VOLUMETRIC BONE MINERAL DENSITY AND BONE STRENGTH BASED ON SPORT IN ELITE FEMALE AND MALE ATHLETES

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ABSTRACT

Introduction: 1 in 2 women and 1 in 4 men will suffer from osteoporosis and/or osteoporotic fracture in their lifetime. Osteoporosis is a condition characterized by low bone mass and bone structural deterioration, which leads to a decrease in bone strength and an increase in fracture susceptibility. Physical activity is a critical element for building a strong skeletal structure and offsetting bone fragility in later life. Thus, there is interest in identifying activities that are osteogenic.

Purpose: To investigate differences in bone mineral density (BMD) and bone strength in elite male and female athletes.

Methods: A total 160 elite collegiate (18-25 years) ice hockey (male=19, female=21), swimming (male=13, female=17), soccer (female=15), and running (male=19, female=22) athletes and non-active controls (male=15, female=19) were studied. Areal (aBMD) and volumetric (vBMD) bone mineral densities and bone strength were assessed via dual energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT).

Results:

Part 1: Females in the weight-bearing sports, soccer and ice hockey, were associated with the highest adjusted total body and lumbar spine aBMD compared to swimmers and controls. At the distal tibia, the soccer group demonstrated significantly greater trabecular vBMD and bone strength index compared to all other groups. At the distal radius, ice hockey players were associated with greater bone strength index compared to swimmers and controls. There were no differences between the soccer and ice hockey groups in any of the tibia measurements.

Part 2: Gender differences were found in relation to bone strength of the tibia between elite male and female athletes. Males in weight-bearing sports (ice hockey and running) had greater section modulus, strength strain index at the tibial shaft and tibial mid-shaft compared to their female counterparts. No differences were evident between males and females of the non-weight bearing swimming group.

Summary:

Weight-bearing sports such as hockey, soccer, and running are beneficial activities to enhance bone mass and strength in males and females. In these populations, skeletal adaptations appear to be influenced by their loading environment. However, in the weight-bearing sports, males had greater bone strength at all sites compared to females. This is congruent with findings from the general non-active population. Even after adjustments the differences existed. Strength differences may help explain the discrepancies in fracture rates between males and females.

TABLE OF CONTENTS

LIST OF TABLES

Review of the Literature/Methods and Materials

Manuscript One

Manuscript Two

LIST OF FIGURES

Review of the Literature

Manuscript One

Manuscript Two

GLOSSARY OF TERMS AND ABBREVIATIONS

TERM	DEFINITION
Areal bone mineral density (aBMD)	The total grams of bone mineral per unit (g/cm^2) of bone area commonly assessed by dual energy X-ray Absorptiometry (also referred to as Bone Mineral Density).
Basic Multi-Cellular Unit (BMU)	During remodeling, involves the *coupled* action of osteoclast resorption and osteoblast formation.
Bending	When force is applied to the bone, the convex surface of the bone experiences tension and the concave surface experiences compression [Khan 2001]. Example: Forearm during a bicep curl.
Bone	A complex system composed of hard connective tissue consisting of cells affixed in a matrix of organic (type I collagen), inorganic (mainly hydorxyapatite crystals composed of calcium and phosphate), and water.
Bone Architecture	Refers to the size and shape of bone. Often describing the properties of cortical and trabecular cone (e.g. cortical thickness).
Bone Geometry	Property of bone that refers to its mineralized tissue configuration, placing great importance on the amount of tissue and their distribution throughout the particular bone (e.g. cross-section).
Bone growth	Enlargement of the skeleton through cellular activity and environmental strain (e.g. mechanical loading and muscle activity).
Bone mineral content (BMC)	The total grams (g) of bone mineral within the scanned region. A major determinant of the material properties of bone. Directly associated with bone strength and stiffness, accounting for more than 80% of the strength component (also referred to as *Bone Mass*).
Bone Modeling	Process in which bone grows and becomes stronger through an organized bone cell activity of osteoblasts and osteoclasts. Increases bone strength by increasing or adding mass and

	improving the existing geometry.
Bone Stiffness	Refers to a bone's elastic properties. Physiologically important because it describes a bone's ability to withstand a load, accommodate the load, then return to its original shape.
Bone Strength	"Ultimate load" bone can withstand until failure. Intrinsic quality or property that is independent of size.
Bone Strength Index (BSI)	Measures of the compression strength at the distal tibia and/or radius. Calculated by multiplying the $Total_{area}$ x $(Total_{density})^2$ of the site of interest.
Bone Structure	Properties of bone including: size, shape and distribution of bone material.
Bone Turnover	Blanket term used to describe the adding or subtracting bone via growth, modeling and remodeling.
Compression Stress	Stress produced when two forces are aimed toward each other along a straight line [Khan 2001]. Causes the bone to both shorten and widen in order to absorb maximal stress. Example: Hip joint during walking.
Cortical Bone	The superficial thin layer of bone, forming the external portion of long bones and consisting mainly of dense, calcifies tissue.
Cortical Area, mm^2	Total amount of cortical bone within the cross-section. Measure obtained from pQCT.
Cortical Density, mg/cm^3	Amount of cortical material averaged over the cross-section. Measure obtained from pQCT.
Cortical Thickness, mm	Thickness of the cortice from edge to edge, not including the marrow area. Measure obtained from pQCT.
Cross-sectional Moment of Inertia (CSMI)	Property of bone that refers to its resistance to bending. The distribution of the material (or area) around the given axis or strength to "lightness" ratio.

Dual Energy X-ray Absorptiometry (DXA)	Bone measurement modality that uses two contrasting X-ray beams to yield a two dimensional representation of the skeleton. DXA calculates the attenuation values of photons that pass from the X-ray tube through the measurement site of interest. Outcome variables include bone mineral content (BMC) and areal bone mineral density (aBMD).
Endosteum	Inner surface of cortical bone that faces bone marrow.
High-impact loading	Characterized by both high rate and high magnitude loadings. Evidence suggests that loadings that include strain at a high rate and high peak force in diverse movements may be the most effective in enhancing bone formation, especially in girls/women. Recent data suggests programs that incorporate high-impact loadings are effective in maintaining and improving bone mass and preventing bone loss due to age.
High-magnitude loading	Characterized by high peak forces of loading. May have a greater influence on bone mass versus large numbers of loading cycles. Bone response can be achieved with high magnitude and a low number of loading cycles.
Lamellar bone	The normal type of adult bone. Replaces immature woven by arranging its collagen in a repeating fashion along the lines of force. Resembles multi-layer plywood.
Low-magnitude loading	Characterized by low peak forces of loading. When low, the number of loading cycles may become an important factor to maintain bone structure.
Macromodeling	Process in which cells and collagen are organized. Mainly responsible for the size, shape, and strength of bones.
Mechanical Loading	Refers to the applied forces placed on the skeleton or individual bones via forms of physical movement or activity.
Micromodeling	Process in which cells and collagen are organized. Responsible for determining what kind of tissue is

	to be formed. Even after growth stops this process continues.
Odd-impact loading	Charactertized by rapid accelerating and decelerating movements with a high magnitude of non-conventional movements of the body and hips.
Osteoblasts	Cell formation responsible for bone formation. Produce a bone matrix composed of collagen and other substances, which ultimately become calcified.
Osteoclasts	A multinucleated bone cell responsible for the removal of bone (resorption).
Osteoporosis	Characterized by low bone mass and micro-architectural deterioration resulting in a reduction in bone strength and increased fracture risk.
Peripheral Quantitative Computed Tomography (pQCT)	CT-type bone measurement modality that provides a three-dimensional representation of a particular site of interest. Measures the attenuation of radiation as it passes from the source to the site of interest. Outcome measures include BMC, BMD, bone and muscle cross-sectional area, bone strength, and unlike DXA, has the ability to differentiate between types of bone and may be more sensitive to changes in bone due to physical activity.
Periosteum	Outer surface of cortical bone facing tissue and muscle. Bone appositional site during growth and development.
Polar moment of inertia (*J*)	Used to determine the amount of stress during torsional loading and is derived by summing any two cross-sectional moments of inertia (I_{max} and I_{min}) around the axes.
Remodeling	Process in which old bone is removed and new bone is formed at the same site at variable time intervals.
Repetitive loading	Characterized by continuous loading patterns for an extended duration. Some exercise studies have shown repetitive loading (e.g. walking) can increase

	bone mass, however others have found no significance.
Repetitive non-impact loading	Loading modality characterized by the ability to elicit great numbers of musculoskeletal movements, however lacking ground or surface impacts.
Resorption	Process in which bone is removed via osteoclast activity.
Second Moment of Inertia	Refers to the amount of deflection under loading conditions.
Section Modulus (Z)	Indicator of long bone bending strength. Measured by pQCT and calculated by dividing *I* (or CSMI) by the maximum distance from the bending axis to the outer surface in the bending plane.
Shear stress	Stress produced when two forces are aimed parallel to one another but not along the same line [Kahn 2001]. Example: Stopping quickly on a soccer field. The foot is stationary, however body mass is still motion, placing stress on the tibia.
Strain	Describes the deformation of bone from its original shape under certain loading conditions. It is equal to the change in length divided by the original.
Strain cycles	Refers to the number of loading bouts. Less important than magnitude, however there are also a certain number of cycles to maintain bone structure. May be more important if magnitude is low. Bone strength is increased if bouts are shorter and separated compared to long continuous bouts.
Strain distribution	Refers to the placement of the strain on particular bone. Bone tends to adapt better if strains deviate from normal loading patterns.
Strain magnitude	Magnitude or size of the load placed on the bone during a particular bout. There is a Minimum Effective Strain (MES) to maintain bone structure (200-2500 $\mu\varepsilon$).
Strain Rate	Rate at which the load is applied. Proportional to

