$$\text{NN CSA}_{ij} \sim N(XB,\ \Omega)$$

$$\begin{aligned}\text{NN CSA}_{ij} = {} & \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.0016241(0.0006210)agec2}_{ij} + \text{-0.0004104(0.0000754)agec3}_{ij} + \text{-0.0034602(0.0021251)HT.FNL}_{ij} + \\ & \text{0.0000396(0.0000023)XB.LEAN.TOT}_{ij} + \text{0.0000009(0.0000014)FAT.TOT}_{ij} + \text{0.0000015(0.0000111)CALCIUM.MG}_{ij} + \\ & \text{0.0000548(0.0000322)VIT.D.IU}_{ij} + \text{0.0087592(0.0067977)Act.Cat}_{ij} + \text{-0.0070444(0.0538622)earlyDum}_{j} + \\ & \text{-0.0099054(0.0740997)lateDum}_{j} + \text{-0.0072871(0.0071664)age*early}_{ij} + \text{-0.0020488(0.0101253)age*late}_{ij}\end{aligned}$$

$$\beta_{0ij} = 1.0286533(0.2915142) + u_{0j} + e_{0ij}$$

$$\beta_{1j} = 0.0362723(0.0070972) + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim N(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} 0.0485081(0.0070849) & \\ 0.0047421(0.0008458) & 0.0006680(0.0001248) \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim N(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} 0.0084891(0.0005632) \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -813.6575409(649 of 812 cases in use)

$$\text{NN Sect Mod}_{ij} \sim N(XB,\ \Omega)$$

$$\begin{aligned}\text{NN Sect Mod}_{ij} = {} & \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.0000012(0.0003845)agec2}_{ij} + \text{-0.0002977(0.0000477)agec3}_{ij} + \text{-0.0002733(0.0012927)HT.FNL}_{ij} + \\ & \text{0.0000245(0.0000014)XB.LEAN.TOT}_{ij} + \text{0.0000017(0.0000009)FAT.TOT}_{ij} + \text{0.0000092(0.0000070)CALCIUM.MG}_{ij} + \\ & \text{0.0000226(0.0000204)VIT.D.IU}_{ij} + \text{0.0007534(0.0043292)Act.Cat}_{ij} + \text{-0.0177765(0.0304895)earlyDum}_{j} + \\ & \text{-0.0216304(0.0419687)lateDum}_{j} + \text{-0.0039723(0.0045138)age*early}_{ij} + \text{-0.0021427(0.0063989)age*late}_{ij}\end{aligned}$$

$$\beta_{0ij} = -0.0390774(0.1767501) + u_{0j} + e_{0ij}$$

$$\beta_{1j} = 0.0221780(0.0044323) + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim N(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} 0.0152022(0.0022666) & \\ 0.0016501(0.0003004) & 0.0002631(0.0000496) \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim N(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} 0.0034520(0.0002289) \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -1424.6607930(649 of 812 cases in use)

IT Site Males

$$\text{IT CSA}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\begin{aligned}\text{IT CSA}_{ij} = {} & \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.0004595(0.0012009)agec2}_{ij} + \text{0.0000212(0.0001429)agec3}_{ij} + \text{0.0233395(0.0041621)HT.FNL}_{ij} + \\ & \text{0.0000516(0.0000044)XB.LEAN.TOT}_{ij} + \text{-0.0000057(0.0000028)FAT.TOT}_{ij} + \text{-0.0000096(0.0000210)CALCIUM.MG}_{ij} + \\ & \text{0.0000455(0.0000609)VIT.D.IU}_{ij} + \text{0.0420283(0.0129154)Act.Cat}_{ij} + \text{-0.0780295(0.1162495)earlyDum}_{j} + \\ & \text{0.2403655(0.1597997)lateDum}_{j} + \text{-0.0118538(0.0132053)age*early}_{ij} + \text{0.0148573(0.0187091)age*late}_{ij}\end{aligned}$$

$$\beta_{0ij} = \text{-2.3847167(0.5745642)} + u_{0j} + e_{0ij}$$

$$\beta_{1j} = \text{0.0119347(0.0134723)} + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0.2300766(0.0330045)} & \\ \text{0.0192527(0.0033574)} & \text{0.0021694(0.0004160)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} \text{0.0302642(0.0020322)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = 31.9722902(635 of 812 cases in use)

