The Timing of Peak Tissue Velocities at the Proximal Femur during Adolescence

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Abstract

Purpose: The objective of this study was to examine the timing of the age and the magnitude of peak lean tissue mass accrual (peak lean tissue velocity, PLTV) as it relates to the age and magnitude of peak cross sectional velocity (PCSAV) and section modulus velocity (PZV) during adolescence. It was hypothesized that the age of PLTV would precede the age of PCSAV and PZV and that there be a positive relationship between the magnitude of PLTV and both PCSAV and PZV

Methods: 41 males and 42 females aged 8-18 years were selected from the Saskatchewan Pediatric Bone Mineral Accrual Study (1991-2005). Participants' total body lean tissue mass was assessed annually for 6 consecutive years using DXA. Narrow neck, intertrochanteric and femoral shaft cross sectional areas and section modulus, measures of bone strength, were determined annually using the hip structural analysis (HSA) program. Participants were aligned by maturational age (years from peak height velocity). Lean tissue mass, CSA, and Z were converted into whole year velocities and the maturational age of peak tissue velocities was determined using a cubic spline curve fitting procedure. A 2x3 factorial ANOVA with repeated measures was used to test for differences between age of PLTV and both, the age of PCSAV and PZV between males and females. Multiple regression analyses was used to determine the relationship between PLTV and both PCSAV and PZV.

Results: There was no sex difference in the ages at which tissue peaks occurred when aligned by maturational age. There were significant differences between the age of PLTV and both PCSAV and PZV at the narrow neck (, p=0.001) and femoral shaft (p=0.03), where the age of PLTV preceded both PCSAV. There were no significant differences at

the intertrochanteric site (p=0.814). PLTV was a significant predictor of the magnitude of both PCSAV and PZV at all sites (p<0.05).

Conclusions: These findings support the hypothesis that the age of PLTV precedes the age of PCSA and PZV at the proximal femur and provides further evidence supporting the muscle-bone relationship suggesting that lean tissue mass accrual influences bone strength.

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Dedication

This thesis is dedicated to my family and friends.

They were there throughout this thesis process, lending an ear during times of frustration and staying up late for last minute revisions. Their support provided me with all the motivation to complete this thesis and they never fail to encourage and push me beyond my limits.

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List of Abbreviations

aBMD	Areal Bone Mineral Density
BMAS	Pediatric Bone Mineral Accrual Study
BMD	Bone Mineral Density
BMC	Bone Mineral Content
vBMD	Volumetric Bone Mineral Density
BSI	Bone Strength Index
CSA	Cross Sectional Area
CSMI	Cross Sectional Moment of Interia
CV	Coefficient of Variance
DXA	Dual Energy X-ray Absorptiometry
FSI	Femur Strength Index
GRF	Ground Reaction Force
HSA	Hip Structural Analysis
ISAK	International Society for the Advancement of Kinanthropometry
ITCSA	Intertrochanter Cross Sectional Area
IT	Intertrochanter
ITZ	Intertrochanteric Section Modulus
LTM	Lean Tissue Mass
MES	Minimum Effective Strain
MRI	Magnetic Resonance Imaging
MVC	Maximal Voluntary Contraction
NN	Narrow Neck

NNCSA	Narrow Neck Cross Sectional Area
NNZ	Narrow Neck Section Modulus
PCSAV	Peak Cross Sectional Area Velocity
PHV	Peak Height Velocity
PLTV	Peak Lean Tissue Mass Velocity
pQCT	Peripheral Quantitative Computed Tomography
PVBMC	Peak Bone Mineral Content Velocity
PVLBM	Peak Lean Body Mass Velocity
PZV	Peak Section Modulus Velocity
QTL	Quantitative Trait Loci
S	Femoral Shaft
SCSA	Femoral Shaft Cross Sectional Area
SSI	Stress Strain Index
SZ	Femoral Shaft Section Modulus
Z	Section Modulus

Introduction

Osteoporosis is a disease characterized by bones becoming porous and brittle resulting in an increased risk of fractures (Borer, 2005). Osteoporotic fractures in the elderly may result in a loss of functional capacity and in some cases death (Osteoporosis Canada, 2007). Over 1.4 million Canadians suffer from osteoporosis, affecting one in four woman and one in eight men over the age of 50 (Osteoporosis Canada, 2007). Although it is natural for aging bones to show decreases in bone mineral content (BMC) after the 3rd-4th decade of life, a one standard deviation decrease in bone mineral density (BMD) leads to a 2-fold increase in the risk of debilitating functional capacity and injury (Borer, 2005). The majority of the data related to osteoporosis are dependent on measures of BMD; however, most recently, the notion of bone strength, as determine by other elements such as bone geometry and tissue architecture, is being recognized as an important factor in determining bone fragility. Bone strength is the bone's ability to resist fracturing and while it incorporates the measures of BMD, it also includes other variables such as bone geometry, architecture, and mineralization (Bonnick, 2007). Optimizing bone mineral accrual during the growing years and maintaining BMD and bone strength can reduce the risk of osteoporosis; however, the most effective means of doing so remains unknown.

Physical activity and play during childhood and adolescence are believed to be an influential factor in maximizing bone mass (Bailey et al., 1996). Physical activity serves as a stimulus for mechanically loading the skeletal system in the form of gravitational forces and muscular contractions. These gravitational forces and muscular contractions place dynamic strains on bone tissue which results in positive osteogenic effects on bone

mass (Proctor et al., 2002; Lima et al., 2001; Fuchs et al., 2001; Vicente-Rodriquez et al., 2005, Wang et al., 2007, Rauch et al., 2004) and bone strength (Faulkner et al., 2003; Vainionpää et al., 2007; Petit et al., 2002; Forwood et al., 2006).

Muscular contractions generate the greatest physiological loads experienced by bones, placing stresses that are several fold greater than that of gravity (Burr, 1997). For this reason, it makes logical sense that there is a strong association between muscle mass and bone mass (Vincente-Rodriguez et al., 2005; Schoenau, 2005; Rauch et al., 2004; Witzke & Snow, 1999). Explaining this association, Frost (1987) proposed a mechanostat theory which postulates that the link between muscle mass and bone mass is a reflection of the skeleton's ability to elicit an adaptive response to mechanical strains. Following Frost's theory, muscular contractions are believed to generate sufficient forces that are above the minimum effective threshold required to elicit bone adaptation, allowing muscular forces to drive bone development. Rauch et al. (2004) investigated this theory by examining the relationship between peak lean body mass (LBM) accretion and peak velocity bone mineral accretion. Results showed the maximal rate of LBM accretion preceded the maximal increase in BMC. Furthermore, the peak rates of change between LBM and BMC were closely correlated (Rauch et al., 2004). These observations provide support for the mechanostat theory, suggesting a functional relationship between the mechanical forces exhibited by muscles and bone development. In addition, these findings support a sequential timing where peak gains in lean body mass precede peak gains in bone mineral. However, Rauch et al. (2004) only identified the relationship between lean body mass and BMC, a single indicator of bone strength. Although it is well established that the decline in BMD is associated with increased risk of fractures

(Melton et al, 1993; Cummings et al., 1993), both BMD and BMC are only surrogates of bone strength. Bone strength is a concept that is multifaceted encompassing the components of geometry, architecture, porosity, and tissue mineralization (Bonnick, 2007). BMD and BMC measures are incorporated in all these components but fail to distinguish between them (Bonnick, 2007). By using BMC and BMD alone to assess bone strength, it remains difficult to understand how the individual elements (bone geometry, architecture, porosity, and tissue mineralization) contribute to bone strength. Unfortunately, the majority of literature has concentrated on BMC and BMD as the primary estimation of bone strength resulting in a paucity of information regarding the other elements of bone strength. In addition, there is limited information regarding the changes in these other parameters over time. Only a few studies have incorporated longitudinal designs, and even fewer have investigated the relationship between total body muscle mass and bone geometry at the proximal femur. The proximal femur remains a clinically significant fracture site in old age and, provided that childhood and adolescence may represent a unique window of opportunity for bone development, it is essential to identify factors influencing bone strength during this unique developmental period. Therefore, the purpose of this study was to explore the longitudinal relationship between muscle mass and bone geometry, specifically, investigating the sequential timing between the peak in lean tissue development and bone geometric development at the proximal femur in healthy adolescents.

1.0 Review of Literature

A comprehensive understanding of bone physiology, the factors that influence bone development, and the concept of bone strength are necessary in order to establish the conceptual framework in investigating the relationship between muscle mass and bone strength. This chapter summarizes basic bone physiology, introduces the concept of bone strength and discusses the influence of genetics, nutrition, maturation, physical activity, and muscle on the concept of bone strength. For the purpose of this thesis emphasis will be placed on the influence of muscle on bone strength, highlighting the effects of muscle activity, muscular strength, and muscle mass on bone adaptation.

1.1 Bone Physiology

Bone is a highly specialized tissue characterized by its rigidity and hardness. Bone's highly specialized components enable it to serve several functions including structural support, movement, protection for vital organs, hematopoesis, and the maintenance of mineral homeostasis. Bone is primarily composed of calcium and phosphate, arranged in a complex matrix of organic and inorganic compounds forming the mechanically rigid and load bearing bone mineral crystal hydroxyapatite (Robey & Boskey, 2006). It is the hydroxyapatite structure that allows bones to act as a reservoir for calcium and phosphate ions.

The skeleton is comprised of cortical and trabecular bone. Cortical bone comprises approximately 80% of the adult skeleton and is characterized by its dense and solid macroscopic structure (Dempster, 2006). The more metabolically active trabecular bone, also known as cancellous bone, is composed of fused plates that give trabecular bone its distinctive honey comb like appearance (Dempster, 2006). Although cortical and

trabecular bone may differ in structure and function, they both consist of three cell types: osteoblast, osteoclasts, and osteocytes. The osteoblasts are the matrix producing cells that regulate bone mineralization (Sommerfeld & Rubin, 2001). Osteoclasts are responsible for bone resorption and regulate the release of calcium and phosphate. Osteocytes, which account for nearly 90% of all the cells in the adult skeleton, are believed to coordinate the spatial and temporal recruitment of cells for bone formation and resorption (Burger & Klein-Nulend, 1999).

1.1.1 Bone Modeling and Remodeling

Bone modeling is the process by which bones are shaped and reshaped by the independent actions of osteoblasts and osteoclasts (Dempster, 2006). This process is predominantly noted during growth, where new bone is being "laid down". Bone modeling is unique to remodeling by the fact that bone formation is not tightly coupled to bone resorption (Dempster, 2006). During bone modeling, bone strength is improved by adding mass and increasing the periosteal and endosteal diameters. The increase in periosteal (the outer surface of the bone, see Figure 1.1) and endosteal (the inner surface lining the medullary cavity of the bone, see Figure 1.1) diameters can be region specific and improves the bone geometric properties, a process called macromodeling (Khan et al., 2001a). In addition, the trabecular bone may undergo minimodeling, where the trabeculae align their orientation in line with the loading forces (Khan et al., 2001a). Both types of modeling are responsible for the strengthening of developing bone.

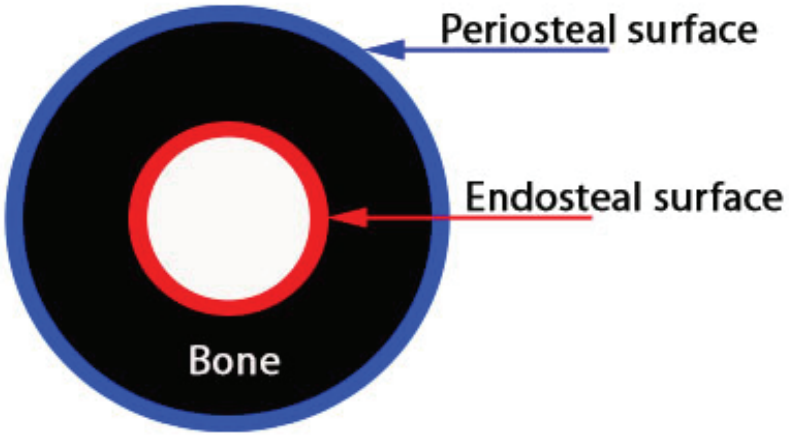


Figure 1.1 – A cross sectional illustration of a long bone diaphysis identifying the periosteal and endosteal surfaces

Bone remodeling, although similar to modeling, is a distinct and unique process. Remodeling consists of four distinct cycles: activation, resorption, reversal, and formation. During the activation stage there is recruitment of the osteoclast precursors in circulation allowing for the infiltration of the bone lining cell layer and the fusion of pre-osteoclasts (Dempster, 2006). The sites selected for activation are unclear, but there is evidence to support that target sites require tissue repair (Burr, 2002). The pre-osteoclasts adhere to 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) (Robling et al., 2006). Specific proton pumps allow H^+ ions to be transferred into the sealing zone allowing the osteoclast to effectively dissolve and digest organic bone matrix leaving a saucer-shaped cavity called Howship's lacunae (Robling et al., 2006). 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 (Dempster, 2006). 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 (Dempster, 2006). In old age, however, the remodeling process eventually results in a net loss, as the coupling mechanisms do not fully replace the bone that has been resorbed (Bailey et al., 1996). Finally, the formation phase is initiated. During this phase 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 maintain in constant contact whilst in the matrix by means of gap junction enabling them to transmit information to one another when necessary (Dempster, 2006). Before the completion of bone formation, the osteoblasts endure one of three fates: incorporated in matrix (becoming osteocytes), remain on surface as bone lining cells, or 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 (Robling, 2006).