	the dynamic load magnitude.
Stress	Refers to the intensity of the load applied. The force applied per unit area (classified as compressive, tensile, or shear). Obtained by dividing the force and the area of bone in which it is applied.
Tanner Staging	A self-report method of assessing the stage of physical maturity for boys (pubic hair development) and girls (breast stage development)
Tensile stress	Stress produced when two forces are aimed away from each other along a straight line [Khan 2001]. Example: The patella of the knee being pulled both by the quadriceps muscle and the patellar tendon at the same time.
Torsion	Occurs when shear stress is experienced along the length of the bone.
Total Area, mm^2	Total bone cross-sectional area. Includes the cortex and the marrow cavity. Measure obtained from pQCT.
Trabecular Bone	Cancellous bone, forming the internal component of bone consisting of both horizontal and vertical plates.
Trabecular Area, mm^2	Total amount of trabecular bone within the cross-section. Measure obtained from pQCT.
Trabecular Density, mg/cm^3	Refers to the density (vBMD) in relation (averaged over) to the area of the measured cross-section. Measure obtained from pQCT.
Total Density , mg/cm^3	Total density. Includes both the cortical and trabecular compartments. Measure obtained from pQCT.
Volumetric Bone Mineral Density (vBMD)	Measure of the volumetric tissue density of appendicular bone. As measured by pQCT can displayed in cortical (CoA), trabecular (TrbD), or total bone mineral density (ToD).
Woven bone	Immature bone characterized by random arrangement of collagen.

Yield point	Represents the transition, above which stresses begin to cause permanent damage (or material failure) to the bone structure.
Young's or the Elastic Modulus	Determines the amount of deformation in a bone for a given applied load.

ABBREVIATION	DEFINITION
DXA	**Dual Energy X-ray Absorptiometry.**
aBMD	Areal Bone Mineral Density.
BMC	Bone Mineral Content. The total grams (g) of bone mineral within the scanned region.
pQCT	**Peripheral Quantitative Computed Tomography (XCT 3000).**
BSI, mg^2/mm^4	Bone strength index. Measure of the compression strength at the distal tibia area. BSI = $Total_{area}$ x $(Total_{density})^2$
CSMI	Cross- Sectional Moment of Inertia. The ratio, or the distribution of the material (or area) around the given axis.
MCSA	Muscle Cross-Sectional Area. Refers to the amount of non-bone tissue in the cross-section (fat/lean mass).
SSI, mm^3	Strength-strain index.
Z, mm^3	Section Modulus. Indicator bending strength.
vBMD	Volumetric bone mineral density. Measure obtained from pQCT.

CHAPTER 1 INTRODUCTION & STUDY PURPOSES

1.1 Introduction

Osteoporotic fractures have become a major global health concern. It is estimated 200 million people worldwide are currently suffering from the disease[1]. In the United States alone, experts predict osteoporosis will affect more than 10 million men and women by 2010 and 14 million by 2020[2]. Furthermore, 30-50% of women and 15-30% of men will suffer an osteoporotic fracture in their lifetime[3]. Healthcare costs related to osteoporosis currently exceed 17 billion per year and are estimated to reach 45 billion by 2020 and could exceed 100 billion by 2040[4,5]. Most importantly, fractures such as those of the hip are a significant cause of morbidity and mortality in older adults.

Osteoporosis is referred to as a “silent” disease, often exhibiting no signs or symptoms until a fracture(s) occurs. The condition is characterized by low bone mass and micro-architectural deterioration which leads to a decrease in bone strength and an increase in fracture susceptibility. It is well established that high peak bone mass is associated with reduced risk of osteoporotic-related fractures later in life[6,7]. Typically, strategies to combat the disease have focused on older populations, and most interventions have been pharmacological in nature, targeting the rate of bone turnover or rate of bone loss. In women, there is some evidence that drug therapy may reduce fracture risk by as much as 50%, but far less is know of its effectiveness in men[8]. In addition, pharmacological approaches appear to have only protective effects for those already suffering from osteoporosis, often possess negative physiological side effects, and are extremely costly. Therefore, it is important to develop safe preventative strategies that are also inexpensive and widely available.

Mechanical loading, primarily through physical exercise, is a viable preventative tool and may be the most cost-effective method to counteract low bone mass. Bones adapt their strength to the mechanical loadings they experience. These loadings are a result from the forces created by movement (e.g. ground reaction forces) and muscle contractions. Certain loading types are of great importance in initiating the adaptive response of bone (increasing bone mass and strength) in both animals and humans[9-12]. Weight-bearing loads, such as those characterized by high-magnitude and high-impact, are generally more osteogenic than

those lacking sufficient weight-bearing movements[9,10,13-16]. It is well established that loadings, specifically inherent in many sports, increases both bone mineral mass and bone strength at loaded sites[14-20]. Athletes participating in high impact and/or odd-impact sports, such as gymnastics[16,21] and soccer[22,23], tend to yield stronger bone structures than those in less or non-weight bearing sports, like cycling[24] and swimming[9,13,25]. However, thus far, the majority of evidence is predominantly based on adult athletes and data (mainly areal bone mineral density or aBMD) derived by dual energy X-ray absorptiometry (DXA). Interpretation of bone structure and strength, using DXA-derived variables (BMC and aBMD) alone, may be somewhat ambiguous[26]. Due to its planar nature, DXA fails to adequately assess the architecture and structure of bone properties that have been suggested to influence fracture rates[26-28]. DXA is also unable to account for changes that occur during growth (bone size and geometry) as well as those induced by mechanical loading[28]. Peripheral quantitative computed tomography (pQCT) is a recently developed bone measurement modality that provides a three-dimensional representation of a particular site of interest. More importantly, pQCT has the ability to differentiate between types of bone, can yield an estimate of bone strength, has the ability to assess the muscle-bone relationship, and may be more sensitive to changes in bone due to physical activity.

1.2 Study Purposes

The purpose of this cross-sectional study was to expand and add to the mostly DXA-based information as well as deepen our understanding of the influence of different physical activities on bone structure in male and female athletes. Exploratory in nature, it sought to examine which of the targeted sports appears to be the most osteogenic, explore differences in bone strength in the specified sites between sports as well as between males and females participating in the same sport. In the following sections, this thesis will discuss relevant background literature (Chapter 2), including the skeleton and its important components, the process of adaptation as well as other important influences (e.g. environmental) that affect skeletal growth and maintenance throughout life. Chapter 3 will present the methods and materials used to conduct the overall study. Chapter 4 will provide the research findings from

two developed manuscripts that will potentially be submitted for publication. Finally, Chapter 5 will integrate and summarize the overall findings and discuss future research potential.

CHAPTER 2 REVIEW OF THE LITERATURE & STUDY HYPOTHESES

2.1 Importance of Peak Bone Mass, Osteoporosis Risk, and Childhood

Two important concepts throughout this body of work in terms of the skeleton are *peak bone mass* and *bone strength*. Both are essential underlying factors that ultimately influence susceptibility to osteoporosis and fracture risk. The following section will focus mainly on the importance of peak bone mass and its influence on osteoporosis, while bone strength will be discussed in greater detail in a later section.

"Peak bone mass" refers to the amount of bone mineral mass accumulated by the end of growth. Its accrual is affected by a multitude of factors including genetics, gender, ethnicity, nutritional status, hormonal factors, exposure to various negative environmental risk factors (e.g. smoking, alcohol consumption, etc.) as well as physical activity levels[29]. It has been suggested that peak bone mass may be the single most important factor for the prevention of osteoporosis and related fractures later in life[30]. An increase in just 10% of mass during growth could reduce the risk of osteoporosis-related fracture by as much as 50% and delay the onset of osteoporosis by as much as 13 years[31-34]. There is also evidence that the risk for fracture after age 60 may be directly related to both structural (e.g. size and shape) and biomechanical (e.g. strength) properties acquired during the first few decades of life[29].

Recent evidence is pointing to childhood as the most critical time period to combat osteoporosis. It is estimated that children accrue approximately 25% of their total adult bone mass in a 2-year period surrounding adolescence, equaling as much as they will lose during later life[35,36]. Furthermore, growing bone appears to have a greater osteogenic (bone forming) response to mechanical loading than mature bone[35,37]. And although over 50% of peak bone mass is governed by heredity, lifestyle factors such as physical activity greatly influence bone mineral accrual during the growing years[29,38]. In the following sections, this thesis will discuss the skeleton, its components, and how it adapts its structure to mechanical loading. In addition,

it will examine the key factors of mechanical loading as well as other important influences essential for the development of a strong, healthy skeletal structure.

2.2 Function and Important Properties of Bone

Bones serve many functions including levers for locomotion, attachment sites for tendons, ligaments and muscles, protect and support vital organs (e.g. the heart), serves as a reservoir for calcium, and also a site for the formation of blood cells[39]. However, the foremost function(s) of bone are to support loads, and provide a means for efficient locomotion; all while resisting fracture. Bone is a complex tissue that oftentimes has to accommodate contradictory needs. Unlike other organs or tissues, it is required to possess somewhat different characteristics simultaneously. At times bone must be stiff and resist deformation to accommodate loads placed upon it by internal and external forces[39-41]. Simultaneously, it also must be flexible and have the capability of absorbing energy by deforming[40]. Bone must possess the ability to shorten and widen under compression as well as lengthen and narrow when subjected to tensile forces[40]. At any time, if it does not have the ability to accommodate to these needs, cracking and/or complete fracture will occur. Finally, it must accommodate all of these needs, and still be light enough for efficient locomotion.