$$\text{IT Sect Mod}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\begin{aligned}\text{IT Sect Mod}_{ij} = {} & \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{0.0019360(0.0013031)agec2}_{ij} + \text{-0.0001096(0.0001692)agec3}_{ij} + \text{0.0252366(0.0042980)HT.FNL}_{ij} + \\ & \text{0.0000655(0.0000048)XB.LEAN.TOT}_{ij} + \text{-0.0000056(0.0000030)FAT.TOT}_{ij} + \text{-0.0000046(0.0000259)CALCIUM.MG}_{ij} + \\ & \text{0.0000507(0.0000725)VIT.D.IU}_{ij} + \text{0.0499707(0.0164090)Act.Cat}_{ij} + \text{-0.0765183(0.0984576)earlyDum}_{j} + \\ & \text{0.2140150(0.1356183)lateDum}_{j} + \text{-0.0054763(0.0136714)age*early}_{ij} + \text{0.0192312(0.0195472)age*late}_{ij}\end{aligned}$$

$$\beta_{0ij} = \text{-4.0986209(0.5884912)} + u_{0j} + e_{0ij}$$

$$\beta_{1j} = \text{0.0020489(0.0151111)} + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0.1551833(0.0235438)} & \\ \text{0.0159698(0.0029267)} & \text{0.0020000(0.0004414)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} \text{0.0504895(0.0033614)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = 226.1107036(635 of 812 cases in use)

Femoral Shaft Site in Males

$$\text{S CSA}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\begin{aligned}\text{S CSA}_{ij} = {} & \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.0023032(0.0008764)agec2}_{ij} + \text{-0.0004463(0.0001060)agec3}_{ij} + \text{-0.0045239(0.0030466)HT.FNL}_{ij} + \\ & \text{0.0000561(0.0000032)XB.LEAN.TOT}_{ij} + \text{-0.0000007(0.0000021)FAT.TOT}_{ij} + \text{-0.0000090(0.0000158)CALCIUM.MG}_{ij} + \\ & \text{0.0000206(0.0000463)VIT.D.IU}_{ij} + \text{0.0229543(0.0096585)Act.Cat}_{ij} + \text{0.0355471(0.0701360)earlyDum}_{j} + \\ & \text{0.0927705(0.0966615)lateDum}_{j} + \text{-0.0089825(0.0086335)age*early}_{ij} + \text{0.0123638(0.0122737)age*late}_{ij}\end{aligned}$$

$$\beta_{0ij} = \text{1.2534263(0.4197795)} + u_{0j} + e_{0ij}$$

$$\beta_{1j} = \text{0.0788301(0.0098169)} + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0.0818607(0.0120623)} & \\ \text{0.0063369(0.0012779)} & \text{0.0008787(0.0001794)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} \text{0.0146160(0.0010677)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -361.4158746(567 of 812 cases in use)

$$\text{S Sect Mod}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\begin{aligned}\text{S Sect Mod}_{ij} = {} & \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.0017105(0.0005938)agec2}_{ij} + \text{-0.0004342(0.0000750)agec3}_{ij} + \text{-0.0001496(0.0020032)HT.FNL}_{ij} + \\ & \text{0.0000351(0.0000022)XB.LEAN.TOT}_{ij} + \text{-0.0000005(0.0000014)FAT.TOT}_{ij} + \text{-0.0000017(0.0000113)CALCIUM.MG}_{ij} + \\ & \text{0.0000140(0.0000328)VIT.D.IU}_{ij} + \text{0.0022246(0.0069697)Act.Cat}_{ij} + \text{0.0146163(0.0423916)earlyDum}_{j} + \\ & \text{0.0281054(0.0584969)lateDum}_{j} + \text{-0.0044416(0.0060385)age*early}_{ij} + \text{0.0073978(0.0086114)age*late}_{ij}\end{aligned}$$

$$\beta_{0ij} = 0.0023330(0.2746035) + u_{0j} + e_{0ij}$$

$$\beta_{1j} = 0.0459290(0.0067972) + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} 0.0288713(0.0044057) & \\ 0.0027054(0.0005452) & 0.0004231(0.0000884) \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} 0.0077045(0.0005605) \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -776.6776192(567 of 812 cases in use)