1.2 Long Bone Strength

In its simplest form, bone strength is the ability of a bone to withstand fracturing; however, several factors influence bone's resistance to failure such as geometry, architecture, porosity, and tissue mineralization. Cadaver studies have shown that BMC

and aBMD provide good predictive potential of bone's ultimate failure at the proximal femur, accounting for ~28% of the variance in failure load (Cheng et al., 1997)

The predictive ability of geometric properties has been reported by Beck et al. (1990) and Manske et al. (2006). Beck and colleges measured the breaking strength of 20 femora, identifying that HSA predicted strength showed better agreement with material testing (r^2=0.89) than femoral neck aBMD (r^2=0.79). Using MRI, Manske et al. (2006) recently noted that cortical CSA, had the highest association with failure load at the femoral neck, explaining 46% of the variance in failure load. These findings suggest that the geometry of cortical bone also significantly contributes to the prediction of ultimate bone failure (Manske et al., 2006). Although the ultimate failure load provides relevant insight into bone fracturing, the clinical application of these tests remains controversial. Areal BMD is currently the measurement used to define osteoporosis and there is evidence to suggest that it is one of the best population based predictors of osteoporotic fractures (Marshall et al, 1996; Gnudi et al, 2007). However, at an individual level, aBMD does not predict hip fractures (Marshall et al., 1996). This limitation of aBMD is a result of its overlapping connection with the geometric, architectural and porosity properties of bone strength. Another explanation for aBMD's poor individual predictive power is that clinical fractures are determined not only by bone fragility, but by the magnitude, type and location of the external forces (Bouxsein et al., 2007). Geometric properties determined by HSA have been reported to predict fracture risk. 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 BMD (Szulc et

al.,2006; Faulkner et al., 2006; Crabtree et al., 2002) While the potential of geometric properties for predicting fracture risk remains undetermined in a clinical setting, their evaluation provides insight into the actions of external and internal forces of hip fractures (Gnudi et al., 2007). As a result, investigating the influencing factors on geometric properties such as CSA and Z may be of clinical importance for determining fracture risk. As will be discussed in later sections, a positive relationship between muscle and bone has been previously acknowledged, where muscular development may stimulate bone development (Rauch et al., 2004). Therefore, understanding the developmental interplay between muscle and bone geometry may provide vital information on the improvement of bone strength, helping better to predict and prevent future fractures.

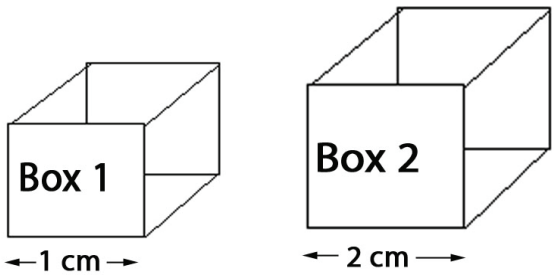
1.3 Imaging Techniques

The following section is dedicated to discussing the major imaging techniques used to assess the properties of bone. This section will concentrate on discussing the advantages and disadvantages associated with DXA, pQCT, MRI and HSA.

1.3.1 Dual Energy X-Ray Absorptiometry (DXA)

Dual energy x-ray absorptiometry (DXA) is currently the most widely and readily available tool used to diagnose the risk of osteoporosis. DXA technology incorporates the principle of differential tissue attenuation to provide a two-dimensional projection image of a scanned region of interest, which commonly includes the whole body, lumbar spine, and proximal femur (Blake et al.,1999). The principle information provided by a DXA scan, as related to the properties of bone, include the estimation of BMD and BMC measured in grams per square centimeter (g/cm^2) and grams (g), respectively. The major

advantage of DXA technology is its versatility to measure bone mineral at a variety of axial and appendicular skeletal sites while maintaining a low level of exposure to ionic radiation (Khan et al., 2001b). The radiation dose is about 10-30 microsievert (μSv), which is less than the exposure found in a Trans-Canadian flight from Toronto to Vancouver (Lewis et al., 2001). This low level of exposure makes DXA a suitable tool for both pediatric and adult assessments. In addition, DXA has proven a reliable and precise method for assessing bone mineral and soft tissue composition (Wallace, 1995; Ellis et al., 1994; Speakerman, 2001). Furthermore, a DXA scanning procedure requires no special preparation from the participant, proving to be a painless and rapid method of assessment (Khan et al., 2001b). Although many clinicians and researchers prefer DXA due to its ease of use, precision and reliability, it is not without its disadvantages. The major limitation of DXA is its two dimensional assessment. As a result, DXA is unable to assess bone properties in three dimensions, providing no information related to bone geometry and architecture. In addition, DXA technology is unable to differentiate between the types of bone (cortical versus trabecular). This makes DXA insensitive to identify changes within the differing types of bone. Moreover, because of its two dimensional imaging, DXA can only estimate areal BMD rather than true volumetric BMD. Consequently, areal BMD is more susceptible to inaccuracy because it is dependent on bone size and orientation. For example, if two bones are made of the same material, but vary in size, the larger bone will have a greater areal BMD (see Figure 1.2)



Measure	Box 1	Box 2
Total mineral density (g/cm^3)	1 g/cm^3	1 g/cm^3
Total BMC (g)	1g	8g
Bone Area (cm^2)	1 cm^2	4 cm^2
Areal BMD (g/cm^2)	1 g/cm^2	2 g/cm^2

Figure 1.2 – Demonstration of size dependency of areal BMD measured by DXA. Even though the both boxes have the same material composition, the large box has greater areal BMD (modified from Khan et al., 2001b)

1.3.2 Peripheral Quantitative Computed Tomography (pQCT)

Peripheral Quantitative Computed Tomography is as imaging machine used primarily to image the peripheral appendicular skeleton. Unlike DXA, pQCT uses a single energy x-ray source for which attenuation discrimination between tissues is determined (Prevrhal et al., 2008). The radiation exposure is smaller than DXA and offers scan times ranging from ~1-4 minutes. pQCT also offers a three-dimensional view of the scanned region, allowing for the estimation of bone geometry and estimated bone strength. The three dimension assessment enables pQCT to estimate cross sectional moments of inertia (CSMI, cm^4), bone-strength index (BSI) and stress-strain index (SSI). Additionally, pQCT is able to asses "true" volumetric BMD (vBMD, in mg/cm^3) rather than areal BMD. As a result, vBMD measures are not affected by bone shape and size (Prevrhal et al., 2008). Finally, pQCT possesses the advantage of differentiating between

trabecular and cortical bone, enabling pQCT to assess bone geometry, strength and density in both types of bone. Despite the clear advantages of pQCT, the clinical impact remains relatively small compared to DXA (Prevrhal et al., 2008). pQCT measurement protocols remain inconsistent between studies making comparison between findings difficult. In addition pQCT technology remains an expensive assessment tool and it is limited to assessing the appendicular skeleton, making it unable to assess the clinically significant region of the proximal femur.

1.3.3 Magnetic Resonance Imagery (MRI)

MRI technology has grown drastically over the last decade. MRI technology uses pulses of magnetic energy 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 a two-dimensional image of a three-dimensional shape. MRI technology allows for a variety of imaging sequences including multi-slice, oblique, spin-echo, and inversion recovery (Hornak, 2007), with spin-echo image sequencing being the most commonly used for muscle and bone assessments. The major advantage of MRI is its ability to produce three dimensional images without exposure to radiation. This makes MRI ideal for assessing bone geometry and strength in pediatric and adult populations. MRI is also able to assess structural properties of bone at a variety of whole body locations (Hornak, 2007), making it able to assess clinically significant fracture sites. However, MRI remains the most expensive tool for tissue imaging. There is limited access to this technology making it difficult to assess special and rural populations. Furthermore, because it is a relatively new technology, there are no standardized assessment protocols for imaging bone, making comparisons

between studies difficult. MRI is a young and growing technology, but until it becomes more cost effective and accessible, its clinical application for bone will remain limited.

1.3.4 Hip Structural Analysis (HSA)

The HSA program will be discussed in further detail in the Methods and Procedures section, but this section is dedicated to outlining the HSA advantages and limitations. The HSA program provides estimation of BMD and structural geometry of the proximal femur using DXA-derived scans (Beck, 2002). 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 is able to estimate a variety of bone strength parameters including estimations of BMD, CSA, CSMI and Z[♣]. 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 bone geometry and strength at a clinically significant fracture site.

[♣] The HSA program is not limited to simply these variables. The variables derived from the HSA program are highlighted in the Methods and Procedures section.

1.4 Factors Affecting Bone Growth

Bone growth and strength is dependent on the delicate interaction between non-modifiable and modifiable factors. The non-modifiable factors, such as genetics, largely influence bone development, but the modifiable factors may facilitate the achievement of their full potential. These modifiable elements include, but are not exclusive to, dietary factors (Vatanparast et al., 2005) and lifestyle choices such as physical activity (Bailey et al., 1996). The following section will outline the effects of the modifiable and non-modifiable factors as they relate to bone and bone strength. Although it is necessary to understand all factors effecting bone, for the specifics of the muscle-bone interplay please begin at section *1.4.5.* entitled *Muscle.*

1.4.1 Genetics

The early works of Smith et al. (1973) displayed a significant relationship between bone mass and first-degree family relationship, emphasizing the influence of heritability and genetics on bone. According to Krall & Dawson-Hughes (1993), who assessed the contribution of genetic and lifestyle factors on familial resemblance in female and male members in 40 families, the genetic and heritable factors account for up to 46-62% of the variability in BMD. Similarly, McGuigan et al. (2002) investigated the contribution of genetics and environmental variables on the regulation of peak bone mass in 460 males and females followed longitudinally. They concluded that environmental factors, in combination with the polymorphism of the vitamin D receptor gene (Bs*m*I) and estrogen receptor α gene (P*vu*II), contributed 14-18% of the variance in BMD. McGuigan et al. (2002) suggested that much of the unexplained variance is due to allelic variations that have yet to be defined and that much of the variance in BMD is controlled

by an overlapping set of environmental and genetic influences. This complexity of the genetic contribution is further emphasized by Peacock et al. (2002), who indicated that there are more than 20 known candidate genes that have been associated with the measure of BMD. In addition, many of these known candidate genes are located across a variety of chromosomes which are associated with other genetic factors responsible for muscle development and physical fitness, which may, in turn, further influence overall bone strength (Peacock et al, 2002). Although the majority of the genetics and bone literature focuses on the measures of BMC and BMD, there is growing evidence suggesting that the genetic determinants of bone geometry are equally as complex. 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 candidate genes and chromosomes. Xiong et al. (2006) recently 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 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 components of bone strength are largely determined by genetics.

1.4.2 Nutrition

The growth and development of bone requires adequate supply of nutritional intake. Bone is primarily composed of calcium and phosphate; thus the dietary intake of

these nutrients is essential. Bonjour et al. (1997) conducted a randomized, double-blind, placebo controlled calcium supplementation on 149 prepubescent girls, in order to investigate the effects of calcium supplementation on bone mass. The authors concluded that the increase in calcium intake led to substantial proliferation in bone mass accumulation in prepubertal girls. Similarly, Prentice et al. (2005) reported that supplementing 143 males during their pubertal growth spurt (16-18 years old) with 1000 mg of calcium per day increased BMC of the whole body, lumbar spine, intertrochanter and hip and was associated with greater height, lean mass and bone area.

The dietary requirements for calcium are primarily determined by the needs of bone development and maintenance (Flynn, 2003). The two years surrounding peak bone accretion is a time of rapid bone development, and represents a period where the need for calcium intake is the greatest (Whiting et al., 2004). Matkovic et al. (2005) identified that calcium supplementation positively affected BMD in pubertal girls, but the effects of supplementation diminished by young adulthood. These results emphasize that the demand for calcium intake is influenced by the rapid growth in bone during the adolescent period.

Several other nutritional factors also affect bone. There is evidence supporting the importance of protein and vitamin D on bone. Protein is an important element in the composition of the organic matrix of bone and it has been shown to stimulate the increase of osteotrophic hormones, such as insulin-like growth factors (Dawson-Hughes, 2003) stimulating bone development. In addition, Vitamin D supplementation of about 20μg (800 UI) or greater is noted to decrease the relative risk of fractures by 30% (Vieth, 2005). In summary, during growth there is evidence to suggest that bone requires

adequate supplementation of calcium, protein and vitamin D. The consumption of these nutrients may be vital to enhance bone strength, thus, it is essential to understand that nutritional quality is an integral component in developing and maintaining bone strength.

1.4.3 Growth and Maturation

Growth and maturation are two distinct entities. Growth refers to the changes in size of an individual, either as a whole or in parts (Baxter-Jones & Sherar, 2006) while maturation refers to the process of progressing towards the mature state (Malina et al., 2004). Although growth and maturation are distinct entities they occur simultaneously and interact (Baxter-Jones & Sherar, 2006). As a result, the process of maturation does influence bone mass and strength.

Faulkner et al. (1996) originally presented the normative growth data for BMC and BMD using DXA measures from children and adolescent boys and girls age 8-17 years from Saskatoon**.** Based of this growth data the authors highlighted that depending on the site, at least 90% of adult BMC is acquired during adolescence. Although Faulkner et al. used a cross sectional database, similar findings are reported by Bailey (1997). Using DXA measures from 113 boys and 115 girls, Bailey (1997) observed that the adolescent growth period is a critical time for bone mineral accretion, noticing that by the time boys and girls reached developmental maturity (peak height velocity) 90% of adult height, 60% of adult total body BMC, and 70% of adult femoral neck BMC was attained.