2.3 The Skeleton

The skeletal system is made up of individual bones and connective tissue and consists of two parts: the axial and appendicular skeletons. The *axial* skeleton includes flat bones such as the skull, scapula, vertebrae, and the pelvis. The *appendicular* skeleton comprises of all long bones including the tibia, femur, and humerus. Typically, the long bones serve as a classical model when looking at both the macro- and microscopic levels of bone. Throughout this body of work, the long bone will be used as the reference model. The long bone of an adult consists of: a *diaphysis* (cylindrical shaft), which is composed mainly of cortical bone; two *epiphyses* (wide ends of bone) composed mainly of cancellous or trabecular bone; a conical or cone-like region called the *metaphysis* which connects the diaphysis to each of the epiphyses;

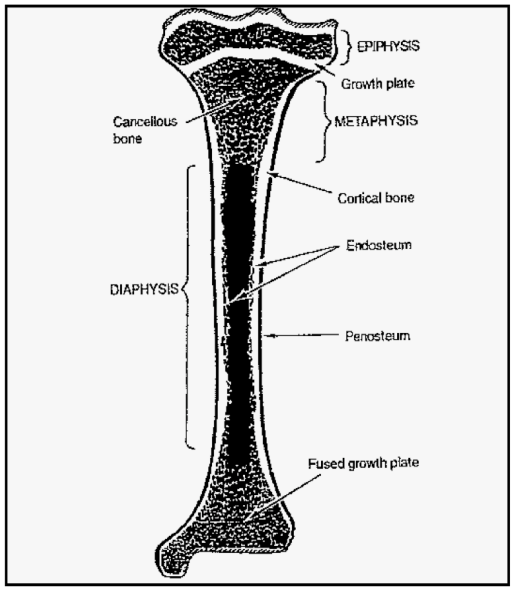


Figure 2-1: Human long bone.
Adapted from Cowin[42].

and a plate of hyaline or articular cartilage known as the *epiphyseal or growth plate* which separates the epiphyses and the metaphyses[27,42].

2.4 Bone Properties: Composition, Tissue Level & Types of Bone

2.4.*1 Composition*

Bone is the main component of the skeleton and is characteristically rigid and hard. These properties allow the skeleton to maintain the shape of the body, protect vital tissues and organs (e.g. brain and heart), and facilitate movement by aiding in the transmission of muscular contraction from one part of the body to another[42]. Bones consist primarily of organic components, inorganic components, and water. The *organic* component (approximately 20-25% by weight) is chiefly made up of Type I collagen fibers, non-collagenous proteins (e.g. osteocalcin) as well as biologically important bone cells[27]. Collagen, which is also found in skin, tendons, and ligaments give bone its flexibility and ability to withstand tensile strains[43]. The *inorganic* component (70% by weight) consists mainly of hydroxyapatite crystals (made up of calcium and phosphate). These crystals are found around collagen fibers, and give bone its rigidity, stiffness, and compressive strength[27,40,43].

2.4.*2 Tissue Level*

At the tissue level, bone can be described as tissue as either woven or lamellar bone. *Woven* bone is immature bone tissue, characterized by a *random* collagen arrangement. It makes up the entire skeleton at birth and is also found in areas of fracture healing as well as sites of extreme mechanical loadings[27]. Woven bone is a provisional material that is eventually replaced (around ages 2 to 3 years) by mature, mineralized lamellar bone[42]. Unlike woven bone tissue, *lamellar* bone is slowly formed and *highly organized*, consisting of parallel layers (resembles the structure of plywood) of collagen arranged anistropically (varying in direction)[27,43,44]. This arrangement gives bone a more optimal structure and increases its resistance to fracture under loading conditions.

2.4.*3 Types of Bone*

Bone tissue is organized into compartments of either trabecular or cortical bone. *Trabecular* bone is a cancellous-type (lattice-like structure) bone that is highly porous and found in primarily in the ends of long bones (e.g. epiphyses and metaphyses) and vertebral bodies[40]. It forms plates in the bone matrix both horizontally and vertically, and is usually organized in the direction of the greatest stresses[43]. Trabecular bone is light and flexible, able to deform when loaded, however lacks the ability to tolerate peak loads. The bulk of human cancellous bone is formed during longitudinal growth (taking place in the growth plates between the epiphyses and the metaphyses) primarily by a process called *endochondral ossification*[45-47]. This process, which will be covered in greater detail in a later section, is also involved in forming the cancellous bone found in the skull, vertebrae, and the pelvis.

Cortical bone is a dense, calcified tissue that makes up the outer component of both long bones and vertebrae. It is highly organized, consisting of overlapping osteons (mineralized bone) and collagen fiber matrix, arranged along the lines of force[27,40]. In contrast to trabecular bone, it is not porous, but dense and rigid and capable of bearing great loads. Cortical bone is arranged in a cylindrical fashion and has two primary surfaces: the periosteum and the endosteum. The *periosteum* is the outside surface (facing the soft tissue) of cortical

bone while the endosteum is a thin layer that lines the marrow cavity of the diaphysis[40,42]. The *endosteum* is biologically important because it contains important bone surface cells (osteoclasts, osteoblasts, osteocytes, and bone lining cells), which carry out essential bone resorption and formation processes. Cortical bone is formed by periosteal apposition, predominantly by (re)modeling processes[47].

2.5 Bone Growth: Longitudinal Growth, Essential Bone Cells, and Modeling & Remodeling

Bone growth is influenced by genetic and circulating systemic factors[42,46]. During growth, bone structure is altered in length and width as well as mass, shape, and tissue density. The following sections will discuss theses changes in relation to longitudinal bone growth (endochondral ossification) and the modeling and remodeling processes as well as the cells that carry out these processes.

2.5*.1 Longitudinal Bone Growth*

Longitudinal bone growth or growth in length is primarily the result of *endochondral ossification* which takes place in the growth plates between the epiphyses and the metaphyses[45,46]. This process involves actions of chondrocytes or cartilage cells that proliferate and deposit new matrix in the form of cartilage. The cells grow and mature, and subsequently calcify, adding to the existing metaphysis, thus increasing length[42,43,46]. As mentioned, this process is essential for forming the bulk of cancellous bone found in the skull, vertebrae, and the pelvis. Diameter growth or growth in width is the result of periosteal apposition in cortical bone carried out by the osteoblasts[44,46]. This is a result of both modeling and remodeling which is the focus of a later section.

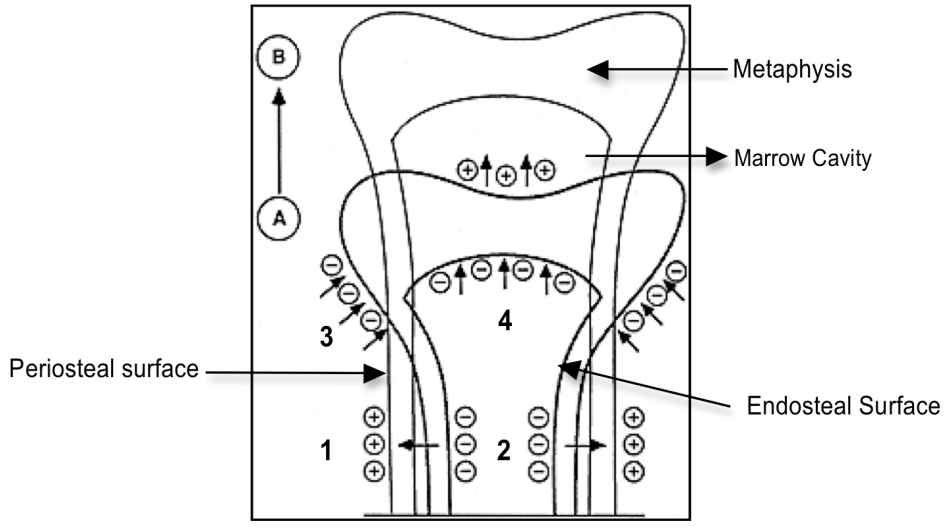


Figure 2-2: Bone formation (+) and bone resorption (-) during longitudinal bone growth. As lengthening occurs (A→B), the diameter of the diaphysis (shaft) expands via periosteal bone formation **(1)**. The marrow cavity of the diaphysis also increases due to bone resorption on the endosteal surface **(2)**. The metaphysis becomes narrower via osteoclast resorption on the periosteal surface **(3)**. The cortex thickens along the cortical endosteal surface of the metaphysis and the marrow cavity expands via resorption of trabecular bone. Adapted from Jee[48]

2.5.*2 Essential Bone Cells*

Within bone there are biologically important bone cells that are responsible for formation and maintenance of the bone matrix. These cells regulate bone metabolism by communicating and responding to biological and/or environmental stimuli (e.g. mechanical loading) by processes known as *modeling* and *remodeling*. The four types of cells consist of osteoclasts, osteoblasts, osteocytes, and bone-lining cells.

Osteoclasts originate from the hemopoietic section of the bone marrow and their main function is to carry out the resorption or removal of old or damaged bone[27,43,44]. Characterized by a ruffled border surrounded by a ring of contractile protein, osteoclasts are usually found in contact with calcified bone tissue and erode targeted bone by first secreting an acid and then dissolving its collagen via enzymatic activation[27,43,44]. *Osteoblasts* are responsible for bone formation. Like osteoclasts, osteoblasts originate from bone marrow, but also develop in the cambium (deep) layer of the periosteal membrane[43]. When bone formation is needed, they are activated and create new bone by the production of osteoids (un-mineralized bone), which

subsequently form the mineralized bone matrix (osteon). Together, both osteoclasts and osteoblasts are responsible for formation and maintenance of bone. They regulate bone metabolism by communicating and responding to biological and/or environmental stimuli (e.g. mechanical loading) by processes known as modeling and remodeling. *Osteocytes* are former osteoblasts that have become entombed in the bone matrix upon osteonal formation. These strain sensitive cells sit in cavities inside the bone matrix called *lacunae* and are responsible for communicating environmental signals to osteoblasts[44]. *Bone lining cells*, like osteocytes, are former osteoblasts. However, they are not embedded in the matrix, rather remain on the bone surface when bone formation ceases. They communicate with osteocytes and are significant because they are thought to initiate the bone remodeling process[48,49]. Both osteocytes and bone lining cells will be discussed in further detail in a later section.