Models with Non-Significant from previous models (above) removed

Narrow Neck Sites

$$\text{NN CSA}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\text{NN CSA}_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.0002566(0.0003676)agec2}_{ij} + \text{0.0000383(0.0000012)XB.LEAN.TOT}_{ij} + \text{-0.0002052(0.0000409)agec3}_{ij}$$

$$\beta_{0ij} = 0.5329100(0.0640169) + u_{0j} + e_{0ij}$$

$$\beta_{1j} = 0.0220214(0.0051846) + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} 0.0487923(0.0070950) & \\ 0.0044649(0.0007938) & 0.0006366(0.0001117) \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} 0.0103911(0.0005966) \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -900.0118471(800 of 812 cases in use)

$$\text{NN Sect Mod}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\text{NN Sect Mod}_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{0.0003238(0.0002301)agec2}_{ij} + \text{0.0000252(0.0000007)XB.LEAN.TOT}_{ij} + \text{-0.0001584(0.0000255)agec3}_{ij}$$

$$\beta_{0ij} = \text{-0.0896376(0.0391114)} + u_{0j} + e_{0ij}$$

$$\beta_{1j} = 0.0150929(0.0032604) + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} 0.0150625(0.0022212) & \\ 0.0015120(0.0002811) & 0.0002679(0.0000465) \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} 0.0039665(0.0002285) \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -1673.7937828(800 of 812 cases in use)

IT Sites Males

$$\text{IT CSA}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\text{IT CSA}_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.0000183(0.0011332)agec2}_{ij} + \text{0.0249115(0.0040224)HT.FNL}_{ij} + \text{0.0000511(0.0000043)XB.LEAN.TOT}_{ij} + \text{0.0391946(0.0127780)Act.Cat}_{ij} + \text{0.0000023(0.0001407)agec3}_{ij}$$

$$\beta_{0ij} = \text{-2.6896241(0.5380601)} + u_{0j} + e_{0ij}$$

$$\beta_{1j} = \text{0.0026417(0.0113811)} + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0.2382252(0.0340855)} & \\ \text{0.0194627(0.0033697)} & \text{0.0021342(0.0004054)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} \text{0.0303828(0.0020311)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = 37.7194449(639 of 812 cases in use)

$$\text{IT Sect Mod}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\text{IT Sect Mod}_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{0.0018495(0.0012350)agec2}_{ij} + \text{0.0258630(0.0041929)HT.FNL}_{ij} + \text{0.0000646(0.0000048)XB.LEAN.TOT}_{ij} + \text{0.0472481(0.0162936)Act.Cat}_{ij} + \text{-0.0001356(0.0001673)agec3}_{ij}$$

$$\beta_{0ij} = \text{-4.2077312(0.5565867)} + u_{0j} + e_{0ij}$$

$$\beta_{1j} = \text{-0.0001740(0.0131367)} + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0.1602074(0.0241986)} & \\ \text{0.0164164(0.0029709)} & \text{0.0020448(0.0004417)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} \text{0.0507569(0.0033677)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = 234.8867289(639 of 812 cases in use)

$S\ CSA_{ij} \sim N(XB,\ \Omega)$

$S\ CSA_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + -0.0013479(0.0006132)\text{agec2}_{ij} + 0.0000527(0.0000018)\text{XB.LEAN.TOT}_{ij} + -0.0004945(0.0000932)\text{agec3}_{ij} + 0.0201756(0.0094644)\text{Act.Cat}_{ij}$

$\beta_{0ij} = 0.6410914(0.1000775) + u_{0j} + e_{0ij}$

$\beta_{1j} = 0.0735886(0.0083525) + u_{1j}$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim N(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} 0.0828166(0.0121901) & \\ 0.0062708(0.0012736) & 0.0008909(0.0001786) \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim N(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} 0.0146665(0.0010642) \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -356.1884301(572 of 812 cases in use)

$S\ \text{Sect Mod}_{ij} \sim N(XB,\ \Omega)$

$S\ \text{Sect Mod}_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + -0.0016183(0.0003832)\text{agec2}_{ij} + 0.0000360(0.0000013)\text{XB.LEAN.TOT}_{ij} + -0.0002729(0.0000461)\text{agec3}_{ij}$

$\beta_{0ij} = -0.0686180(0.0654319) + u_{0j} + e_{0ij}$

$\beta_{1j} = 0.0344165(0.0051899) + u_{1j}$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim N(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} 0.0264113(0.0040471) & \\ 0.0021784(0.0004513) & 0.0003470(0.0000692) \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim N(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} 0.0110769(0.0006972) \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -781.4072499(694 of 812 cases in use)

MLwiN Models for HSA variables between female maturation groups
Controlling for age center, height, lean tissue mass, fat mass, calcium, vitamin D, physical activity and interactions.