Males, on average, experience PHV, a common maturational landmark, 2-years after females (Bailey et al., 1997). This results in observable sex differences in bone after puberty. There is little discernable difference in bone size, mass, and structure between sexes early in life (Khan et al., 2001c); however, the difference in timing and tempo of

maturation results in larger and longer bones in males with higher BMD (Bailey et al., 1999). These larger and longer bones often confer strength advantages in bone. Macdonald et al. (2005) used pQCT to examine the changes in tibial bone geometry, strength and muscle-bone strength relationship across maturity in boys and girls. They noted that although males and females benefited from age-related gains in bone strength, greater increases in bone bending strength, as determined by section modulus, was observed for males.

McKay et al. (1998) examined the relationship between peak BMC and maturational development in 53 females between the ages of 8 and 14. When examining the timing of peak height velocity (PHV) and age of menarche, McKay and colleagues observed that peak BMC and age of menarche preceded PHV by about 1 year. In addition, there was a significant negative relationship between age of menarche and peak BMC, where girls with an earlier age of menarche had enhance peaks in BMC, suggesting that early maturation in females may potentiate bone mass benefits.

Similarly, Macdonald et al. (2007) conducted a 16 month randomized, controlled school-based intervention assessing the changes in tibial bone strength between prepubertal and pubertal males and females. Using 257 boys and girls in grade 4 and 5, the authors identified that only prepubertal boys tended to have greater increases in bone strength index and that there were no significant differences between the increases in bone strength index between later pubertal groups. These findings further emphasize that maturation and sex may play a role in the bone strength adaptation to exercise.

Bass et al. (2002) used magnetic resonance imaging (MRI) to determine the impact of mechanical loading on the bone dimensions in 47 pre-, peri-, and post-pubertal

female tennis players. Using the non-dominant arm as a control, the authors observed that growth resulted in a 14% increase in cortical area. When assessing the differences in bone dimensional changes amongst maturity status, the authors suggested that prior to puberty, periosteal apposition primarily accounts for the increase in cortical area while late in puberty, periosteal and endocortical apposition contribute equally to cortical area (Bass et al., 2002). Since the timing of puberty is delayed in males, there is a longer duration in which periosteal apposition contributes to cortical area. This periosteal apposition increases section modulus, contributing to observable sex differences in bone strength.

In summary, the literature suggests that there is a sex difference in the timing and tempo for maturation between males and females, which potential effects bone strength. Therefore, when identify the influence of muscular development on bone strength development, sex differences must be considered and maturational status standardized amongst participants.

1.4.4 Physical Activity

Physical activity mechanically loads the skeleton, placing intense physical demands on the loaded bones. These increased loads serve as mechanical stresses which produce forces that result in increased bone deformation or bone strain. The dynamic strains placed on the bone tissue create a hydrostatic pressure gradient that delivers shear stresses to the bone cells. It is these shear forces that drives bone deformation, stimulating osteogenesis (Turner & Robling, 2003). As a result, it is believed that physical activity, especially during childhood and adolescence, is associated with enhanced bone mineral accrual (Bailey et al., 1996, Bailey, 1997, MacKay et al., 1998, and Bailey et al., 1999).

Using DXA measures from 113 boys and 115 girls, Bailey et al. (1999) assessed the influence of physical activity on bone mass, highlighting that physically active males and females had a 9% and 17% increase respectively in total body BMC, compared to their less active counterparts, supporting that claim that physical activity may enhance bone mass during this critical growth period. Recently, Janz et al. (2006) provided further support for the importance of physical activity during growth. Janz et al. (2006) examined the relationship between habitual physical activity levels and BMC accrual in 171 boys and 199 girls age 4-6 years. They reported that children maintaining higher levels of habitual physical activity, as assessed by accelerometers, had 13% greater trochanter BMC compared to children maintaining lower levels of physical activity. As a result of the enhanced BMC at the trochanter, the authors concluded that this suggests there is enhanced bone remodeling specifically at the locations of muscle insertions.

Not only is there evidence to suggest that individuals who are more physically active develop greater bone mass, there are also data supporting the potential for physical activity to enhance bone geometry. Forwood et al. (2006) 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 cross sectional area (CSA) and section modulus (Z) were significantly predicted, after controlling for biological age, height and weight, by physical activity in both males and females. Janz et al. (2007) reported similar findings in children age 5-8 years old. Using accelerometers to assess physical activity, Janz et al. (2007) examined the longitudinal associations between physical activity and hip strength during childhood concluding that the loading

conditions imposed by physical activity resulted in physically active males and females having greater CSA and Z than their less active peers throughout childhood.

In addition to the literature highlighting the potential benefit of physical activity on bone mass and bone geometry in a normal population, athlete models have also shown similar results. Meyer et al. (2004) compared areal BMD (aBMD), using DXA, in 40 female Olympic winter games athletes with healthy age-matched controls and observed a 5-13.8% positive difference in aBMD for total body, lumbar spine, proximal femur, and femoral neck in the Olympic athletes. Similarly, Vicente-Rodriguez et al. (2004) investigated the osteogenic benefits of soccer participation in 17 prepubertal male soccer players. Their results showed that the prepubertal soccer players had greater BMC in their legs and 8-16% greater BMD at the lumbar spine, hip, and lower extremities than their non-athletic controls. These studies provide support for athletic participation as a method for enhancing bone mass; however, not all sports are equivalent in their osteogenic potential. Bellew & Gehrig (2006) compared the calcaneus BMD of 64 female athletes involved either in swimming, soccer, and weightlifting. These data showed that soccer participation resulted in greater calcaneus BMD compared to both swimmers and weight lifters, but BMD in the swimmers was not different from weight lifters. Andreoli et al. (2001) investigated the effects of different high intensity activities on BMD and estimated appendicular muscle mass. Of the 62 male participants who were highly trained in judo, karate or waterpolo, all had significantly greater total body BMD than age and sex matched controls except for water polo. Between the athletic groups, the judo group had significantly greater BMD at the arms and legs than the karate and water polo groups. In addition, the authors demonstrated that the athletes had a higher appendicular muscle

mass compared to the control group which they stated was a reflection of the significant physical training but may also potentially explain the increases observed in BMD and BMC in the athletic groups.

As demonstrated by Bellew & Gehrig (2006) and Andreoli et al. (2001) soccer and judo training may help to stimulate osteogenesis as a result of the dynamic and multidirectional forces they produce; however, the majority of the forces produced in soccer and judo are confounded to the lower extremities. Gymnastic training produces large dynamic strains in both the upper and lower extremities, making it an ideal sport to examine the effect of physical activity and loading on bone mass and strength. Proctor et al. (2002) examined the effects of gymnastics training on BMD, specifically in the upper limbs, of 25 elite female gymnasts aged 18-25 years and compared them to healthy age and weight-matched controls. Despite groups being matched for body weight, gymnasts were still significantly younger, shorter, and leaner than controls. Gymnastic training also resulted in higher BMD at the lumbar spine, proximal femur, whole body, and both the dominant and non-dominant arms compared to controls. Even after adjusting for the effects of size and lean mass, gymnasts remained significantly higher in BMC/LBM ratio for spine, proximal femur and upper extremities compared to controls, indicating that the intense physical nature of gymnast training is beneficial to bone mineralization (Proctor et al., 2002). Similarly, Faulkner et al. (2003) investigated the effects of gymnastic training in 30 premenarcheal females on the structural properties at the proximal femur using HSA. When compared to age-matched controls, the gymnasts had greater size-corrected BMC, enhanced BMD, increased CSA and Z at the narrow neck. These results are comparable to the findings of Vainionpää et al. (2007), who evaluated the effect of

different loading levels on bone geometry using spiral QCT. Employing a high-impact jumping exercise intervention with 120 premenopausal women, Vainionpää et al. (2007) identified that impact accelerations equivalent to or exceeding $1.1g$[1] significantly predicted changes in bone geometry, specifically bone circumference and cortical CSMI at the mid-femur. These findings suggest that it is the loading component of physical activity that may be the important aspect for initiating alterations in bone strength. To further investigate the effects of loading on bone, Lima et al (2001) separated loading into two forms: impact and active loading. Impact loading consisted of activities that produced ground reaction forces (eg. gymnastics, tennis, running) while active loading consisted of non gravitational mechanically loaded activities (eg. swimming, cycling). Using this loading division, Lima et al. (2001) cross sectionally investigated the effects of loading patterns on BMD and bone biochemical markers in 45 elite male athletes age 12-18 years. The athletes were separated into an impact loaded and an active loaded group, and contrasted with a healthy control group. The results identified that the impact loaded group had the highest lumbar spine, femoral neck and total body BMD while the control group had the lowest total body BMD. Although no difference in lumbar spine and femoral neck BMD was observed between the active loaded and control group, the active group had greater total body BMD which may be a reflection of the high intensity muscular activity imposed at sites not measured (Lima et al., 2001). This conjecture was supported from biochemical marker findings. Bone formation and bone remodeling markers were significantly higher in the active load group compared to both the impact and control group (Lima et al. 2001), suggesting that the benefits of active loading may

[1] The acceleration from gravity. $g = 9.81$m/s

be represented at a site not measured or in the form of alterations to other elements not measured (eg. bone geometry).

Intervention studies, which incorporate a multitude of loading variation, have also been applied to observe the influence of mechanical loading on bone mass and bone geometry. Fuchs et al. (2001) conducted a 7 month high intensity jumping intervention in prepubescent boys and girls in order to examine the effects of high intensity loading forces (> 4-times body weight) on hip and lumbar spine bone mass. Fuchs et al. (2001) concluded that high intensity loading produced significant improvements to femoral neck BMC and lumbar spine BMC and BMD. Likewise, Petit et al. (2002) examined the geometric and structural adaptation of bone following a 7-month high-impact circuit training exercise intervention in pre and early pubertal females. The natural progression of growth and development over the 7 months resulted in a 14-30% change in CSA and a 10-18% change in Z, but after controlling for maturation, only the more mature females (early pubertal group) had an additional 2.6% and 1.7% increase in bone geometry at the femoral neck and intertrochanteric regions, respectively. Similar benefits have also been reported in prepubescent males. MacKelvie et al. (2004) examined the effectiveness of a school based bone-loading exercise intervention on augmenting bone mass and geometry in prepubescent males. Incorporating a randomized controlled design, whole schools were randomly assigned to an exercise intervention, consisting of a progressive 10-12 minute program of diverse weight-bearing exercises scheduled during regular physical education classes, or served as a control, consisting of the normal physical education curriculum. The intervention was implemented for 2 years, assessing participants at baseline, 8 months, 12 months and 20 months using DXA and HSA. After 20 months, the

intervention group had a significantly greater change in BMC and bone area at the femoral neck compared to controls. In terms of geometric changes, the intervention group conferred significantly greater subperiosteal and endosteal expansion, enhanced CSMI and Z at the narrow neck. According to the findings of Fuchs et al. (2001), Petit et al. (2002), and MacKelvie et al. (2004) progressive weight-bearing exercise programs spanning as little as 7-months may be an effective method for increasing bone structural integrity at the proximal femur.

In summary, physical activity has been shown to be an influential factor for enhancing bone mass and bone geometry. Physical activity enhances bone mass and geometry by imposing dynamic mechanical loads on bone tissue. The literature highlights the importance of the loading component of physical activity, emphasizing the contribution of gravitational forces, in the form of high impact and weight bearing activity, on providing a loading stimulus at the bone tissue. However, there is evidence to suggest that the muscular forces, generated through muscular contractions, act as a separate muscular load, which also contributes to the loading component of physical activity. It is this muscular loading that may be as, if not more influential, than physical activity itself for providing a stimulus in enhancing bone mass and bone geometry.

1.4.5 Muscle

Muscle mass and girth increase with age from infancy through adolescence. During this growth period, lean tissue mass contributes substantially to total body mass, accounting for about 65-80% of total body mass in late childhood and adolescence (Malina et al., 2004); However, the relative water content within muscle tissue declines with age, reaching adult levels of adult by adolescence. Rauch et al. (2004) have

previously reported that the peak in lean body mass accrual, assessed using DXA, occurs after PHV at approximately12 years of age in females and 14 years of age in males. The peak in lean mass accrual corresponds to the maximum increment in strength reported by Carron & Bailey (1974). Using a unique system of tensiometers, Carron & Bailey observed that in adolescent males the maximum incremental increase in strength occurred between 13-14 years of age. Although maximal increment in strength may occur during early adolescence, absolute peak muscular strength occurs during the 3rd - 4th decade of life. There are no significant sex differences in lean tissue and muscle mass during childhood; however in adolescence the triggering of maturation provokes marked sex differences. Tanner et al. (1981) reported that muscle widths in males and females remain similar during childhood until ~14-15 years of age when males begin to show significant increases. Similar results are observed between males and females performing a maximal isometric contraction using a hand held dynamometer. Beenakker et al. (2001) observed that by age 14 there is a significant discrepancy in muscular force output in favor of the males. Despite these gender discrepancies, muscular contractions generate the greatest physiological loads experienced by bones in both sexes, placing stresses that are several fold greater than that of gravity (Burr, 1997). The muscle attachments are generally located closest to the joint which in turn creates an inefficient lever arm that requires a large amount of generated force to produce movement. In fact, it takes more than 2kg of muscle force on bone to move 1kg of body weight (Burr, 1997). As a result of this large dynamic physiological strain, it is logically theorized that bone adaptation is elicited primarily due to the dynamic loads of muscular forces.

Frost (1987) proposed a theory of bone adaptation called the mechanostat theory which postulates that bone mass is a reflection of the skeleton's ability to elicit an adaptive response to mechanical strains that are above a set threshold. Mechanical strains that are below a minimum effective strain (MES) fail to elicit bone adaptation, while mechanical strains above the MES propagate adaptation (see Figure 1.3). According to Frost's theory, muscular contractions elicit bone adaptation because the force generated exceeds the MES, providing the necessary stimulus to drive bone development.