2.5.*3 Modeling & Remodeling*

The ultimate goal(s) of bone's adaptive responses is one of structural optimization and strength. Bone is a dynamic tissue that is capable of adapting its structure to environmental stimuli and repairing damage that occurs. Nearly 4 decades ago, H. M. Frost postulated two unique processes by which certain bone cells work independently or together to achieve structural optimization. These mechanisms or processes, *modeling* and *remodeling*, work in conjunction to structurally optimize and strengthen the skeleton as well as repair compromised regions[44]. During growth, the processes serve to establish the skeleton's peak bone strength, while in adulthood their function is to maintain existing bone strength[40].

M*odeling* and growth are intertwined. It provides a mechanism by which the skeleton adapts or alters its structure to accommodate to new stresses placed on it by influences such as an increase in muscle growth, changes in body size, and the lengthening of the long bones[44]. This process involves selectively adding or removing bone tissue (referred to as "formation and resorption drifts") with the primary goal of optimizing bone geometry (size, shape, position)[42-44]. Bone modeling involves either (but not both) osteoclast activation and bone resorption or osteoblast activation and bone formation. During growth bones get wider because osteoblast formations add new bone tissue to the periosteum faster than osteoclasts

remove it from the endosteal surface[42]. Modeling essentially makes bone stronger in two ways by: (1) significantly increasing cross-sectional area, which helps counteract compressive forces by distributing them over larger areas (helps reduce stress across the loaded area)[39]; and (2) augmenting the resistance to bending and twisting by increasing bone's *second moment of inertia* or deflection under loading[39]. By adding bone, especially to the endosteum, forces are displaced farther away from the axis of bending, thus increasing its ability to resist such strains[39]. Several studies have indicated that modeling increases the second moment of inertia in response to mechanical loading in younger individuals, mainly by increasing periosteal apposition and the prevention of endosteal resorption[39,50]. Bone modeling slows down with age, however during the younger years this process is essential for controlling the strength, size, shape, and overall growth of bone and joints.

Bone *remodeling* is a process by which the skeleton adapts to mechanical stimuli and repairs old or damaged tissue. Different from modeling, remodeling involves the *coupled* action of osteoclast resorption and osteoblast formation. Together these two mechanisms work as one unit (referred to as the basic multicellular unit or BMU) to produce and maintain a skeleton that is mechanically and metabolically competent[42,44,51,52]. Remodeling has two types, Haversian and Trabecular, and always follows three distinct steps: activation, resorption, and formation. Briefly, during *Haversian* or intracortical remodeling, the BMU takes a 3-dimensional form and moves through the diaphysis longitudinally. In response to mechanical stimuli and/or apparent damage, osteoclasts are activated and begin boring through the targeted long bone in a "tunnel-like" manner. The tunnel reaches approximately 250-300**μ**m (micrometers), which defines the cross-sectional size of the new bone that will be subsequently formed[44]. Mononuclear cells then follow and line the tunnel, smoothing the scalloped edges caused by the osteoclasts, creating a "reversal zone" (a thin, mineral deficient, sulfur rich layer of matrix). *Side Note:* When remodeling ceases, the reversal zone separates an osteon (newly mineralized bone) from other collagen or interstitial lamellae[44]. Osteoblasts are then activated and adhere to the reversal zone and begin depositing (in a concentric manner) multiple layers of osteoid (un-mineralized bone). At a certain point mineralization occurs, deposition ceases, and the new bone or *secondary osteon* is formed. In human cortical bone, this process takes approximately 120 days: 20 days spent initiating and increasing the diameter of the tunnel by

the osteoclasts; 10 days for the reversal zone to be formed; and 90 days for the deposition of bone matrix by the osteoblasts[44]. The remodeling process and time to formation is similar in trabecular bone; however bone is removed and replaced in "pancake-like" packets (instead of tunnels) on the surface of the bone material. Figure 2-3 is a schematic of cortical bone remodeling.

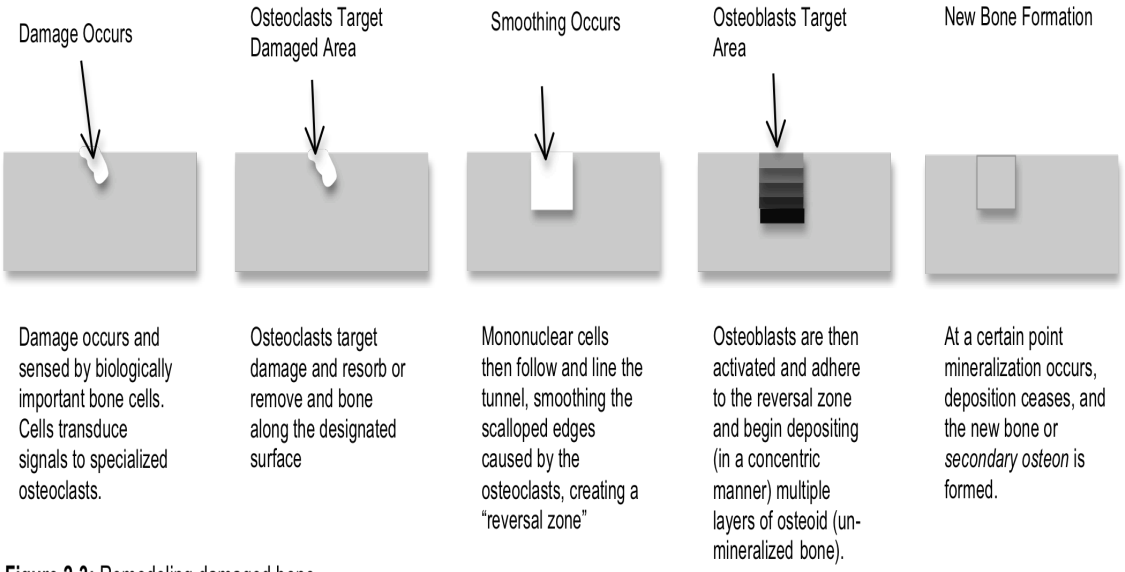


Figure 2-3: Remodeling damaged bone

The following sections will provide the different biomechanical and material properties of bone and the organic and inorganic components that are crucial for bone strength.

2.6 Biomechanical Properties of Bone

Perhaps the most important of the biomechanical properties of bone is its strength. Bones must be *strong* in order to bear loads and resist fracture. The concept of bone strength refers to the "ultimate load" bone can withstand until failure. It is the product of its intrinsic *material properties* (density, stiffness, strength, and mass) and *structural characteristics* (size, shape, cortical thickness, cross-sectional area, and trabecular structure)[40,42,43]. Bone strength can be increased by altering its microstructure (e.g. Haversian remodeling), changing its geometry (to accommodate stress), and/or adding bone mass to the periosteal surface, while

at the same time removing it from the endocortical surface[41,44]. The latter increases the size of the bone and positions the cortex further away from the neutral axes, thus augmenting the second moment of inertia (or resistance to bending) and/or ability to accommodate torsional loading[44] (See Figure 2-5). New bone formation is the most effective when apposition occurs in the area of greatest stress, thereby reducing stress "hot spots" and subsequently reducing the risk of structural failure[44].

2.7 Material Properties

The *material properties* of bone are the traits or characteristics at the tissue level that influence the overall bone strength. They include the organic and inorganic components as well as properties derived from the stress/strain relationship. In order to better understand this relationship, it is important to examine the underlying fundamental biomechanics related to bone and its strength.

Under stress, bone deforms or strains to absorb energy. *Stress* is the force applied per unit area. It is calculated by dividing the force by the bone cross-sectional area[42,53]. Stress is expressed in pascals (Pa) in which one pascal is equivalent to one Newton (N) per square meter (m^2) or N/m^2. Typically in bone, interesting physiological values tend to be of great magnitude and expressed in millions of pascals or megapascals (MPa) [53]. *Strain* refers to the relative amount of deformation or the proportional change in length caused by the imposed stress[27,42,53]. It is calculated by dividing the measured deformation by the original length and is expressed in microstrain (or 10^{-6} mm/mm) or as a percentage[43,54]. Strain is the greatest at the highest point of loading stress and dissipates along the length of the bone[27]. It is from this relationship between stress and strain that other important material properties can be assessed.

2.7*.1 Organic & Inorganic Components*

In essence, the organic and inorganic components determine bone's material properties. These components are responsible for providing tensile strength and resistance to

compression[27]. The *organic* component consists primarily of Type I collagen fibers, non-collagenous proteins, and essential bone cells. Collagen gives bone its flexibility and ability to withstand tensile strains[43] (See Figure 2-6 for types of loadings placed on bone in nature). The organic components are the main determinants of the structure and the mechanical and biomechanical properties of bone[27]. The *inorganic* component consists primarily of calcium and phosphate crystals (hydroxyapatite). They give bone its rigidity and stiffness, and the ability to resist compressive forces[27,40,43]. At the material level, the strength of bone is determined by the properties and the orientation of the arrangement of collagen fibers and the percentage of hydroxyapatite crystals that surround each fiber[55]. It is important to note that material properties are all independent of bone size.