Females

NN Site in females

$$\text{NN CSA}_{ij} \sim N(XB,\ \Omega)$$

$$\begin{aligned}\text{NN CSA}_{ij} = {} & \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.000873(0.000562)agec2}_{ij} + \text{-0.000448(0.000069)agec3}_{ij} + \text{0.004599(0.002151)HT.FNL}_{ij} + \\ & \text{0.000039(0.000003)XB.LEAN.TOT}_{ij} + \text{0.000007(0.000001)FAT.TOT}_{ij} + \text{-0.000019(0.000016)CALCIUM.MG}_{ij} + \\ & \text{0.000034(0.000041)VIT.D.IU}_{ij} + \text{0.007419(0.006217)Act.Cat}_{ij} + \text{0.042752(0.057648)earlyDum}_{j} + \\ & \text{-0.025113(0.052235)lateDum}_{j} + \text{-0.008645(0.006133)agec*late}_{ij} + \text{-0.006119(0.006819)agec*early}_{ij}\end{aligned}$$

$$\beta_{0ij} = \text{-0.272516(0.286541)} + u_{0j} + e_{0ij}$$

$$\beta_{1j} = \text{0.027406(0.004104)} + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim N(0,\ \Omega_u) \ :\ \Omega_u = \begin{bmatrix} \text{0.034707(0.004886)} & \\ \text{0.002284(0.000460)} & \text{0.000267(0.000059)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim N(0,\ \Omega_e) \ :\ \Omega_e = \begin{bmatrix} \text{0.007661(0.000492)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -975.753238(691 of 903 cases in use)

$$\text{NN Sect Mod}_{ij} \sim N(XB,\ \Omega)$$

$$\begin{aligned}\text{NN Sect Mod}_{ij} = {} & \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{0.000105(0.000282)agec2}_{ij} + \text{-0.000250(0.000036)agec3}_{ij} + \text{0.003450(0.001072)HT.FNL}_{ij} + \\ & \text{0.000019(0.000002)XB.LEAN.TOT}_{ij} + \text{0.000004(0.000001)FAT.TOT}_{ij} + \text{-0.000012(0.000009)CALCIUM.MG}_{ij} + \\ & \text{0.000018(0.000022)VIT.D.IU}_{ij} + \text{0.003738(0.003317)Act.Cat}_{ij} + \text{0.000029(0.028462)earlyDum}_{j} + \\ & \text{-0.003599(0.025679)lateDum}_{j} + \text{-0.002911(0.003366)agec*late}_{ij} + \text{-0.004330(0.003752)agec*early}_{ij}\end{aligned}$$

$$\beta_{0ij} = \text{-0.500870(0.141458)} + u_{0j} + e_{0ij}$$

$$\beta_{1j} = \text{0.015016(0.002174)} + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim N(0,\ \Omega_u) \ :\ \Omega_u = \begin{bmatrix} \text{0.008187(0.001173)} & \\ \text{0.000670(0.000127)} & \text{0.000085(0.000018)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim N(0,\ \Omega_e) \ :\ \Omega_e = \begin{bmatrix} \text{0.002199(0.000141)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -1868.213752(691 of 903 cases in use)

IT Site females

$$\text{IT CSA}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\text{IT CSA}_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + 0.000030(0.001004)\text{agec2}_{ij} + -0.000104(0.000117)\text{agec3}_{ij} + 0.026689(0.003905)\text{HT.FNL}_{ij} + 0.000059(0.000005)\text{XB.LEAN.TOT}_{ij} + 0.000001(0.000002)\text{FAT.TOT}_{ij} + -0.000025(0.000026)\text{CALCIUM.MG}_{ij} + 0.000064(0.000066)\text{VIT.D.IU}_{ij} + 0.009295(0.009879)\text{Act.Cat}_{ij} + 0.150149(0.124679)\text{earlyDum}_{j} + 0.019193(0.113811)\text{lateDum}_{j} + -0.019611(0.011025)\text{agec*late}_{ij} + 0.003997(0.012210)\text{agec*early}_{ij}$$