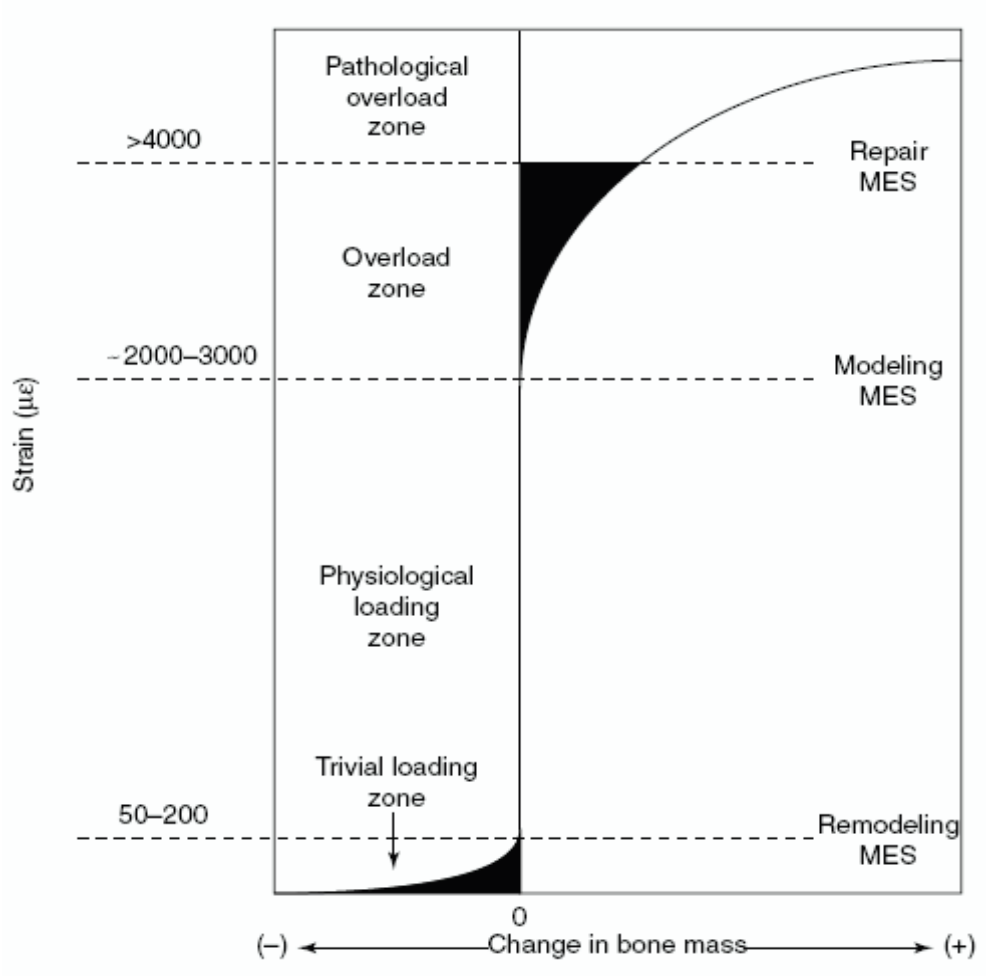


Figure 1.3. A schematic representation the Frost's mechanostat theory and the minimum effective strain (MES) limits proposed to elicit osteogenic adaptation. Adapted from Bachrach (2001)

Muscle-Bone Relationship

The following section summarizes the literature investigating the relationship between muscle mass, muscle strength, and bone.

Snow-Harter et al. (1990) examined the relationship of muscular strength on bone mineral density (BMD) at various body sites in 59 healthy women. Assessing muscular strength using one repetition maximum, the authors concluded that muscular strength was an independent predictor of BMD, accounting for 15-20% of the variance in BMD (Snow-Harter et al., 1990). Similarly, Madsen et al. (1993) investigated the relationship between quadriceps muscular strength, using an isokinetic dynamometer, and site specific BMD. These authors reported similar conclusions, stating that quadriceps muscular strength was the only significant predictor of tibial BMD. Following suit, Valimarsson, Ö et al. (1999) using a cross sectional design, investigated the relationship between physical activity and muscle strength on BMD at the lumbar spine, femoral neck, forearm, and total skeleton in 254 healthy Icelandic females 16-20 years of age. Lean tissue mass, an estimate of muscular strength, had the greatest correlation with BMC, bone area, and BMD at all measurement sites. In addition, lean tissue mass explained 58.5% of the variance in total body BMC and BMD and remained the most important factor for predicting BMC and BMD at all measurement sites. Likewise, Witzke & Snow (1999) incorporated a variety of muscular fitness measures (lean mass, leg power and leg strength) to identify the relationship between BMC and BMD with muscular fitness in healthy post-pubertal adolescent females. Lean tissue mass was estimated from DXA, leg strength, via leg extension, and leg power, through Wingate anaerobic test. Their results indicated that only bone-free whole body lean tissue mass independently accounted for

25-59% of the variance in whole body, lumbar spine and femoral shaft BMD. Height and lean mass were also significant predictors of whole body BMC, but lean mass accounted for ~ 60% more variance than height alone. These findings are similar to the results of Vicente-Rodriquez et al. (2005), who investigated the affect of changes in soft tissue body composition (lean mass, fat mass and total body mass) over a 3 year period during growth on femoral bone mass in 42 prepubertal males. The males were separated into two groups (active and controls) based on physical activity levels because participation at higher levels of physical activity produces greater muscle hypertrophy which has the potential of eliciting greater strain on bones (Vicente-Rodriquez et al., 2005). The active group consisted of males who participated in regular activity that was additional to the compulsory physical education sessions offered in their schools. The controls consisted of males whose physical activity was limited to the compulsory physical education sessions. Tissue composition and bone mass were assessed at baseline and ~3 years later. At baseline, the active group had significantly greater trochanteric BMC and femoral neck BMD. Approximately 3 years later, the active males had a 2 fold increase in femoral neck and intertrochanteric BMC compared to controls. Similarly, the change in femoral BMD in the active group was twice that of the controls. In addition, the increment in lean mass had the greatest correlation and had the highest predictive value for both the increases in femoral BMC and BMD, explaining 43% and 48% variance, respectively.

Wang et al (2005) examined the contribution of lean tissue and fat mass in predicting bone mass in 921 young women age 20-25 years old from an variety of ethnic backgrounds (African Americans, Asians, Caucasians, and Latinas). Using one time DXA measures for tissue body composition and bone mass, there was a significant

difference amongst ethnic group in terms of weight, fat mass, lean tissue mass and bone mass. Despite the discrepancies between ethnic groups, lean tissue mass, fat mass and weight were all correlated with femoral neck BMD, whole body BMD and whole body BMC. Their multiple regression analysis revealed that lean tissue mass and fat mass significantly predicted BMD and BMC at all sites, when analyzed separately, but when analyzed together lean tissue mass had a significantly greater effect on BMD and BMC than fat mass. Based on these results, the authors concluded that fat mass contributes to the effect of skeletal loading but the effect is lesser than that of lean tissue mass.

Recently, Wang et al. (2007) investigated the association of weight bearing and muscle loading of the upper and lower limbs in 258 healthy pubertal females. They acknowledged that, for any given amount of limb muscular strength, more bone mass was accrued. In addition, they observed that the maximum isometric voluntary contraction (MVC) of the upper and lower flexors was highly associated with BMC in the arm and leg, respectively, and that the correlation coefficients of MVC and BMC in the arm and leg did not differ. These similar correlation coefficients suggest that muscular strength affects bone mass in a similar manner in both the arms and legs (Wang et al., 2007), and further emphasizes the contribution of muscular contractions in physiologically loading bones. These previous studies outline the strong association between muscle strength and bone mass seen in both males and females; however, they failed to identify the influence of muscle mass and strength on actual bone development.

Rauch et al. (2004) conducted a longitudinal study investigating the association between muscle and bone development. Utilizing data from the Pediatric Bone Mineral Accrual Study (BMAS), Rauch et al. (2004) aligned individuals by maturational status,

using PHV, and observed a sequential timing where the peak in lean body mass velocity (PVLBM), a surrogate of muscular strength, preceded the maximal peak in bone mineral content velocity (PVBMC). Additionally, through multiple regression modeling, Rauch et al. (2004) noted that only PVLBM was independently associated with PVBMC after controlling for sex and maturity. These findings provide support for Frost's (1987) mechanostat theory, revealing a sequential timing and a strong relationship between the development of muscle mass, as represented by lean tissue, and the development in bone mineral accrual. However, like the majority of literature acknowledging a muscle-bone relationship, Rauch et al. (2004) only identified the relationship between muscle mass and bone mass, a single indicator of overall bone strength.

As mentioned previously, bone geometry is a direct component of bone strength and, as a result, is a better representation of overall bone strength compared to the measure of bone mass. When considering the literature related to muscle mass, muscle strength and bone there is also evidence to suggest that muscle force is related to bone geometry. Daly et al. (2004) investigated the relationship between muscle area and bone during growth in 47 pre-, peri-, and post pubertal competitive tennis players age 8-17 years. The authors assessed the relationship between muscle area on total bone area, medullary area, cortical bone area, and polar second moments of area (I*p*, a measure reflecting the bone's resistance to bending) of the dominant and non-dominant playing humeri at each pubertal stage using MRI. At each stage of puberty, there was a linear relationship between muscle area and total bone area, medullary area, cortical bone area, and I*p*. They also observed that there was more bone mass and cortical area for any given amount of muscle area, specifically in the post-pubertal athletes. This result

suggests that there may be a different temporal patterning of growth for bone and muscle, in which bone mass may 'lag' behind muscle growth during pre- and peri-pubertal stages, and only later in puberty, fully develops. In comparing side to side differences, there was a 6.7% greater muscle area in the dominant arm which accounted for 11.8-15.9% of the variance in side to side differences in total bone area, cortical area, and I*p.* Although the variance accounted for was small, this may be a result of a small sample size and other factors that mediate the association between muscle and bone, such as genetic regulation, nutrition, and hormonal factors (Daly et al., 2004).

Schoenau et al. (2000) used pQCT to investigate the interaction of muscle area and cortical bone area in 318 healthy children and adolescents who took part in the Dortmund Nutritional and Anthropometric Longitudinally Design Study. After assessing muscle cross sectional area and bone geometry at the forearm the authors observed a strong correlation between muscle and cortical area of the radius in all children and adolescents (r^2=0.77), and reported that 85% of the variance in cortical area was explained by muscle area. Similar correlations were found when using MRI to investigate the relationship between region specific muscle cross-sectional area and total cortical bone area in 17 prepubertal and early pubertal girls (Heinonen et al., 2001). Heinonen and colleagues assessed muscle cross sectional area in the lower limbs, separating the cross sections into three anatomical divisions; the SI sector consisting of the medial-anterior portion of the lower leg, SII consisting of the lateral area, and SIII consisting of the medial posterior section. Based on these anatomical divisions, Heinonen and colleagues found that total muscle cross-sectional area was significantly correlated with total cortical area at the tibia in both legs (r^2 =0.54). Specifically, the highest

significant correlation between muscle cross sectional area and cortical area was in SII. In addition, in assessing the ground reaction forces (GRF) and cortical area, it was noted that jumping GRF were not significantly related to cortical cross sectional area, but they were significantly correlated to muscle area. These findings suggest that the GRF may be more beneficial towards increasing muscle area, which in turn may be beneficial for increasing cortical area at specific muscle insertion points such as the anterior-lateral tibia.

MacKelvie et al. (2004), as discussed earlier, introduced a 2 year progressive weight-bearing intervention on prepubescent males, reporting that it was an effective method for increasing CSMI and Z at the narrow neck as assessed by HSA. In addition to these findings, MacKelvie and colleagues observed that the changes in lean body mass were positively related to changes in Z at the narrow neck and intertrochanteric regions, highlighting the importance of lean mass as a mediator for mechanically loading bone tissue.

Petit et al. (2005) used HSA to compare the bone geometry of overweight and healthy weight children, investigating the influence of lean mass in determining bone strength. The authors observed that obese children had greater Z than healthy children but the absolute lean mass did not differ between groups. When bending strength at the proximal femur was adjusted for lean tissue mass, there was no longer a difference between obese and healthy children. Furthermore, lean mass, and not fat mass, was a significant and independent predictor of Z at the femoral shaft and narrow neck regions in both groups (Petit et al., 2005). These findings indicate that body weight in itself, although an important loading component, does not serve as the dominant primer for

osteogenic adaptation and that lean tissue mass may play a decisive role in enhancing bone strength.

As previously described, Forwood et al. (2006) used the HSA program to investigate the effect of physical activity on bone strength at the femoral neck in boys and girls. In addition to finding a positive influence of physical activity on bone strength, the authors observed that the difference between active and inactive participants was accounted for when the data was normalized for mineral-free lean tissue mass. This finding provides further evidence to suggest that mineral free lean tissue, a surrogate for muscular strength, may be influencing the strength differences seen at the femoral neck between inactive and active adolescents. Similarly, when Forwood et al. (2004) used HSA to examine the bending and axial strength at the proximal femur during growth in 70 adolescent males and 68 females they concluded that, compared to males, females had a lower bending strength at the femoral neck and that this sexual dimorphism can be accounted for by the greater increase in lean body mass of males during adolescence; again, indicating that the mechanical loads imposed by muscular forces may influence bone development and strength.