2.7*.2 Material Stiffness, Toughness & Strength*

The material properties of *stiffness*, *strength*, and *toughness* can be obtained from the stress/strain relationship, using the *stress/strain curve*. Below (Figure 2-4) is a representation of a standard stress/strain curve of a bone specimen produced during mechanical testing. The curve has two basic regions, the elastic and plastic regions, which are separated by the *yield point*. The yield point represents the transition, above which stresses begin to cause permanent damage (or material failure) to the bone structure[42]. Typically, when stress is first applied to the bone it follows a linear path. If the stress is released before a certain point, the bone remains elastic and returns to its original length. However, if the stress is not released and further loading occurs, beyond a certain point, the material becomes more plastic in nature, less stiff, and permanently deformed[39,55,56]. At this point, bone is now compromised and fracture may occur (referred to as the *failure point*). The point at which the stress/strain relationship becomes non-linear and bone shifts from its elastic to plastic properties is defined as the *yield point*[42]. The slope of the stress/strain curve (within the elastic region) is an important material property called *Young's or the elastic modulus*. This slope represents the intrinsic *stiffness* of the material or its ability to resist loading[27,42,55]. More specifically, it determines the amount of deformation in a bone for a given applied load[57]. Stiff materials tend

to have a steeper slope than more compliant ones. Mathematically, stiffness can be represented by:

$$\boldsymbol{E}=\mathbf{S}/\varepsilon.$$

in which ***E*** = Young's modulus, **S** = stress, and ε = strain. It is important to note that the stress/strain curve allows for the comparison of different material properties by providing a representation of "stiffness" or resistance to loading[27]. The term stiffness is in quotes because it actually refers to the property of the whole structure. Oftentimes, material stiffness is determined in a region of bone not the whole, therefore a more appropriate term is *modulus of elasticity or elastic modulus*[53]. The area under the curve represents the *material toughness*. It is probably best defined by the amount of energy a material can absorb before failure[53]. Tough materials tend to exhibit a large amount of post-yield deformation (or damage), but this allows for the specimen to absorb a considerable amount of energy before fracture occurs[53]. Typically, a tougher bone will be more resistant to fracture. *Material strength* is determined at the yield or failure points and is routinely defined as the "ultimate load" bone can withstand until failure. It is also designated by the "ultimate strength", "greatest stress", "the load necessary to cause material yield", and/or "the load at the yield or failure points" [53,55]. Strength is ultimately determined by the arrangement and properties of the collagen fibers and the amount of mineral present[27,55]. It is important to note that material strength, as it is defined by the stress/strain curve, is an intrinsic property of bone. More specifically, the strength values are independent of size and shape and the forces required (or ultimate loads) for fracture are different from bone to bone[27,42].

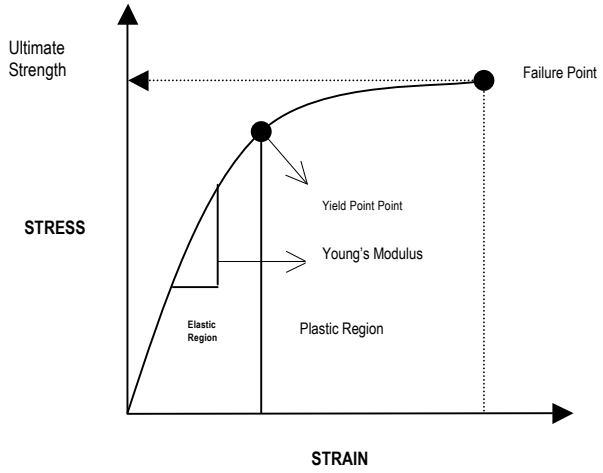


Figure 2-4: Schematic: Standard stress/strain curve of a loaded bone. Adapted from Khan[27].

2.7.*3 Bone Mineral Mass (BMC)*

Bone mineral mass (or bone mineral content, BMC) is a determinant of bone material properties. It is the distribution of mass and the structural properties that influence the strength and stiffness of bone[27]. Both stiffness and strength are a function of bone density, in which stiffness is proportional to the density cubed and the strength squared[27,53]. Although bone strength is determined by more than one component, bone mineral mass explains more than 80% of the variable[27]. The following sections will discuss bone structural and geometric characteristics, define important biomechanical and material measures of bone strength as well as briefly present the concept of the bone-muscle relationship.

2.8 Structural/Geometric Characteristics

The *structural properties* of bone that influence strength include size, shape, cortical thickness, cross-sectional area, and trabecular structure. The structural properties are greatly influenced by their loading environment (e.g. adaptive processes). The loading on bone occurs in one or a combination of four ways: *compression, bending, twisting, and shearing*[39,58] (See Figure 2-6). In long bones particularly, resistance to such forces are more a result of the cross-sectional properties (or distribution of bone tissue) than the bone density or mass[41]. Specifically, the bone adapts by altering its microstructure, changing its geometry, and/or periosteal apposition and endosteal resorption[27,44,53]. This increases its size and positions the cortex further away from the neutral axes, thus increasing its resistance to such forces found in bending and/or torsion, and maximizing the strength to lightness *ratio*[41,42,44].

2.8.*1 Cross-Sectional Moment of Inertia, Polar Moment of Inertia, Section Modulus, Bone Strength Index*

This *ratio*, or the distribution of the material (or area) around the given axis, is best described by the *cross-sectional moment of inertia* (CSMI or *I*) [42]. To better understand this concept, it might be helpful to picture the long bone as a “hollow” cylinder. Conceptually, this form provides an ideal structure with the least mass but the greatest strength during both

bending and torsional loadings. To design a structure that is both strong and light, it is most effective if it is made hollow with the diameter as large as possible. This is accomplished when the cross-sectional area is "designed" (e.g. via modeling/remodeling) as far away from the neutral axis as possible[27]. The structure is therefore both stronger and stiffer due to the bone mass, although less in quantity (both mass and area), being distributed further away from the center of mass of the cross-section[27]. Even a small amount of new bone tissue on the periosteal surface can significantly increase the CSMI of the structure[27,55]. The CSMI is an important measure because it can be used to ascertain other essential bone strength components such as the polar moment of inertial, the section modulus, and the bone strength index. The *polar moment of inertia* (*J*) is used to determine the amount of stress during torsional loading and is derived by summing any two cross-sectional moments of inertia (I_{max} and I_{min}) around the axes (42). *Section modulus* (Z) refers to the bending strength of long bone. It is calculated by dividing *I* (or CSMI) by the maximum distance from the bending axis to the outer surface in the bending plane[55]. Bone strength index (BSI) is an indicator of bone's compressive strength at the distal sites, and is calculated by multiplying the $Total_{area}$ times the $Total_{density}$ squared.

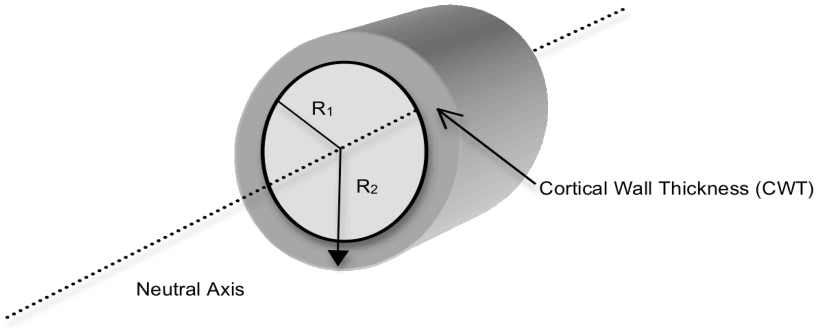


Figure 2-5: Schematic of Cross-sectional moment of inertia (CSMI) of a long bone. CSMI increases when the mass (CWT) is as far from the neutral axis as possible: R_1 = inner radius, R_2 = outer radius, CWT = cortical wall thickness. In the skeletal long bone, a larger CSMI = the stronger and stiffer the bone. Adapted from Khan[27].

2.8.*2 Volumetric & Areal Bone Mineral Densities*

At the tissue level, bone can be separated into trabecular and cortical compartments[55]. The separate compartments are composed of trabeculae and secondary osteons, which make up approximately 10-35% and more than 90% of the total tissue respectively[55]. Differentiating between the two compartments is helpful in examining certain metabolic effects (e.g. bone modeling) that influence bone adaptation and development. More specifically, determining volumetric densities (vBMD) of each compartment can the help track the movements of the endocortical surface (influences the size of the trabecular compartments) and periosteal expansion as well as endocortical resorption and/or expansion (cortical compartment)[59]. In the trabecular compartment, vBMD is typically determined by the total number of trabeculae (in the measured space), thickness of the trabeculae, the spacing between them, and to some extent, by tissue mineralization[55,59]. In the cortical compartment, vBMD is ascertained by the porosity (or number and size) of the osetonal canals as well as by the mineralization density[55]. *Total volumetric bone mineral density* (Total vBMD) can then be determined by summing the cortical and trabecular vBMD's and by the relative sizes of the compartments[59]. *Areal bone mineral density* (aBMD) or also known simply as bone mineral density (BMD) is the total grams of bone mineral per unit (g/cm^2) of bone area. The measurement of BMD has been very important in the clinical management of osteoporosis because the reduction of bone density is closely related to fracture risk later in life.

2.9 Bone-Muscle Strength Indices

The concept of examining the muscle-bone relationship is not novel. However, recently it has been gaining a great deal of attention, especially in pediatric research. Many experts believe the effects of mechanical loading on bone cannot be examined independent of muscle mass and muscle strength. Bones adapt their strength to the mechanical forces they experience. Realistically, these loadings are a result from not only environmental forces (e.g. ground reaction forces), but also from more internal forces, such as muscle contractions[60-62]. It has been suggested that these internal musculoskeletal forces are 2 to 10 times greater than

external ones[61]. During *growth*, muscle force and contraction is the primary driving force for bone adaptation[61,62]. In addition, it appears that muscle development outpaces that of bone development, strongly supporting the position that lean mass plays an enormous role in the accumulation of bone mineral (especially during growth) [61,62].