$$\beta_{0ij} = -3.112339(0.525599) + u_{0j} + e_{0ij}$$

$$\beta_{1j} = 0.002470(0.007127) + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} 0.172890(0.023514) & \\ 0.009569(0.001835) & 0.000982(0.000194) \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} 0.018771(0.001213) \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -247.791585(688 of 903 cases in use)

$$\text{IT Sect Mod}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\text{IT Sect Mod}_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + 0.002486(0.001013)\text{agec2}_{ij} + 0.000148(0.000122)\text{agec3}_{ij} + 0.033299(0.003874)\text{HT.FNL}_{ij} + 0.000063(0.000005)\text{XB.LEAN.TOT}_{ij} + -0.000004(0.000002)\text{FAT.TOT}_{ij} + -0.000010(0.000028)\text{CALCIUM.MG}_{ij} + 0.000076(0.000071)\text{VIT.D.IU}_{ij} + 0.006543(0.010869)\text{Act.Cat}_{ij} + 0.075455(0.106308)\text{earlyDum}_{j} + 0.081957(0.096629)\text{lateDum}_{j} + -0.011363(0.010053)\text{agec*late}_{ij} + 0.006634(0.011159)\text{agec*early}_{ij}$$

$$\beta_{0ij} = -5.143357(0.520034) + u_{0j} + e_{0ij}$$

$$\beta_{1j} = -0.032447(0.007081) + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} 0.120934(0.016787) & \\ 0.006399(0.001363) & 0.000651(0.000155) \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} 0.023299(0.001500) \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -192.973317(688 of 903 cases in use)

Femoral Shaft Site in females

$$\text{S CSA}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\text{S CSA}_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + -0.002546(0.000798)\text{agec2}_{ij} + -0.000333(0.000096)\text{agec3}_{ij} + 0.012820(0.003064)\text{HT.FNL}_{ij} + 0.000048(0.000004)\text{XB.LEAN.TOT}_{ij} + 0.000003(0.000002)\text{FAT.TOT}_{ij} + -0.000038(0.000022)\text{CALCIUM.MG}_{ij} + -0.000011(0.000055)\text{VIT.D.IU}_{ij} + -0.000281(0.008299)\text{Act.Cat}_{ij} + 0.104918(0.078163)\text{earlyDum}_{j} + -0.030481(0.070409)\text{lateDum}_{j} + -0.013918(0.008554)\text{agec*late}_{ij} + 0.002031(0.009631)\text{agec*early}_{ij}$$

$$\beta_{0ij} = -1.093069(0.410863) + u_{0j} + e_{0ij}$$

$$\beta_{1j} = 0.046676(0.005682) + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} 0.062762(0.008876) & \\ 0.003603(0.000836) & 0.000538(0.000115) \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} 0.012748(0.000847) \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -545.476909(661 of 903 cases in use)

$$\text{S Sect Mod}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\begin{aligned}\text{S Sect Mod}_{ij} = {} & \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.000809(0.000468)agec2}_{ij} + \text{-0.000209(0.000057)agec3}_{ij} + \text{0.010701(0.001793)HT.FNL}_{ij} + \\ & \text{0.000027(0.000002)XB.LEAN.TOT}_{ij} + \text{0.000002(0.000001)FAT.TOT}_{ij} + \text{-0.000025(0.000013)CALCIUM.MG}_{ij} + \\ & \text{-0.000005(0.000033)VIT.D.IU}_{ij} + \text{-0.003006(0.004965)Act.Cat}_{ij} + \text{0.063846(0.045170)earlyDum}_{j} + \\ & \text{-0.002190(0.040632)lateDum}_{j} + \text{-0.008629(0.005041)agec*late}_{ij} + \text{0.004703(0.005678)agec*early}_{ij}\end{aligned}$$

$$\beta_{0ij} = \text{-1.426512(0.239925)} + u_{0j} + e_{0ij}$$

$$\beta_{1j} = \text{0.019924(0.003358)} + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0.020747(0.002947)} & \\ \text{0.001248(0.000284)} & \text{0.000184(0.000040)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} \text{0.004583(0.000305)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -1236.386387(661 of 903 cases in use)