Janz et al. (2007) further emphasized the relationship of physical activity and lean tissue mass on bone strength at the femoral neck during childhood. As mention ned earlier, using participants from the Iowa Bone Development Study, Janz et al. (2007) identified that physically active children had greater CSA and Z then their less active peers. When controlling for the effects of total body lean mass in their multilevel modeling analysis, the authors found that physical activity continued to be a significant predictor of CSA and Z in males, but with a reduced slope, and it was no longer a

significant predictor of CSA and Z in females. These results suggest that lean body mass is mediating the relationship between physical activity and the measures of bone strength.

Although there appears to be a growing amount of literature identifying the relationship between muscle mass and bone geometry, there are currently no studies looking at the influence of muscle mass development on bone geometry development, especially at the clinically significant site of the proximal femur.

1.5 Summary/Purpose

Dynamic muscular loads induce mechanical strain on bone that instigates adaptive physiological responses such as increasing bone mass. Adolescence is considered a time where a window of opportunity exists for optimizing bone adaptations and there is data highlighting the positive relationship between muscle and bone accrual during this time period (Rauch et al., 2004; Macdonald et al., 2006; Ruff, 2003; Vicente-Rodriguez et al. 2005). Unfortunately, there is a paucity of information regarding other parameters of bone strength. HSA is currently one of the best non-invasive methods for estimating mechanical bone strength at the clinically significant site of the proximal femur and may provide further insight into these other parameters of bone strength. Of the limited pediatric bone research that has utilized HSA, a few studies have suggested the importance of lean tissue mass contribution on the strength properties at the femoral neck; however, none have examined the relationship between lean tissue mass accrual and CSA or Z at the proximal femur. CSA and Z are important variables to identify because they provide a better mechanical representation of bone strength then the measures of BMD and BMC. There is also limited information regarding the muscle-bone relationship, in terms of muscle and bone geometry, that have utilized longitudinal

data. As a result, the previous literature has controlled for the effects of maturation through estimation, using techniques such as self identified Tanner stages. Although this is a widely practiced method in pediatric research, it does not compare to the accuracy of longitudinal methods such as peak height velocity (PHV)[†]. By using PHV, the influence of maturation on bone development can be controlled with greater precision, allowing for a more accurate depiction of the muscle-bone relationship. Similarly, there is currently no literature examining the sequential timing of lean tissue mass accrual and bone geometry. If muscle drives bone development, as proposed by Frost's mechanostat theory, then lean tissue mass, a surrogate of muscle mass, should precede developments in bone geometry. By using longitudinal data, whole year tissue velocities, specifically, peak lean tissue mass velocity (PLTV)[†], peak cross sectional area velocity (PCSAV)[†] and peak section modulus velocity (PZV)[†] can be determined as well as the age that they occur. These velocities can serve as markers in establishing the temporal sequence of developmental events (Rauch et al., 2004). Therefore, the purpose of this study was to determine, using a longitudinal dataset, if there is a sequential timing between the age of peak lean tissue mass velocity and the age of peak CSA and Z velocities during the pubertal growth spurt.

[†] PHV, PLTV, PCSA, and PZV will be explained in further detail in the *Tissue Velocities and Growth Curves* section

2.0 Hypotheses

Hypothesis 1

Peak lean tissue mass velocity will precede peak bone strength velocities at both the proximal femur and femoral shaft.

Sub hypotheses

i) Peak lean tissue mass velocity will precede peak bone cross sectional area velocity at the narrow neck, intertrochanter and femoral shaft

(ii) Peak lean tissue mass velocity will precede peak bone section modulus velocity at the narrow neck, intertrochanter and femoral shaft.

iii) There will be no gender differences in the sequential timing of peak lean tissue mass velocity and peak bone strength velocities

Hypothesis 2

There will be a positive significant relationship between the magnitude of lean tissue mass velocity and the magnitude of bone cross sectional area and section modulus velocities at the narrow neck, intertrochanter and femoral shaft.

3.0 Methods and Procedures

3.1 Participants

The BMAS consisted of 375 male and female students from grades three to eight attending two elementary schools in the city of Saskatoon. Of the 375 participants, 228 parents provided written consent for their children's involvement in the study (113 boys, 115 girls). A number of participants joined the ongoing study from 1991-1993 and by 1993 the original study recruited over 251 participants consisting of overlapping cohorts from age 8-21. Of the original 113 boys and 115 girls, 41 males and 42 females had complete longitudinal data for all variables across the adolescent growth period. Thus data from these individuals were used for subsequent analysis. All participants were of Caucasian descent and from middle class socioeconomic neighborhoods (Bailey et al, 1999). In addition, all subjects included had no history of chronic disease, medication use, or medical conditions known to affect growth (Bailey, 1997).

3.2 Anthropometry

A comprehensive set of 37 anthropometric measures were collected semi-annually for all participants (Bailey, 1997). Height, weight, lengths, skin-folds, girths and breadths were measured by instructors certified by ISAK (International Society for the Advancement of Kinanthropometry) following the anthropometric standards outlined by Ross & Marfell-Jones (1991). Height was recorded without shoes to the nearest 0.1cm using a wall stadiometer. Weight was measured on a calibrated electronic scale to the nearest 0.01 kg.

3.3 Dual Energy X-Ray Absorptiometry (DXA)

Body composition assessment was performed annually by a trained technician using DXA (Hologic QDR-2000, fan beam mode) following the procedures as outlined in the Hologic Quantitative Digital Radiography Operators manual and user's guide (Hologic, 1991). DXA is a scanning technique that incorporates a 3-component model in assessing body composition, measuring fat mass (grams), lean mass (grams) and bone mass (grams and grams·cm^{-2}) simultaneously (Ball & Altena, 2004). Wallace (1995) concluded that DXA in our lab is a precise method for assessing tissue mass with coefficients of variations being 2.95% and 0.54% for total fat mass and total lean tissue mass respectively. However the precision of regional measurements of lean tissue mass are diminished as a result of the scan inability to assess the soft tissue directly above the bone and therefore making an estimation of the tissue mass (Madsen et al., 1997). Wallace (1995) also concluded that DXA is a reliable method for assessing tissue mass with internal consistency at our lab being r = 0.99 for both total body BMD and lean tissue. Ellis et al (1994) and Speakman et al. (2001) tested the validity of DXA determined body composition to chemical analysis using pig, dog and cat carcasses, respectively. Both Ellis et al. (1994) and Speakman et al. (2001) found a strong correlation between DXA scores and chemical analysis (r^2>0.98-0.99), concluding DXA to be a valid technique for determining tissue composition.

3.4 Hip Structural Analysis (HSA)

Geometric analysis was conducted using HSA. HSA is a method of estimating the structural geometry of long bones at locations within the proximal hip region extracted from DXA images. The DXA scans produce pixel values that are expressed as areal mass

(Beck, 2002), and the HSA program employs the principle that a line of pixel values across the bone axis correspond to a cut plane traversing the bone at that location, providing information about the cross-section (Beck et al., 1990). The HSA program 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 (Beck, 2002; see Figure 3.1). From each region, the HSA program produces ten output variables, only two of which are were assessed for this thesis (presented in bold text): 1) **Cross Sectional Area (CSA)–** the estimated amount of bone surface area in the cross section after excluding all the trabecular and soft tissue space, 2) Cross Sectional Moment of Inertia – an index of structural integrity that reflects the distribution of mass about the center and is used to determine section modulus, 3) Subperiosteal Width – the estimated outer diameter of the bone, 4) Endocortical Diameter – the estimated inside diameter of the cortex, 5) Average Cortical Thickness – the estimated mean cortical thickness, 6) **Section Modulus (Z)** – an indicator of bending strength calculated as the CSMI / the maximum distance between the center of mass and outer cortex, 7) Profile Center Distance-the distance from profile Center of mass to your margin of the cortex, 8) Center of Mass Position-the location of the center of mass based on the cross-section from the medial cortical margin, 9) Buckling Ratio- the relative thickness of the cortex has an estimate of cortical stability in buckling, and 10) Bone Mineral Density (Beck, 2002). By providing information on variables such as CSA and Z, the HSA program can estimate the mechanical bending and torsional strength of a bone. The major benefit of the HSA program is the ability to estimate the bone geometry

of the proximal femur at three clinically significant locations: 1) the Narrow neck, 2) Intertrochanteric, and 3) the Shaft.

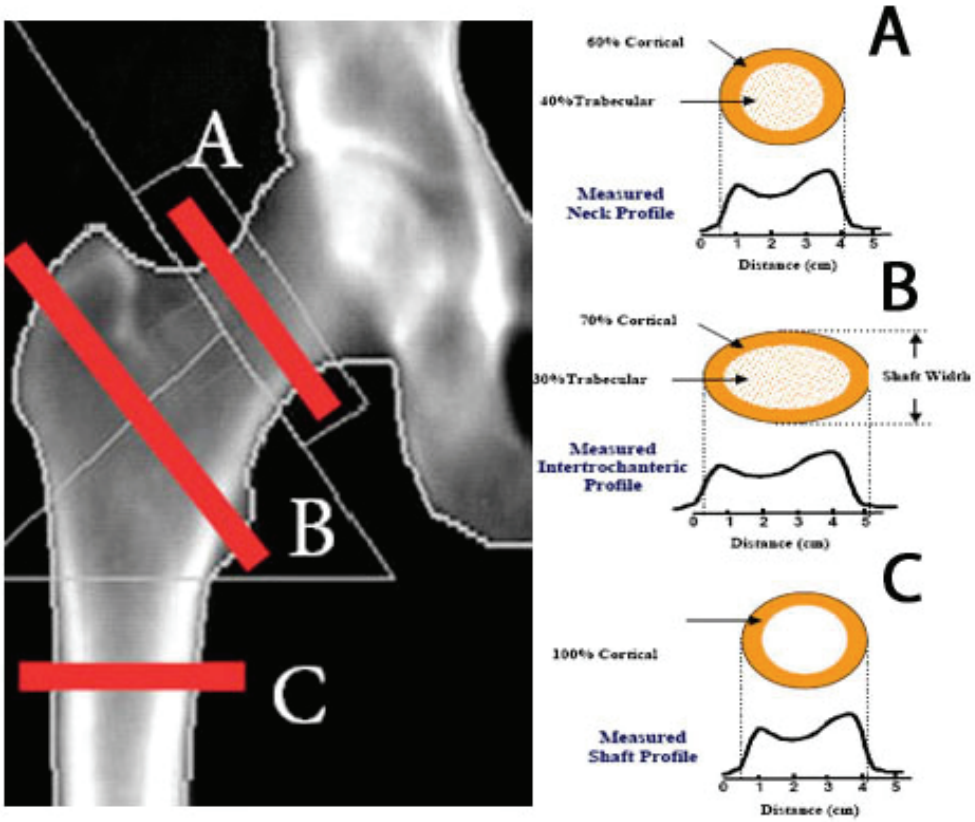


Figure 3.1 – A hip scan from a Hologic DXA scanner. The red lines indicates the position of analysis for the narrow neck (A), intertrochanter (B) and the shaft (C) sites of the proximal femur using the HSA program and the associated bone mass profiles used to estimate the geometric properties. Adapted from (Beck, 2003).

The HSA program is a reliable method provided that the hip positioning and DXA scanners are consistent across subjects (Beck, 2003). Each DXA scanner model is unique in its scanner-dependent errors, making measurements from different models difficult to compare, but maintaining measurements from the same model reduces these inconsistencies (Khoo et al. 2005). In addition, the HSA algorithms require a correctly positioned hip, ensuring the entire margin of the proximal femur is included (Beck,

2003). Scans that fail to do so, may negatively impact the performance of the algorithms, altering the fundamental geometric principles from which they were derived. As a result, of major concern is the accurate placement of the cut planes. These are critical for the comparison of dissimilar individuals, but the use of regional templates have been shown to increase the consistency between scans (Beck, 2003). The short-term precision of HSA derived cross-sectional area and section modulus are comparable to conventional BMD, BMC, and bone area measures at the femoral neck (Khoo et al., 2005). The short-term precision for CSA and Z derived from a hip scan from a Hologic QDR 2000 range from 2.3% to 2.8% and 2.8% to 3.4%, respectively (Khoo et al., 2005). All HSA analyses were completed by a single technician at Johns Hopkins University under the direction of Dr. Thomas Beck.

3.5 Tissue Velocities and Growth Curves

Peak lean tissue velocity (PLTV), peak cross sectional area velocity (PCSAV), peak section modulus velocity (PZV), and peak height velocity (PHV) were determined for each subject. PLTV is the maximum rate of lean tissue mass accrual over time; PCSAV is the maximum rate of change in cross sectional area over time; PZV is the maximum rate of change in Z over the time; and PHV is maximum rate of change in height over time and is a benchmark for maturation.

In order to determine the tissue velocities, a minimum of two reference points was necessary (Baxter-Jones & Sherar, 2006). The raw scores for each tissue at each time point (age of test) served as reference points which provided distance data for height, lean tissue mass, CSA, and Z. The distance values were converted into whole year velocities by dividing the difference between distance values by the time interval (see Table 3.1).

Table 3.1 – Demonstration of how NNCSA velocity was calculated from the distance values. Brackets ( ) identify the equations used to determine the scores within the respective cell.

Age at Test	Whole Year Difference	NNCSA Raw Score	NNCSA Difference	NNCSA Velocity
9.927		1.140		
10.811	(10.811 – 9.927) =0.884	1.202	(1.202 - 1.140) =0.062	(0.062/0.884) =**0.0701**
11.772	(11.772 – 10.811) =0.961	1.263	(1.263 - 1.202) =0.061	(0.061/0.961) =**0.0634**
12.766	(12.766 – 11.772) =0.994	1.42	(1.42 – 1.263) =0.157	(0.157/0.994) =**0.1579**

Each tissue velocity was independently inputted into a cubic spline curve fitting procedure from which PHV, PLTV, PCSAV and PZV were derived (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 at peak tissue growth (see Figure 3.2). The peak tissue velocities were determined as the maximal rate of change over time as determined using this procedure. Upon determining the peak tissue values, the age (from PHV) at which these peaks occurred was determined.