Currently, there appears to be strong associations between lean body mass and muscle strength and bone mass. However, it is important to note that neither the cause and effect relationship nor the main effect of muscle on bone mass has been definitively established[60]. Regardless, recent data has shown significant site-specific relationships between muscle force and bone geometry[64,65]. Using dual energy X-ray absorptiometry (DXA), strong correlations between lean body mass and total body BMC are also evident[66]. Several studies have also shown that muscle strength can explain the variations in bending strength of bones. Results have indicated that as much as 76% of the variation in bone strength at the distal radius and 67% of the resistance to bending at the ulna can be explained by both grip and bicep strength respectively[56,62]. Similar findings have been reported for hamstring and quadriceps strength in relation to femoral BMD[60]. However, others have not found the same, implying that bone strength cannot be determined without taking other factors into consideration (e.g. age, weight, height, hormone levels) [60].

Only a few studies have incorporated muscle force into the evaluation/calculation of bone strength. The majority have included estimates of bone strength (section modulus, bone strength index, or BMC) and muscle force (LBM or muscle CSA), and the height or length of the designated limb[55,67]. Incorporating these variables into a regression model and/or ratio yields a bone-muscle strength index (or BSMI's), which appears to give an adequate estimate of bone strength relative to the muscle force(s)[55,67]. In the following section, this thesis will present how bone adapts to its mechanical environment. More specifically, it will discuss the role of mechanical loading and the process in which bone senses and tranduces signals from its environment to initiate the adaptive responses. In section 2.11, it will present the Mechanostat Theory, the roles of essential loading characteristics, and the rules that govern bone's adaptive processes.

2.10 Mechanical Loading: Adaptation to Mechanical Stimuli

The basic structure of the skeleton is genetically determined. However, its final strength and bone mass are influenced by other factors such as mechanical loading, gender, age, muscle mass, lifestyle factors, medications, hormones, and nutrition. Although skeletal development is globally determined by genetic factors early in life, in adolescence and young adulthood, bone primarily adapts its strength and material properties from the mechanical demands from growth, changes in muscle force, and physical activity. It is well established that mechanical loading (e.g. physical activity) results in increases in BMD and bone strength at most loaded sites[28]. Bone is mechano-sensitive and has the ability to adapt its size, architecture, and mass to changes in its loading environment[44]. Mechanical loading refers to the applied *stresses* placed on the skeleton or individual bones via forms of physical movement or activity. As mentioned previously, the loading on bone occurs in one or a combination of four ways: from compression, bending, twisting, and shearing[39,58] (See Figure 2-6 below). When these stresses are applied, via internal (e.g. muscle contraction) and/or external forces (e.g. ground reaction forces), bone deforms in order to absorb energy[41]. This relative amount of deformation is termed *strain*. It is postulated that strain is the primary stimulus in which bone responds by altering its material and structural properties to maintain or increase its strength required by its mechanical environment.

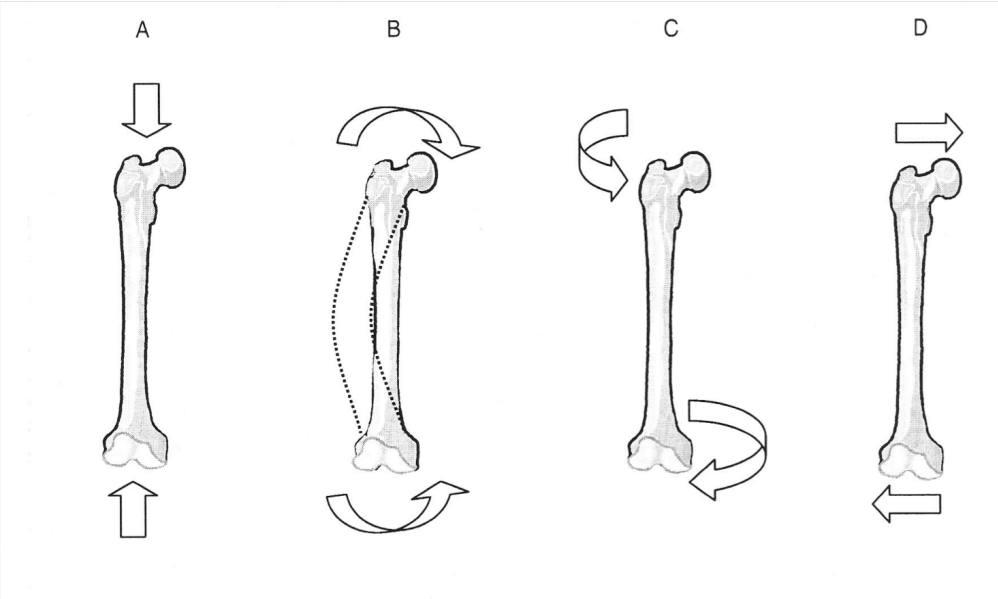


Figure 2-6: The loadings applied to bone in nature. The arrows mark applied forces on the bone: (A) Compression, (B) Bending, (C) Twisting or Torsion, and (D) Shear. Adapted from Pearson and Lieberman[39].

Once bone experiences some kind of mechanical strain and the osteocyte detects significant deformation, the next step is the activation of osteogenic cells, which respond in four specific ways. First, if the signal is/was too weak (below the threshold value) or the response was repressed (occurs often with aging effects), there is no response or quiescence (39). A second result is *modeling,* in which osteocytes recruit *osteoblasts* in the periosteum or endosteum in order to add bone to either surface. A third outcome is bone resorption modeling, in which *osteoclasts* are recruited to resorb or remove bone along the designated surface. And finally, the fourth outcome is *remodeling* or bone turnover in which both osteoclasts and osteoblasts are recruited to remove old or damaged bone and replace it with new tissue.

2.10.*1 Wolff's Law*

For many years, how the skeleton adapts to its mechanical environment has been a mystery to researchers. Even though the subject dates back to Galileo, the first scientist

credited with the idea that bone essentially changes to accommodate the stresses it experiences was German anatomist, Julius Wolff. In the late 1800's, he provided mathematical laws to explain the relationship between bone structure and function. In essence, Wolff's Law states that bone is able to sense mechanical loadings and adapt its structure to ensure that the mass and material properties are appropriate to withstand the forces applied[43]. Throughout the years, what is termed Wolff's Law has been refined by other scientists and now incorporates several other important concepts that Wolff had not included: (1) bone is deposited and resorbed to increase strength but remains light in weight; (2) trabeculae organize/align themselves in direction of the greatest stress; and (3) these adaptations are the result of self-regulating bone cells that respond to a mechanical stimuli acting upon bone tissues[39,43]. In the following sections we will examine the sensing processes or how bone senses and transduces strain, thus initiating the adaptation processes. In addition, it will discuss several factors, specifically loading characteristics, which surround mechanical loading and adaptation.

2.10.*2 Mechanotransduction*

The purpose of bone mechanical adaptability is to optimize its mass and structure to mechanical usage, so that it efficiently and effectively bears loads. As we saw above with Wolff's Law (and later when we examine the *Mechanostat Theory*), it is postulated that bone has the ability to "regulate" its structure and material properties to withstand certain strain thresholds that are applied. The actual physiological process in which bone perceives or senses, and how it responds or adjusts to, these thresholds, is termed *mechanotransduction*. Mechanotransduction is thought to be carried out by a highly complex connected cellular network (CNN) in which specialized bone cells (osteocytes) detect mechanical loads (via bending or some form of deformation) acting upon the bone site, which in turn transmit signals to other bone cells (e.g. osteoblasts and osteoclasts) that add or remove bone at the appropriate sites[39,68,69]. The focus of the following section is to examine the "what's and how's" in bone adaptation. More specifically, *what* senses the changes in the mechanical environment, and *how* does bone respond to these stimuli.

2.10.2.1 The Osteocyte & Bone-Lining Cell

Mechanotransduction is postulated to be carried out by an extensive 3-dimensional cellular network that lies embedded in and on the surface of the bone matrix[27,42]. It is hypothesized that specialized bone cells called osteocytes sense strain and transduce signals that then initiate adaptive processes. *Osteocytes* are the most abundant, making up over 90-95% of all bone cells in the adult animal[68]. There are approximately 10,000 cells per cubic millimeter of bon, and at any given time, no part of bone is more than a few microns away from an osteocyte[70]. These strain-sensitive cells are former osteoblasts that have become entombed in the bone matrix upon osteonal (mineralized bone) formation and are theorized to be responsible for: (1) translating mechanical strain to biochemical signals between osteocytes and other bone cells, thus initiating modeling and remodeling; and (2) regulating mineral metabolism as well as altering the properties of the surrounding bone matrix[44,68]. Osteocytes sit in cavities within bone called *lacunae* and are connected with other osteocytes through small fluid-filled tunnels called *canaliculi*[42,43,68]. There are approximately 15,000 lacunae per cubic meter of bone, and osteocytes may have as many as 80 canaliculi that extend from its cell body[39]. Together with the canaliculi, lacunae make up only about 1% of the total bone volume, however the surface area in which the lacuno-canaliculi system occupies is rather large (adult male skeleton: 1200 m^2), which may indicate the importance of the osteocyte network in both transporting mineral into and out of bone, and its sensitivity to mechanical stress[43,68]. The connection/communication processes between each osteocyte and other biologically important bone cells (osteoclasts, osteoblasts, and bone-lining cells) are facilitated by: (1) *gap junctions* within “finger-like” cytoplasmic processes or *dendrites*; and (2) through the release of signaling molecules (transmitter proteins) into the fluid-filled tunnels of the lacuno-canaliculi system[39,43,68].