Models with NS variables Removed

Narrow Neck Sites

$$\text{NN CSA}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\text{NN CSA}_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.000081(0.000478)agec2}_{ij} + \text{-0.000235(0.000046)agec3}_{ij} + \text{0.005687(0.001892)HT.FNL}_{ij} + \text{0.000037(0.000003)XB.LEAN.TOT}_{ij} + \text{0.000005(0.000001)FAT.TOT}_{ij}$$

$$\beta_{0ij} = \text{-0.359685(0.255162)} + u_{0j} + e_{0ij}$$

$$\beta_{1j} = \text{0.020833(0.003138)} + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0.034675(0.004794)} & \\ \text{0.002161(0.000388)} & \text{0.000220(0.000042)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} \text{0.009137(0.000498)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -1173.017426(881 of 903 cases in use)

$$\text{NN Sect Mod}_{ij} \sim \text{N}(XB,\ \Omega)$$

$$\text{NN Sect Mod}_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{0.000404(0.000233)agec2}_{ij} + \text{-0.000161(0.000024)agec3}_{ij} + \text{0.004257(0.000933)HT.FNL}_{ij} + \text{0.000018(0.000001)XB.LEAN.TOT}_{ij} + \text{0.000003(0.000001)FAT.TOT}_{ij}$$

$$\beta_{0ij} = \text{-0.583138(0.123879)} + u_{0j} + e_{0ij}$$

$$\beta_{1j} = \text{0.011356(0.001657)} + u_{1j}$$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0.008116(0.001139)} & \\ \text{0.000652(0.000110)} & \text{0.000074(0.000013)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} \text{0.002558(0.000139)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -2320.258762(881 of 903 cases in use)

IT Sites females

$\text{IT CSA}_{ij} \sim \text{N}(XB, \Omega)$

$\text{IT CSA}_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.000154(0.000884)agec2}_{ij} + \text{-0.000122(0.000080)agec3}_{ij} + \text{0.028029(0.003470)HT.FNL}_{ij} + \text{0.000056(0.000004)XB.LEAN.TOT}_{ij}$

$\beta_{0ij} = \text{-3.190281(0.476988)} + u_{0j} + e_{0ij}$

$\beta_{1j} = \text{-0.002082(0.004982)} + u_{1j}$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0.164860(0.022210)} & \\ \text{0.007224(0.001402)} & \text{0.000680(0.000124)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} \text{0.022277(0.001220)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -275.067163(877 of 903 cases in use)

$\text{IT Sect Mod}_{ij} \sim \text{N}(XB, \Omega)$

$\text{IT Sect Mod}_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{0.002107(0.000866)agec2}_{ij} + \text{-0.000033(0.000080)agec3}_{ij} + \text{0.036705(0.003380)HT.FNL}_{ij} + \text{0.000057(0.000004)XB.LEAN.TOT}_{ij} + \text{-0.000004(0.000002)FAT.TOT}_{ij}$

$\beta_{0ij} = \text{-5.420174(0.464354)} + u_{0j} + e_{0ij}$

$\beta_{1j} = \text{-0.032032(0.005174)} + u_{1j}$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0.119730(0.016321)} & \\ \text{0.004805(0.001014)} & \text{0.000442(0.000091)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim \text{N}(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} \text{0.024872(0.001359)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -255.841308(877 of 903 cases in use)

$S\ CSA_{ij} \sim N(XB, \Omega)$

$S\ CSA_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.002361(0.000674)agec2}_{ij} + \text{-0.000192(0.000063)agec3}_{ij} + \text{0.010386(0.002659)HT.FNL}_{ij} + \text{0.000052(0.000003)XB.LEAN.TOT}_{ij}$

$\beta_{0ij} = \text{-0.800865(0.362167)} + u_{0j} + e_{0ij}$

$\beta_{1j} = \text{0.042055(0.003907)} + u_{1j}$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim N(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0.057983(0.008040)} & \\ \text{0.002868(0.000614)} & \text{0.000354(0.000068)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim N(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} \text{0.014780(0.000832)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -672.455884(841 of 903 cases in use)

$S\ Sect\ Mod_{ij} \sim N(XB, \Omega)$

$S\ Sect\ Mod_{ij} = \beta_{0ij}\text{cons} + \beta_{1j}\text{agec}_{ij} + \text{-0.000635(0.000403)agec2}_{ij} + \text{-0.000120(0.000038)agec3}_{ij} + \text{0.009240(0.001587)HT.FNL}_{ij} + \text{0.000029(0.000002)XB.LEAN.TOT}_{ij} + \text{0.000002(0.000001)FAT.TOT}_{ij}$