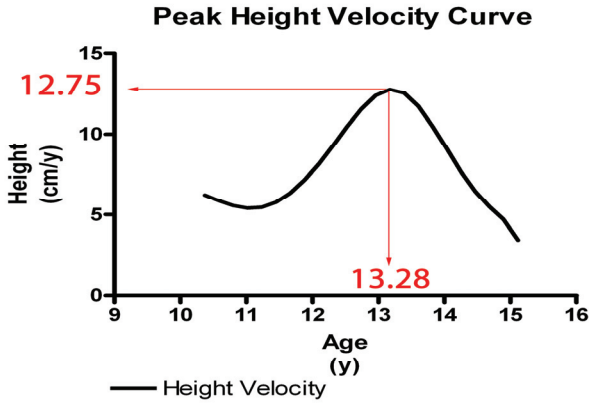


Figure 3.2 – The height velocity curve for subject 1002 created from the cubic spline procedure. The cubic spline estimates the magnitude of the peak velocity (12.75cm/y), including the age at which this peak occurred (13.28 y).

3.6 Statistical Analyses

3.6.1 Variables

From the tissue velocity and growth curves, the ages of PCSAV, PZV and PLTV were determined. These age values were used for the subsequent analyses. The independent variables were gender and tissue type. There are 3 levels for the tissue type factor which included PCSAV, PZV, and PLTV. Each tissue type was assessed as the age from PHV. Three sites were included as the dependent repeated measures. These included the NN, IT and S of the proximal femur.

3.6.2 Hypothesis 1

To take advantage of the between-within subject design, a 2x3 (gender x tissue) factorial MANOVA with repeated measures[♦] was used to test for differences in the age of PLTV, age of PCSAV and age of PZV between males and females at the narrow neck, intertrochanter and femoral shaft sites of the proximal femur. If no significant gender by tissue interaction and no significant gender main effect were observed, males and females were pooled for a subsequent one-factor (tissue type) MANOVA with three dependent repeated measures (sites) to examine tissue main effects. If a significant multivariate main effect was found, a univariate ANOVA was conducted for each site. Site specific differences between the ages of peak tissue velocities were evaluated post hoc by paired t-test comparisons. Data were checked for skewness, kurtosis and sphericity violations. Sphericity violations were assessed using Mauchley's test of sphericity. Sphericity violations were adjusted using the Greenhouse-Giesser method. A p value of 0.05 or below was considered significant. All analyses were performed using SPSS 15.0 for Windows (SPSS, Chicago, IL, USA).

[♦] The sites of assessment (narrow neck, intertrochanter, and femoral shaft) were inputted as the dependent within-subjects repeated measures.

3.6.3 Hypothesis 2

A stepwise multiple regression analysis was used to assess the relationship between peak lean tissue raw scores and the raw scores of each measure of bone strength (CSA and Z) at the narrow neck, intertrochanter, and femoral shaft sites for a combined total of six multiple regression analyses. For each regression analysis, predictor variables were entered in two steps. The age and height at PHV were inputted first, to control for maturation and size, respectively, with peak lean tissue velocity raw score inputted second. The *p* value was set at 0.05.

4.0 Results

4.1 Participants

Participant's characteristics and magnitudes of tissue velocities are presented are presented in Table 4.1.

Table 4.1 – Descriptive statistics of participants and the magnitude of their tissue velocities. (Mean ± SD)

	Males n = 41	Females n = 42
Age PHV (y)	13.15 ± 0.89	11.70 ± 0.98
Height at PHV (cm)	172.1± 18.6	158.3 ± 16.9
Weight at PHV (kg)	57.3 ± 5.6	48.7 ± 4.4
PHV (cm/y)	10.52 ± 1.39	8.57 ± 1.13
NN PCSAV (cm^2/y)	0.39 ± 0.08	0.30 ± 0.08
IT PCSAV (cm^2/y)	0.79 ± 0.22	0.59 ± 0.14
S PCSA V (cm^2/y)	0.59 ± 0.18	0.48 ± 0.23
NN PZV (cm^3/y)	0.25 ± 0.06	0.16 ± 0.04
IT PZV (cm^3/y)	0.95 ± 0.29	0.64 ± 0.15
S PZV (cm^3/y)	0.44 ± 0.21	0.29 ± 011
PLTV (g/y)	8989 ±1571	5272 ± 1090

4.2 Tissue Timing (Hypothesis 1)

As shown in Table C.1, there was no gender by tissue interaction. Thus, genders were pooled for tissue main effect analyses. The subsequent analyses are shown in Appendix C.

There was a significant difference in tissue timing at the NN (p<0.05). As shown in Figure 4.1, the age of PLTV significantly preceded the ages of both PCSAV and PZV (p<0.05) at the NN. The age of PCSAV occurred about 0.19 years subsequent to the age of PLTV, while PZV proceeded the age of PLTV by about 0.33 years.

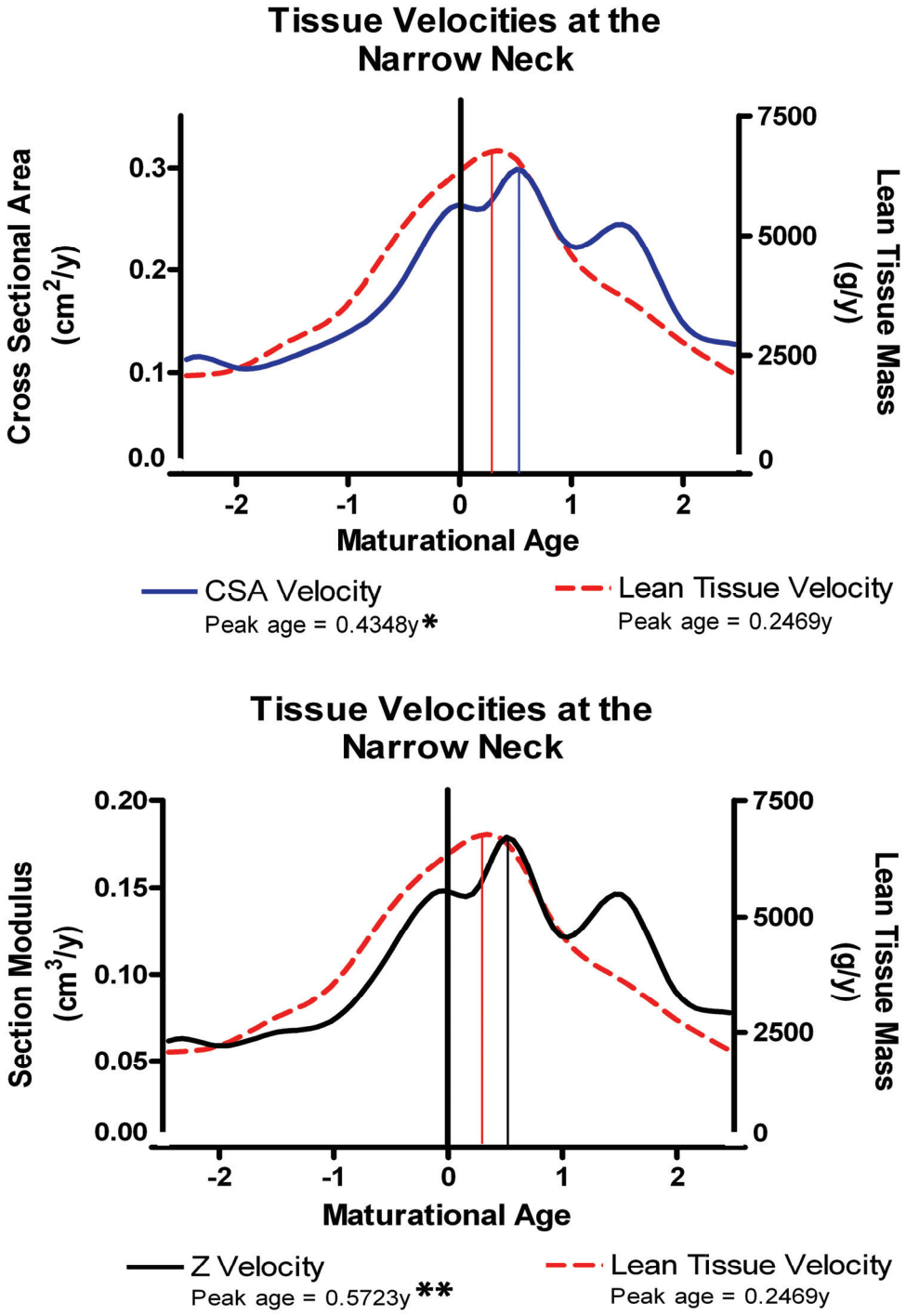


Figure 4.1 – Tissue velocity curves for lean tissue mass, CSA (A) and Z (B) at the NN aligned by maturation. The maturational age of zero (0) represent the age of PHV. The solid drop down lines landmark the maturation age at which the peak tissue velocities occurred.

* indicates a significant difference between the age of PLTV and PCSAV

** indicates a significant difference between the age of PLTV and PZV

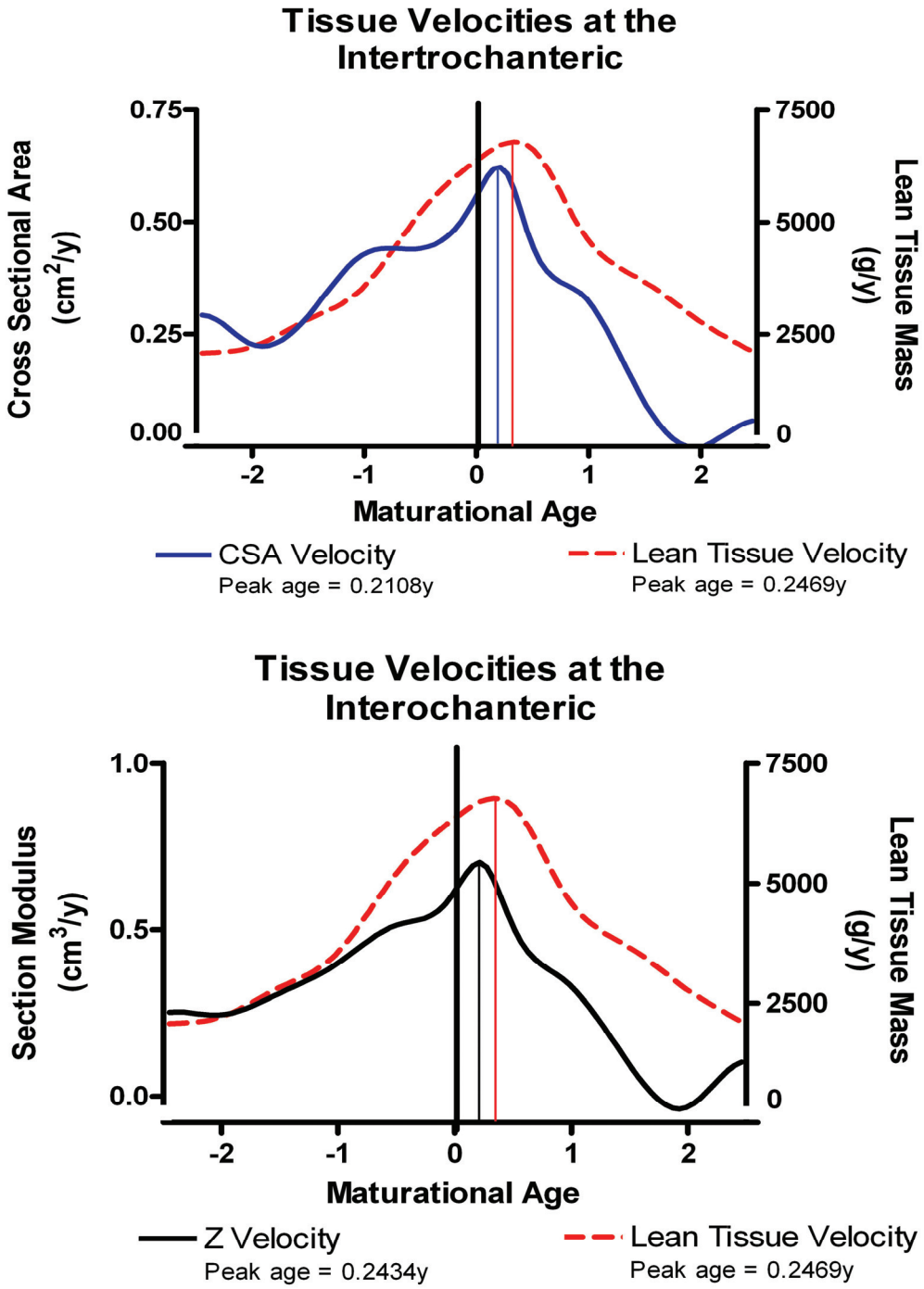


Figure 4.2 – Tissue velocity curves for lean tissue mass, CSA (A) and Z (B) at the IT aligned by maturation. The maturational age of zero (0) represent the age of PHV. The solid drop down lines landmark the maturation age at which the peak tissue velocities occurred.