Bone lining cells, like osteocytes, are retired or “quiescent” osteoblasts. However, they are not embedded in the matrix, rather they rest on the bone surface. When the production of bone matrix stops, bone-lining cells become still and flattened against the bone surface[43]. In adult humans, bone-lining cells are found on the surfaces of trabeculae, the periosteal and endosteal surfaces, and the Haversian and Volkmann canals of old and newly formed

mineralized bone[42]. Similar to osteocytes, they are thought to be responsible for sensing mechanical strains and the transfer of mineral in and out of the bone unit[44]. It is also suggested that bone-lining cells play a pivotal role in bone remodeling by mediating the activation of the process in response to various chemical and mechanical stimuli[49,69]. They are connected to each other and surface osteocytes via cell processes. However, unlike osteocyte processes, they are not dendritic (finger-like) in nature, but flatter and broader[42]. The cell processes of osteocytes (internal) and bone-lining cells (external) are connected by gap junctions, which in turn, form this 3-dimensional cellular network[42].

2.10.*2.*2 Sensing and Transducing: Role of the Osteocyte

It is established that bone has the ability to sense and adapt to changing mechanical demands. It is also clear that the mechanosensory mechanism within the bone unit follows a distinct pattern in response to mechanical loading. The sequence includes: (1) the cell system is stimulated by external mechanical stimuli applied to the bone; (2) the cell system then transduces the mechanical stimuli to a communicable signal; and finally (3) the system transmits the signal to effector cells (osteoclasts and osteoblasts) to initiate modeling and remodeling[42]. It is believed that the osteocyte is the main mechanosensory cell of bone, however there is little conclusive evidence to show *how* osteocytes and the CNN actually sense mechanical loading[39,42,68].

Currently, there are two predominant theories (plus a third and a fourth that are receiving some attention) as to how osteocytes sense strain. They all surround the idea of interstitial fluid flow caused by the mechanical strain(s). Theoretical and experimental evidence suggests that shear stress, caused by fluid flow in response to mechanical loading, may affect the osteocyte, which in turn mediates the activation of the adaptive responses (modeling/remodeling). The first idea proposes that, when bone is deformed, the strain creates pressure gradients within the lacuno-canaliculi system, which causes the interstitial fluid to move. As the fluid flows past the cell membranes of osteocytes, it creates fluid shear stress within the membrane of the cell, which causes deformation of both the cells and their dendrites within the lacuno-canalicular system. This deformation causes the release of anabolic signals

(including nitric oxide, prostaglandins, and adenosine tri-phosphate or ATP), which are then thought to trigger communication at gap junctions, thus initiating the activation of the remodeling process[44,68]. A second idea proposes that this fluid flow through the canalicular space deforms the shape of important "tethering" elements attached to the internal cytoskeleton of the osteocyte, which then cause the release of biochemical signals[27,68]. Bone cells are attached to the matrix by binding to integrins. Integrins are forms of glycoprotein that stretch across the membrane of the cell. They are attached to the internal cytoskeleton, which connects to the extracellular matrix[27,68]. When shear stress is created by the fluid flow (upon bone deformation), it induces mechanosensation in osteocytes via perturbations of these integrins (thus deforming the cytoskeleton), which then causes the release of prostaglandin and the initiation of the adaptive response[68]. A third possibility is the idea of functional cilia that surround the osteocyte. It has been shown that cilia exist on the surfaces of osteocytes as well as osteoblasts. These cilia are thought to function as sensors of things such as odors, light, and movement[71]. It is proposed that cilia sense the fluid flow, thereby initiating communication between cells—remodeling. Finally, yet another hypothesis that is receiving some attention, is the idea that fluid flow within the bone matrix induces or generates small changes in electrical charge that then initiate the adaptive responses[39].

2.11 Mechanostat Theory

Within the past two decades the idea of bone's adaptability to loading has been even more refined by H.M. Frost and others. Frost's (1987) *mechanostat* theory is based on the idea that there is a specific range of mechanical strain (magnitude) values that regulate bone mass and architecture[42]. According to the theory, there is a "set point" or *minimum effective strain* (MES) range, which is essential to simply maintain bone integrity[52,72]. Specifically, the set point is a range of strain values that will basically produce no adaptive response, but will maintain the existing structure[72]. However, it is postulated that values above this MES range will cause positive adaptations in both muscle and bone mass and bone strength, but those below (as seen in bed rest and hypogravity environments) result in negative adaptations or bone loss[42,72]. In recent years, Lanyon and others have "updated" the mechanostat to show

that in addition to the magnitude of the strain, other factors such as *strain rate,* the *strain frequency, strain distribution,* the *number of strain* or *loading cycles*, and the *rest* between loading cycles are also important in determining bone's adaptations[73].

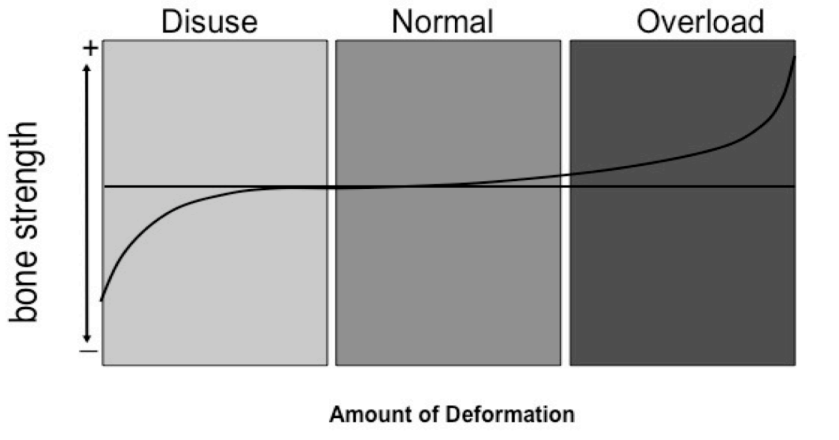


Figure 2-7: Mechanostat Theory. The *Normal* region represents the minimum effective strain (MES) that is needed for bone to maintain bone integrity. *Overload* represents the strain that is required to cause positive adaptations in mass and strength. The *Disuse* region represents where loading strain is too low and negative effects occur (e.g. space flight). Adapted from Frost[52].

2.11.*1 Strain Magnitude*

Strain magnitude is simply the amount of change in bone length or extent of deformation under mechanical loading conditions[27,58]. The role of strain magnitude and bone formation was established by the early work of Lanyon and others. Using animal models, results indicated that there was a distinct relationship between increased bone formation and larger strain magnitudes[58,75-77]. Specifically, bone formation is linear with strain magnitudes greater than 1000 microstrain ($\mu\varepsilon$)[75,76]. Based on the work by Frost, Lanyon, and colleagues (studies in the 1970's and 1980's), the *mechanostat* theory was formulated and/or further refined, and viable strain ranges that are essential for bone maintenance and formation were quantified. As we saw in the previous section, the MES or set point was the range at which bone integrity is preserved. Quantitatively, this range is suggested to lie between 200-2000 microstrain ($\mu\varepsilon$)[27,42]. Within this range remodeling is at a steady state and bone strength is maintained[27,42]. However, strains below 200$\mu\varepsilon$, in which little or no mechanical stimulus influences bone, initiates, but net bone loss results over time[27,42]. Conversely, strains greater

than the MES (1500-3000με) produce a positive response leading to an increase in bone mass[42]. It is important to note that the set point is suggested to be genetically determined as well as highly influenced by past loading and biochemical factors[27]. In addition, the response to mechanical loading is site specific, which can lead to an increase in bone in one area and a net loss in another[27].

2.11.*2 Strain Rate*

Strain rate refers to the change in loading magnitude per second or the rate at which strain is applied and released[58,77]. It is suggested that strain rate, a combination of both strain magnitude and strain frequency, is a major factor in determining bone response[58,77]. Several studies have reported that loadings characterized by high magnitude strains and high strain rates have a greater osteogenic effect than those that are characteristically low[54,77,79]. Mosley and Lanyon (1998) axially loaded rat ulnae at differing rates (3 groups: low, moderate, high rate) to determine the influence of strain rate on the adaptive modeling response. Results indicated the high strain rate group had a 54% greater osteogenic response than the moderate group who had a 13% larger response than the low strain group[77]. Quantitatively, it has been shown that activities that produce strain rates less than 4000 microstrain per second are ineffective in initiating adaptive formation, however those that elicit high (~4000-200,000με) strain rates (e.g. running and jumping) are highly osteogenic[12,51].

2.11.*3 Strain Frequency*

Strain frequency is the number of strain cycles per second (expressed in Hz). The osteogenic effects influenced by strain frequency are harder to detect, due to the failure of many experiments to control for the influence of strain rate with higher frequency loadings[78]. Some results do indicate mechanical loading will not have any effect on adaptive processes if both strain magnitude and strain frequencies are low. In fact, there appears to be a direct relationship between frequency and magnitude. For instance, if magnitude is high, frequency need not be and vice versa. Umemura et al (1997) showed similar gains in bone mass within

immature bone subjected to 5 jumps per day compared to 40. Further, 100 jumps per day did not result in sizable gains versus 5 jumps, indicating that younger bone may not need to be subjected to a great number of loadings to increase bone formation[12,80].

Although the number of loadings has not been clearly established in humans, frequency is important and should be considered when researching and/or designing mechanical loading-type programs. It has been shown that positive effects are attained if strain frequencies are 0.5Hz or greater[44]. Work by Hsieh and Turner (2001) have demonstrated that loading rat forelimbs resulted in a tenfold increase in bone formation in those subjected to 10Hz versus 1Hz. Others have reported that as frequency increases from 0 to 2Hz bone formation also increases; and when the frequency increases 1 to 30Hz, the strain threshold needed for bone maintenance decreases from 1200 to 100 microstrain[76]. Work by Rubin and colleagues (1994) have also indicated there an inverse relationship between frequency and magnitude, showing bone-forming effects with magnitudes of single digits, but frequencies above 30Hz[51]. Thus, it has been proposed that the anabolic effect of mechanical loading on bone is altered by loading frequency[81].