$\beta_{0ij} = \text{-1.274643(0.216422)} + u_{0j} + e_{0ij}$

$\beta_{1j} = \text{0.015886(0.002471)} + u_{1j}$

$$\begin{bmatrix} u_{0j} \\ u_{1j} \end{bmatrix} \sim N(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0.018640(0.002603)} & \\ \text{0.000845(0.000195)} & \text{0.000109(0.000022)} \end{bmatrix}$$

$$\begin{bmatrix} e_{0ij} \end{bmatrix} \sim N(0,\ \Omega_e)\ :\ \Omega_e = \begin{bmatrix} \text{0.005432(0.000305)} \end{bmatrix}$$

*-2*loglikelihood(IGLS Deviance)* = -1534.439197(841 of 903 cases in use)

Appendix E

Study Two: Maturational timing does not predict HSA estimated adult bone geometry at the proximal femur. As published in the journal *Bone*.

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Maturational timing does not predict HSA estimated adult bone geometry at the proximal femur

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ABSTRACT

Late maturational timing is documented to be detrimental to bone strength primarily at the distal radius. Studies at the proximal femur have focused on bone mass and the results remain controversial. The purpose of this study was to examine the long term relationship between the onset of maturation and the development of estimated cross sectional area (CSA) and section modulus (Z) at the proximal femur.

Two hundred and twenty six individuals (108 males and 118 females) from the Saskatchewan Pediatric Bone Mineral Accrual Study (PBMAS) were classified into maturity groups based on age of attainment of peak height velocity. CSA and Z were serially assessed at the narrow neck (NN), intertrochanter (IT) and proximal shaft (S) sites using hip structural analysis (HSA). Multilevel models were constructed to examine the development of CSA and Z by maturity group.

Cross sectional observations indicated that during adolescence, early maturing males had significantly greater CSA and Z than late maturing males at all sites of the proximal femur, while early maturing females had greater Z at the NN and S, and greater CSA at the NN, IT and S sites compared to late maturing females. When age, body size, body composition, physical activity and dietary intake were controlled no significant effects of maturational timing were found at the NN, IT or S regions ($p>0.05$) in either males or females.

In this population of healthy individuals there appears to be no effect of the onset of maturation on estimated CSA and Z development at the proximal femur in both males and females. This may be a result of the proximal femur's loading environment. Future research is required to determine the role of loading on the relationship between maturational timing and bone structure and strength development at the proximal femur.

Introduction

Loss of bone mass is an inherent part of the aging process in both men and women. The attainment of peak bone mass is considered an important aspect in the prevention of osteoporosis and the debilitating fractures that coincide with this disease [1]. The adolescent period is crucial for bone mass development with approximately 40% of peak bone mass laid down during the 5 years surrounding the pubertal growth spurt [2]. It is purported that the timing of maturational onset may influence the development of peak bone mass; however this relationship between maturational timing and bone mass accrual remains controversial. The few studies that have investigated this maturation-bone relationship have focused primarily on females [3–6] leaving a paucity of information in males. We have previously shown, in a cohort of boys and girls followed continuously for 15 years, there appears to be a deleterious effect of a naturally delayed maturation on total body bone mineral content (BMC) development in females but not males, with late maturing females developing less bone mass throughout adolescence and into adulthood than their early and late maturing peers [7]. These findings suggest a potential sex-dependent effect of maturational timing on bone mass development and perhaps sex difference in the development of bone structure and strength at the hip. Further supporting this supposition, Forwood et al. [47] observed apparent sex difference in cross sectional area (CSA) and section modulus (Z) at the proximal during adolescence, even after maturational difference were controlled. However, Forwood and colleagues did not identify whether the timing of maturation (i.e. being an early, average, or late maturer) influenced adult proximal femur bone strength and whether these differences were sex dependent.

In general, clinicians do not use BMC as a clinical assessment tool, rather areal bone mineral density (aBMD) is used as a measure to diagnose osteoporosis and predict fracture risk [8]. However, aBMD is only a single component of whole bone strength. Although it remains difficult to assess bone strength in vivo, it can be estimated from knowledge of geometry, material composition and the loading configuration [9,10]. There is a growing amount of literature highlighting the importance of geometric measures, such as CSA and Z, which

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