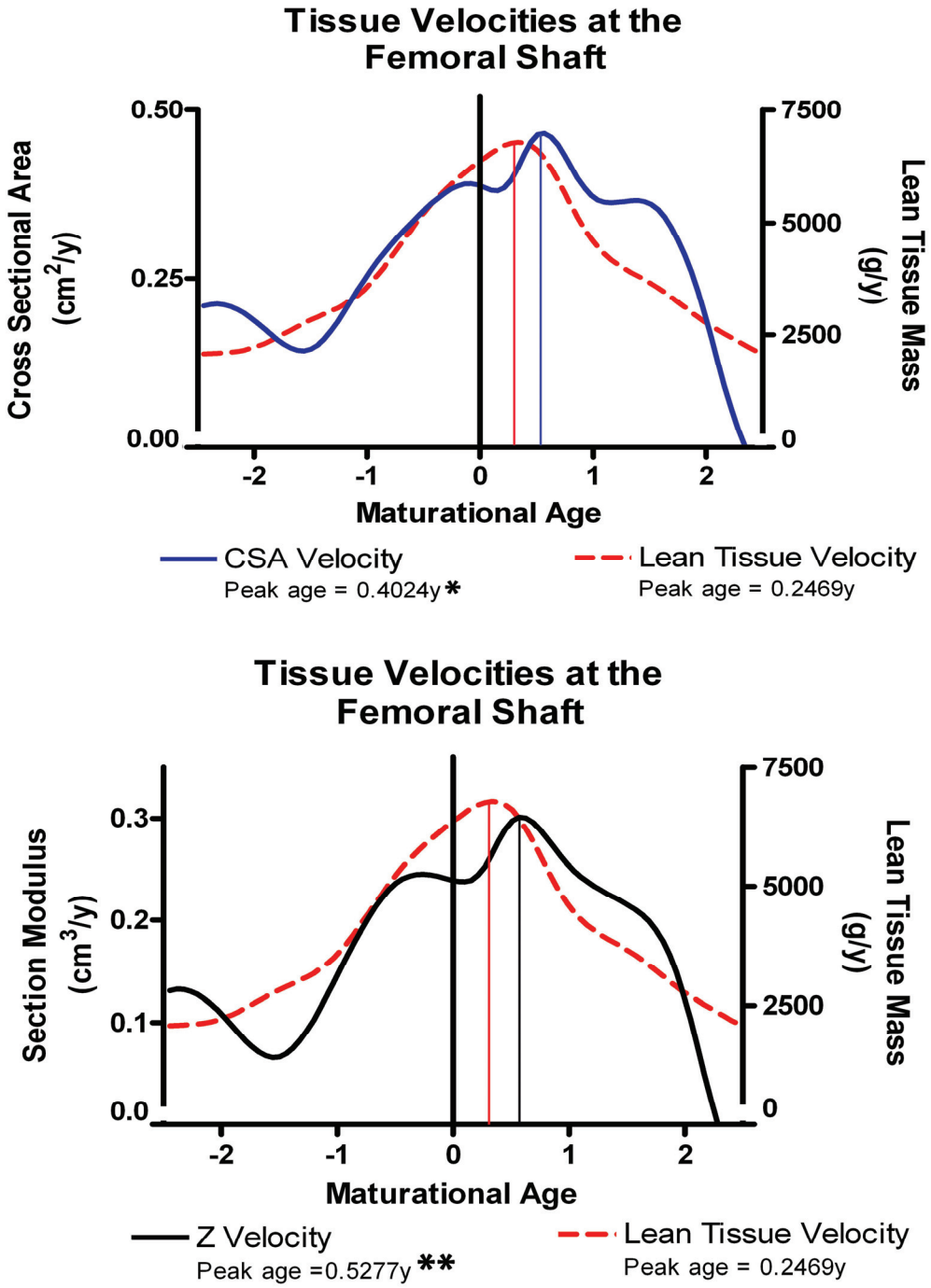


Figure 4.3 – Tissue velocity curves for lean tissue mass, CSA (A) and Z (B) at the S aligned by maturation. The maturational age of zero (0) represent the age of PHV. The solid drop down lines landmark the maturation age at which the peak tissue velocities occurred.

* indicates a significant difference between the age of PLTV and PCSAV

** indicates a significant difference between the age of PLTV and PZV

The tissue velocity curves at the IT are shown in Figure 4.2. There was no significant difference in tissue timing observed at the IT.

Figure 4.3 shows the velocity curves at the S site. Similar to the NN, the age of PLTV preceded the age of both PCSAV and PZ at the femoral shaft ($p < 0.05$). The age of PLTV preceded the age of PCSAV and PZV by 0.16 and 0.28 years, respectively.

4.3 Tissue Magnitude (Hypothesis 2)

After controlling for PHV and height at PHV, multiple regression analysis revealed that PLTV was a significant predictor of both the PCSAV ($p<0.05$) and the PZV ($p<0.05$) at the NN (Table D.1 and D.2). PLTV accounted for 33.8% of the variance of the peak in CSA and 52.7% of variance of the peak in Z at the NN.

Similar to the NN, PLTV was a significant predictor of both PCSAV ($p<0.05$) and PZV ($p<0.05$) at IT site (Table D.3 and D.4). The peak in PLTV accounted for 32.4% and 40% of the variance of the PCSAV and PZV, respectively at the IT site.

PLTV was also a significant predictor of the PCSAV ($p<0.01$) and PZV ($p<0.01$) at S site (Table D.5 and D.6). However, at this site PLTV accounted for 11.1% and 10.8% of the variance seen in PCSAV and PZV, respectively.

5.0 Discussion and Conclusions

5.1 Discussion

The purpose of this study was to examine the relationship of the timing and magnitude between peak lean tissue mass accrual and PCSAV and PZV in males and females at the NN, IT and S regions of the proximal femur. Following Frost's (1987) mechanostat theory, I hypothesized that if the mechanical loading of muscular forces stimulate bone development there would be a differential developmental pattern in the sequential timing between PLTV and both PCSAV and PZV. Secondly, I hypothesized that there would be a positive association between the magnitude of PLTV and PCSAV and PZV. My results support both hypotheses and the theory that the mechanical loading imposed by muscular forces promotes subsequent bone development.

I observed that PCSAV occurred about two months prior to PLTV and PZV occurred about three and a half months after the PLTV. This delay in PCSAV and PZV supports the mechanostat theory, indicating the latency in bone adaptation is a reflection of the skeleton's ability to undergo an adaptive response to the imposed mechanical strains. The positive relationship between lean tissue mass, muscle cross sectional area and muscle strength have been previously reported (Maughan et al., 1983; Madsen et al, 1997; Sale et al., 1987), thus, the increase in muscle mass, represented by PLTV, contributes to greater muscular strain exhibited at the bone. These increased forces deliver greater mechanical strain on the bone, stimulating bone adaptation. Although bone undergoes constant adaptation through the processes of modeling and remodeling, mechanical strains stimulate physiological mechanism that may influence bone formation. Firstly, the mechanical strains, if above the minimum effective threshold

(MES, described earlier), will deliver a stress that is translated to the osteocytes via mechanotransduction (Kohrt et al, 2004). The mechanotransduction triggers a cascade of events, which include, but are not limited to, the excitation of osteocytes, the increase in gene expression of c-*fos* and insulin like growth factors (IGF), and the increase of nitric oxide (NO) and prostaglandin production (Chow, 2000). The release of IGFs, NO, and prostagladins are shown to induce the proliferation and differentiation of osteoblastic cells (Chow, 2000). Osteoblastic cell activity produces osteocalcin and releases collagen precursors which synthesize new bone matrix proteins (Aubin et al, 2006). However this is not an expedient process. The gene expression for bone matrix protein synthesis is maximal 3-5 days after the mechanical strain (Chow, 2000) and the formation of new bone takes between 4-6 months. Thus, a delay between the mechanical strain (increased lean tissue mass) and bone formation is expected. My findings highlight this latency suggesting that the increased mechanical strain, imposed by increased lean tissue mass, stimulates osteogenesis resulting in enhanced estimated bone strength development. Furthermore, this delay in PCSAV and PZV suggests that the increase in muscular forces drives the increases in bone strength development. These results are compatible with the findings of Rauch et al. (2004), who observed that peak lean tissue mass accrual occurred 0.36 and 0.51 years prior to the peak in total body BMC accrual in males and females, respectively. I found that PLTV significantly preceded PCSAV and PZV by 0.19 and 0.33 years, respectively, at the NN and 0.16 and 0.28 years at S. This relationship was not found at the IT. At the NN, lean tissue mass may contribute to increased compressive and bending strains. The increase in lean tissue mass results in greater total body weight which places larger compressive and bending forces on the NN during regular activity.

As observed by Petit et al. (2005), Z was enhanced at the proximal femur in overweight children when compared to their normal weight counterparts, but this improved bending strength was adapted relative to overweight individuals' lean mass with fat mass providing no addition influence. Petit et al. (2005) findings emphasize the contribution of lean mass, rather than fat mass towards enhanced bending strength. Thus, it is likely that the mechanical loading on bone resulting from the considerable addition of lean tissue mass may affect skeletal adaptation at the NN. At the S the influence of lean tissue mass may be more apparent due to direct muscle-bone interactions. There are eight muscles that directly attach to the femoral shaft. Thus it is likely that the direct mechanical loading from the muscle attachments contribute to the geometric adaptations. These findings are consistent with previous data reported by Petit et al. (2008) who examined the changes in bone geometry and structural strength with weight gain in late adolescence. After dividing the groups into stable weight and weight gainers, Petit and colleagues reported both groups had enhanced bone CSA and Z at the femoral shaft and that these geometric changes were appropriately adapted to relative lean tissue mass. Petit et al (2008) suggest that the geometric changes are better reflected by the contribution of lean tissue mass, which is further confirmed with my findings. However at the IT site no significant difference between PLTV and both geometry variables was observed. This result was unexpected as the IT site is a major anatomical landmark for nine muscular attachments. It was theorized that the IT site would result in similar findings observed at the S site. It is possible that the results at the IT may be partially explained by the inherent limitations of the HSA program. The HSA program only assesses the geometric properties within the frontal plane (Beck et al., 1990). The bone adaptation may not be

apparent in the frontal plane, but rather in the sagittal or transversal plane which the HSA program is not designed to assess. In addition, the precision error associated with this region may come into question. According to Khoo et al. (2005), the precision error (CV%) at the IT site for CSA and Z are 2.5% and 3.4%, respectively. The average measurement difference observed in my results was 11.4% for CSA and 17.8% for Z. The error associated with HSA precision is less than the difference noticed between the measurements; therefore, the inability to detect a difference in the peak velocities may not be a result of poor HSA sensitivity. Additionally, the sequential timing between PLTV and both PCSA and PZV did not follow the same pattern observed at the NN and S; the peak bone strength velocities preceded PLTV. The IT is composed of both cortical and trabecular bone. CSA and Z are measures better reflective of the cortical adaptations; however the influences of LTM at the IT may be better represented by trabecular adaptations. Using a finite element model that included ligamentous and muscular forces, Rudman et al. (2006) demonstrated that under physiological loading the majority of the proximal femur undergoes compression and these compressive forces were transferred to the femoral shaft by means of the internal trabecular structure at the IT. The trabeculae at the IT arrange themselves to form an arch-like structure, which transfers the forces to the femoral shaft. LTM may potentiate the alignment of trabeculae, helping transfer the compressive forces. HSA is unable to differentiate between the independent adaptations of cortical and trabecular bone, thus CSA and Z may not truly identify the adaptations occurring at the site. Future research is needed to investigate the independent relationship between LTM and both cortical and trabecular bone.

The second purpose of this study was to examine the relationship between PLTV and both PCSAV and PZV. Results showed that when controlling for maturation and size at PHV, PLTV was a significant and independent predictor of both measures of bone strength velocity at all sites of the proximal femur. PLTV accounted for approximately 11-34% of the variance in PCSAV and 11-53% of the variance in PZV. These findings are similar to those observed by Travison et al. (2008) and Petit et al. (2005). Travison and colleges identified that lean tissue mass was strongly associated with CSA, Z and BMD at the proximal femur in adult males. This relationship was actually enhanced in magnitude once fat mass was entered into the predictive models (Travison et al., 2008). Similarly, Petit et al. (2005) observed that lean tissue mass was a significant and independent predictor of Z at both the NN and S after adjusting for gender, biological maturation and moment arm. Janicka et al. (2007) also reported similar findings using DXA and computed tomography measure at the mid-femoral shaft. These authors noted that lean tissue mass had a strong positive and independent effect on femoral BMC, BMD, CSA, cortical bone area, and cortical bone density. This close relationship between muscle and bone is not surprising as it has been previously theorized that muscle and bone may act as a singular "muscle-bone unit", where bone structure and mass are adapted accordingly to muscle development (Frost, 1987; Schoenau, 2005). At the most basic level, the idea of the muscle-bone unit would suggest that when there is a change in muscle development there will be an equivalent change in bone, whether it is structure or mass. Results of my study support the concept of the "muscle-bone unit" as we showed a significant positive relationship between the magnitude of PLTV and both bone strength velocities.

5.2 Strengths and Limitations

This study was unique because of the longitudinal design and subsequent development of growth curves for the bone strength variables. Cross sectional studies mask the unique feature of individual variability during growth which may result in inaccurate representation of temporal patterning during growth. Secondly, this study controlled for maturation by calculating and aligning subjects on their age at PHV. PHV is the most commonly used maturational indicator in longitudinal studies because it is inexpensive, accurate, and requires no specialized equipment (Baxter-Jones & Sherar, 2006). Many studies use Tanner staging, which bases maturity on the development of secondary sex characteristics. In Tanner staging, the determination of sexual maturity is typically assessed by self assessment, where children and adolescence compare themselves to standardized photos or drawings. The correlations between self reported assessments and physician ratings are moderate to high (Baxter-Jones & Sherar, 2006). However, males typically overestimate their sexual maturation while females underestimate. Tanner stage is commonly used in pediatric literature because it does not require longitudinal measurements and is cost effective. Despite these features, Tanner staging remains unable to align males and females on maturation because the timing of sexual development is considerably different between sexes. PHV serves as a maturational benchmark that exists in both males and females, allowing for gender comparisons of maturity (Baxter-Jones & Sherar, 2006). Thus, PHV remains an unparalleled estimation of maturation in longitudinal studies to estimate and control for maturation. Controlling for maturation is important because individuals mature at

differing rates. By aligning individuals by maturity status, one is able to control for the differential rat, and make comparisons between individuals.

Although there are numerous unique features to this research, the conclusions are limited by several factors. Firstly, total body lean tissue mass was used as a surrogate measure of muscular strength. Although lean tissue mass is correlated with muscular strength (Maughan et al., 1983; Madsen et al, 1997; Sale et al., 1987) it may not truly reflect the development of muscular strength. The HSA program also has inherent limitations. As mentioned previously, DXA images are often noisy and blurred resulting in the difficulty of locating precise edge margins (Beck, 2007). In addition, the positioning of femur is important as small changes in femur rotation have a large effect on the geometric dimensions (Beck, 2007). Positioning of the proximal femur was done with care to limit these potential errors; nevertheless, it is difficult to position the hip consistently in repeated measures over time. Furthermore, during childhood and adolescence rapid growth occurs, making accurate reposition and assessment more variable. Lastly, HSA calculations are based on two major assumptions related to the shape and mineralization of the bone. At the NN and S, the geometric properties are modeled using a circular annuli. At the IT, the geometric properties are modeled as an elliptical annulus (Beck, 2002). As a result, CSA and Z measurements may be underestimated. Similarly, mineral properties are assumed using adult bone mineralization. This assumption may further underestimate CSA and Z specifically in a pediatric population (Bonnick, 2007).

5.3 Future Research

Further research is required to investigate the timing and tempo of bone strength as it relates to muscle development. These future endeavors should investigate the contributions of periosteal and endosteal development on PCSAV and PSZ. As the methods of assessing bone strength become more accessible and cost effective, it would be beneficial to confirm these findings with other, potentially more accurate tools, to further investigate the muscle-bone strength relationship. In addition, future research is required to examine the relationship between the other elements of bone strength, such as tissue architecture and porosity, and muscle development, preferably using a longitudinal design that will capture the changes that occur during growth.

5.4 Summary/Conclusions

The following were the hypotheses examined in this study.

Hypothesis 1

Peak lean tissue mass velocity will precede peak bone strength velocities at both the proximal femur and femoral shaft.

Sub hypotheses

i) Peak lean tissue mass velocity will precede peak bone cross sectional area velocity at the narrow neck, intertrochanter and femoral shaft

(ii) Peak lean tissue mass velocity will precede peak bone section modulus velocity at the narrow neck, intertrochanter and femoral shaft.

iii) There will be no gender differences in the sequential timing of peak lean tissue mass velocity and peak bone strength velocities

Hypothesis 2

There will be a positive significant relationship between the magnitude of lean tissue mass velocity and the magnitude of bone cross sectional area and section modulus velocities at the narrow neck, intertrochanter and femoral shaft.

My findings support the first hypotheses and sub hypotheses, identifying that the peak in lean tissue mass velocity occurred prior to the peak in both CSA and Z velocities at the NN and S sites of the proximal femur, and this sequential timing was not significantly different between genders.

My findings also support the second hypothesis, indicating there was a significant positive relationship between the magnitude of PLTV and both PCSAV and PZV at all the sites of the proximal femur.

The mechanostat theory proposes that the mechanical loading of muscle stimulates subsequent bone development. This theory was examined by exploring the relationship between muscle mass and bone geometry, specifically, investigating the timing and magnitude of PLTV and PCSAV and PZV in males and females at the NN, IT and S regions of the proximal femur. A differential developmental pattern was observed between the sequential timing of PLTV and both PCSAV and PSZV. This developmental pattern was also similar in both genders when aligned by maturation. Additionally, the magnitude of PLTV significantly predicted PCSAV and PZV. These findings support my hypotheses and provide evidence supporting the mechanostat theory.

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Appendix A
Consent Form

Parent's statement:

I understand the purpose and procedures of this study as described above and I voluntarily agree to allow my child to participate. I understand that at any time during the study, he or she will be free to withdraw without jeopardizing any medical management, employment or educational opportunities. I understand the contents of the consent form, the proposed procedures and possible risks.

I have had the opportunity to ask questions and have received satisfactory answers to all inquiries regarding this study.

Signature of Parent or Guardian Date

Subject's Statement:

I understand the purpose and procedures of this study as described above and I voluntarily agree to participate. I understand that at any time during the study, I will be free to withdraw without jeopardizing any medical management, employment or educational opportunities. I understand the contents of the consent form, the proposed procedures and possible risks.

I have had the opportunity to ask questions and have received satisfactory answers to all inquiries regarding this study.

Signature of Subject Date

Skeletal fragility in older adults appears to be a function of peak bone mass attained in early adult years. Nutritional factors and mechanical loading factors during the growing years may have an impact on the attainment of an optimal level of bone mass, this is the rationale behind the current study on children.

Heredity is another factor that may be involved in osteoporosis. For this reason provision will be made for any mother, who is interested, to have her own bone density status evaluated. This will provide each participating mother with the most accurate indication of bone density status currently available, and when linked with the data on their child it will provide us with valuable generational information.

As the mother of a child in the bone density study, I am interested __ I am not interested ____, in having my own bone mineral content and skeletal status evaluated at some time during the next year.

Please return this form to Mr. Kikcio, Principal, Prince Philip School by Wednesday April 17, 1991. If you were unable to attend the parent information meetings and have questions about the proposed study, Mrs. Barb Mooney at Alvin Buckwold School (374-0811) can provide you with additional information or you can contact Dr. Don Bailey, the chief investigator for this study, at the University of Saskatchewan (966-6524).

Appendix B
Ethics Approval

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB)

Certificate of Approval

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

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
College of Kinesiology
105 Gymnasium Place
Saskatoon SK S7N 5C2

SPONSORING AGENCIES
CANADIAN INSTITUTES OF HEALTH RESEARCH (CIHR)

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

APPROVAL DATE	EXPIRY DATE	APPROVAL OF
20-Oct-1989	31-Oct-2007	Letter to Participant and Study Results Report (Oct-2007)

Full Board Meeting ☐

Delegated Review ☒

CERTIFICATION
The study is acceptable on scientific and ethical grounds. 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

OCT 15 2007
Signature Date

Please send all correspondence to:

Ethics Office
University of Saskatchewan
Room 305 Kirk Hall, 117 Science Place
Saskatoon SK S7N 5C8
Telephone: (306) 966-4053 Fax: (306) 966-2069

Appendix C
Tables of the Factorial ANOVA Analyses

Table C.1 – 2x3 (Gender x Tissue) Factorial MANOVA with repeated measures Multivariate Analyses Output

Effect			Value	F	Hypothesis df	Error df	Sig.
Between Subjects	Intercept	Pillai Trace	0.376	15.840	3	79	0.000
		Wilks' Lambda	0.624	15.840	3	79	0.000
		Hotelling's	0.602	15.840	3	79	0.000
	Gender	Pillai Trace	0.007	0.175	3	79	0.913
		Wilks' Lambda	0.993	0.175	3	79	0.913
		Hotelling's	0.007	0.175	3	79	0.913
Within Subjects	Tissue	Pillai Trace	0.175	2.681	6	76	0.021
		Wilks' Lambda	0.825	2.681	6	76	0.021
		Hotelling's	0.212	2.681	6	76	0.021
	Tissue * Gender	Pillai Trace	0.088	1.228	6	76	0.302
		Wilks' Lambda	0.912	1.228	6	76	0.302
		Hotelling's	0.097	1.228	6	76	0.302

Table C.2 – Mauchly's Test of Sphericity of tissue velocities separated by site

Within Subjects Effects	Measure	Mauchly's W	Approx. Chi-Square	df	Sig	Epsilon Greenhouse-Geisser
Tissue	NN	.933	5.657	2	0.059	.937
	IT	.890	9.428	2	0.009	.901
	Shaft	.697	29.261	2	>0.001	.767

Table C.3 - Gender pooled one-factor (Tissue) MANOVA with repeated measures Multivariate Analyses Output

Effect			Value	F	Hypothesis df	Error df	Sig.
Between Subjects	Intercept	Pillai Trace	0.376	16.046	3	80	0.000
		Wilks' Lambda	0.624	16.046	3	80	0.000
		Hotelling's	0.602	16.046	3	80	0.000
Within Subjects	Tissue	Pillai Trace	0.174	2.707	6	77	0.019
		Wilks' Lambda	0.825	2.707	6	77	0.019
		Hotelling's	0.211	2.707	6	77	0.019

Table C.4 - Gender pooled one-factor (Tissue) MANOVA with repeated measures Univariate Analyses Output

Source	Measure	Sum of Squares	df	Mean Square	F	Sig.
Tissue	NN	4.430	2	2.215	7.739	.001
	IT *	.066	1.802	.036	.179	.814
	Shaft *	3.286	1.535	2.141	4.005	.030
Error(Tissue)	NN	46.939	153.636	.306		
	IT *	30.056	147.764	.203		
	Shaft *	67.285	125.845	.535		

* indicates site values were adjusted using Greenhouse-Giesser method

Table C.5 – Post Hoc Test at the Narrow neck and Femoral Shaft (Paired T-Test)

Paired Test	Mean Difference	Std. Deviation	Std. Error Mean	t	df	Sig. (2-tailed)
NNCSAV-PLTV	0.188	0.655	0.072	2.615	82	0.01
SCSAV - PLTV	0.156	0.897	0.099	1.579	82	0.01

Appendix D
Tables of the Multiple Regression Analyses

Table D.1 – Regression model summary for cross sectional area velocity at the narrow neck

Model

Model	R	R Squared	Adjusted R Squared	Std. Error of the Estimate
1	0.588[a]	0.346	0.338	0.074

a – Predictors(Constant), Peak lean tissue velocity

ANOVA [b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	0.231	1	0.231	42.775	>0.01
	Residual	0.438	81	0.005		
	Total	0.669	82			

b – Dependent variable: Peak cross sectional area velocity

Coefficients [b]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	0.179	0.026		6.805	>0.001
	PLTV	>0.001	>0.001	0.588	6.540	>0.001

b - Dependent variable: Peak cross sectional area velocity

Table D.2 – Regression model summary for section modulus velocity at the narrow neck

Model

Model	R	R Squared	Adjusted R Squared	Std. Error of the Estimate
1	0.730[a]	0.533	0.527	0.500

a – Predictors(Constant), Peak lean tissue velocity

ANOVA [b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	0.231	1	0.231	92.468	>0.01
	Residual	0.203	81	0.003		
	Total	0.434	82			

b – Dependent variable: peak section modulus velocity

Coefficients [b]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	0.041	0.018		2.274	0.026
	PLTV	>0.001	>0.001	0.730	9.616	>0.001

b - Dependent variable: peak section modulus velocity

Table D.3 – Regression model summary for cross sectional area velocity at the intertrochanter

Model

Model	R	R Squared	Adjusted R Squared	Std. Error of the Estimate
1	0.577[a]	0.332	0.324	0.170

a – Predictors(Constant), Peak lean tissue velocity

ANOVA [b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.160	1	1.160	40.332	>0.01
	Residual	2.331	81	0.029		
	Total	3.491	82			

b – Dependent variable: peak cross sectional area velocity

Coefficients [b]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	0.327	0.061		5.386	>0.001
	PLTV	>0.001	>0.001	0.577	6.351	>0.001

b - Dependent variable: peak cross sectional area velocity

Table D.4 – Regression model summary for section modulus velocity at the intertrochanter

Model

Model	R	R Squared	Adjusted R Squared	Std. Error of the Estimate
1	0.636[a]	0.407	0.400	0.213

a – Predictors(Constant), Peak lean tissue velocity

ANOVA [b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.528	1	2.528	55.583	>0.001
	Residual	3.683	81	0.045		
	Total	6.211	82			

b – Dependent variable: peak section modulus velocity

Coefficients [b]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	0.256	0.076		3.350	0.001
	PLTV	>0.001	>0.001	0.638	7.455	>0.001

b - Dependent variable: peak section modulus velocity

Table D.5 – Regression model summary for cross sectional area velocity at the femoral shaft

Model

Model	R	R Squared	Adjusted R Squared	Std. Error of the Estimate
1	0.350[a]	0.122	0.111	0.205

a – Predictors(Constant), Peak lean tissue velocity

ANOVA [b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	0.472	1	0.472	11.277	0.001
	Residual	3.390	81	0.042		
	Total	3.862	82			

b – Dependent variable: peak cross sectional area velocity

Coefficients [b]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	0.304	0.073		4.140	>0.001
	PLTV	>0.001	>0.001	0.350	3.358	0.001

b - Dependent variable: peak cross sectional area velocity

Table D.6 – Regression model summary for section modulus velocity at the femoral shaft

Model

Model	R	R Squared	Adjusted R Squared	Std. Error of the Estimate
1	0.344[a]	0.118	0.108	0.174

a – Predictors(Constant), Peak lean tissue velocity

ANOVA [b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	0.331	1	0.331	10.880	0.001
	Residual	2.463	81	0.030		
	Total	2.794	82			

b – Dependent variable: peak section modulus velocity

Coefficients [b]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	0.167	0.062		2.670	0.009
	PLTV	>0.001	>0.001	0.344	3.298	0.001

b - Dependent variable: peak section modulus velocity