2.11.*4 Strain Distribution*

Strain distribution refers to the way strain is allocated across the bone site. It has been suggested that mechanical loads, characterized by unusual strains of unbalanced distribution(s), may be more important for osteogenesis than either strain magnitude or repetition number (27). Studies by Lanyon (1996) and recently by Nikander et al (2006) have shown that the more unusual the strain distribution at a particular skeletal site, the greater increase in bone mass at that site. In addition, others have shown that if loading diverges from its original pattern(s), bone will respond, even at lower strain magnitudes[13,27,73].

2.11.*5 Number of Strain Cycles*

The number of strain cycles is simply the number of loading repetitions (or duration of exercise) the bone experiences at a given strain magnitude. With this variable, there lies the

misconception: “more is always better”. Conversely, with bone, extending the number of loading cycles or duration does not result in proportional increases in bone mass[44]. In fact, increasing the number of loading cycles tends to suppress bone’s “mechanosensitivity” or the ability of osteogenic bone cells to sense and respond to loadings, thus reducing bone’s adaptability. In animals, Robling et al (2006) reported that bone loses more than 95% of its mechanosensitivity after only 20 loading cycles. Others have found similar results, and thus it appears that the number of repetitions/strain cycles is not important to stimulate the osteogenic process once some cycle threshold has been surpassed[12,44,51].

2.11.*6 Importance of Rest*

Rest between loadings can also influence the osteogenic effect of mechanical loading[55,82]. As we saw in the previous section, repeated loadings may cause bone cells to desensitize, thus reducing their ability to respond to changes in their mechanical environment[44,45]. However, by inserting rest periods between bouts, recent findings suggest that bone cells will “re-sensitize” and allow for adequate bone formation[55]. In addition, splitting the number of loading cycles into several shorter bouts (separated by rest periods) has been shown to increase bone formation *in vivo*[44,84]. *Important note:* Resensitization can be a matter of seconds or hours depending on the features of the loading stimulus[44]. For instance, in rats, rest periods less than 9 and greater than 15 seconds are not effective and the longer rests do not provide any greater benefit(s) [51]. Why this is so is not clear, however it is hypothesized that it may be due to a lag in fluid flow through bone interstices (induced by the loading stimulus) and/or processes related to the mechanosensation and transduction[51,85].

2.11.*7 Customary Bone Strain Stimulus (CSS)*

It is clear that not just one loading parameter (i.e. loading magnitude) is responsible for bone formation and adaptation as once thought. Recently, Skerry (2006), proposed replacing the idea that the “biological target of loading” should not be referred to as a single customary bone strain (i.e. magnitude), rather a combination or *customary bone strain stimulus* (CSS).

Specifically, CSS includes strain magnitude, but also strain rate, frequency, rest periods, strain cycles or duration, subsequent loading events, and timing of application[51]. He also suggests that each bone is different in relation the its "set-point" or MES threshold, indicating that there is a site-specific customary strain stimulus (SSCSS) that exists for each bone site within the skeleton[51]. Together, these strain parameters determine where the modeling and remodeling processes are aimed so that the architecture/structure is optimized[51].

2.11.*8 Rules of Mechanical Loading*

A unique feature of bone is that it adapts its structure in places where mechanical demands are the greatest, thus increasing bone strength without a substantial increase in bone mass[28]. Mechanical stimuli improve strength by altering bone in three ways: (1) Mechanical loading strengthens long bone by causing the apposition of new tissue on the periosteum, thus increasing the second moment of inertia (or resistance to bending) and/or torsional loading capabilities. (2) Mechanical loading causes trabecular bone to become anistropic (aligned in the direction of the greatest stress), thus increasing the load-carrying capacity and structural efficiency, without increasing its mass. And (3) mechanical loading improves cortical bone strength by influencing collagen alignment. For example, in areas of tensile forces, fibers are aligned along the long bone axis. In areas of compression, collagen fibers are arranged transverse the long bone axis[44].

Several variables must be present for mechanical loading to be osteogenic. Along with strain magnitude, the strain rate, strain distribution, the number of loading cycles, the strain frequency, and the rest between loading cycles are all-important for adaptation[55,73]. The loadings must also follow three rules:

Rule 1: The mechanical stimulus must be dynamic in nature. Studies have shown bone only adapts when under dynamic conditions. Robling et al (2001) compared static vs. dynamic loading on the periosteal and endosteal surfaces, and found the latter yielded a 78% and 300% increase in bone formation rate of the loaded limb[86]. Conversely, the static condition suppressed periosteal bone formation by as much as 41%[86].

Rule 2: The mechanical stimulus must also be of the appropriate duration. Extending the loading duration beyond what is appropriate will *not* yield proportional increases in bone mass[44]. Rather, this will cause the bone cells to desensitize, thus reducing their ability to sense stimuli[44,82].

Rule 3: Bone cells must have some experience with previous loading. In other words, bone cells must have some memory of previous loading conditions thereby creating an initial "stimulus threshold" [44]. When the loading causes this threshold to extend beyond the already "stored" threshold, the mechanical signal initiates cellular response (modeling/remodeling). Previous loadings will alter the mechanosensitivity of the bone cells (cellular re-organization), thus allowing the cell to accommodate to the new stimulus[44]. The following sections will discuss other factors that influence the skeleton. More specifically, it will discuss heredity, the role of hormones, nutritional influences as well as gender and age and how they affect growth and maintenance.

2.12 Heredity and Other Factors Affecting Skeletal Development

Studies reveal a relationship between the risks of osteoporosis within the family structure, with the passing of this genetic predisposition from mothers or fathers to their children[87-89]. In females, this genetic predisposition in bone mineral is evident before the onset of puberty. This "genetic" effect appears to have greater influence in areas where the proportion of trabecular bone is greatest (e.g. vertebral bodies) compared to those dominated by cortical structures[90]. In addition, several twin and family studies indicate that as much as 70% of the variance of bone mineral mass (aBMD) is attributable to heredity[91]. This means approximately 20-40% of bone mineral variability is likely explained by environmental and other factors[29]. It is important to note that there is a small relationship between birth-weight and adult bone mass, and maternal smoking, diet and physical activity during pregnancy and bone mineral accrual during the intrauterine stage[92]. Futhermore, childhood diseases (e.g. osteogenesis imperfecta), growth hormone deficiencies, and maternal vitamin D insufficiency have also been associated with poor bone mineral accrual and fracture risk later in life[92,93].

The following sections will examine the more predominant environmental and/or "other" factors that are known to affect skeletal health. In addition, this thesis will discuss two common methods of assessing bone, their capabilities as well as their inherent strengths and weaknesses. Finally, it will examine exercise and physical activity as an osteogenic stimulus.

2.12*.1 Hormones: Estrogen, Testosterone, Growth Hormone, Insulin-Like Growth Factor-1*

Hormones play a vital role in bone biology. The endocrine status of an individual is known to have an important influence on bone development, indicating a great association between compromised bone strength and hormone deficiencies. The major hormones that influence bone development are: the sex hormones, estrogen and testosterone, growth hormone (GH) and insulin-like growth factor-1 (IGF-1).

2.12*.1.*1 Estrogen

Estrogen has long been known to influence bone and its properties in both men and women. It is strong predictor of vBMD and overall bone geometry and is known to influence bone size and BMD[94]. It is an integral part of bone mineral accrual in boys and girls by influencing the rate of formation and resorption during the remodeling process. More specifically, it essential for epipyseal fusion in both boys and girls and it is thought to lower the *mechanostatic* set point in girls during loading[74,95-97]. Estrogen has both direct and indirect effects on bone. During times of need, it is directly related to osteoblast formation and osteoclast suppression, thus maintaining bone mass by limiting bone resorption[27]. Estrogen may also be indirectly related by increasing renal calcium retention and the stimulation of calcitonin[27]. Calcitonin limits bone turn-over and loss as well as influences calcium conservation[27]. In relation to exercise, it is suggested that bone accrual is dependent on the presence of estrogen[94].

Estrogen deficiency during growth and adulthood has a strong association with the development of osteoporosis. Lack of the sex hormone increases resorption on the

endocortical surface, thus decreasing cortical thickness and significantly reducing bone strength[98]. In addition, estrogen deficiencies, via menstrual disturbances especially during puberty, inhibit peak bone mass accrual, increasing the susceptibility for fracture in later life[98].

2.12.*1.*2 Testosterone

The action of this sex steroid is not well understood. However, *testosterone* does contribute to periosteal bone apposition and may be associated with larger peak bone size in men compared to women[27]. It is also positively associated with increased BMD at the hip and spine[27]. It was once thought that testosterone alone, mediated skeletal growth in men, however it is now accepted that both estrogen and testosterone are critical for bone formation, and without estrogen, males cannot optimize their skeleton[94].

2.12.*1.*3 Growth Hormone & Insulin-Like Growth Factor-1

Growth hormone (GH) is the most important factor in relation to longitudinal growth at the epiphyseal growth plate[27]. It is secreted by the pituitary gland and is necessary for the production of cartilage cells as well as promotes the secretion of *insulin-like growth factor-1* (IGF-1) from the liver[99]. Together GH and IGF-1 directly influence osteoblast formation and osteoclast resorption leading to an increase in bone accrual[27]. GH may also play a role in the maintenance of bone during puberty and/or young adulthood[27]. Both GH and IGF-1 decrease with age and these deficiencies may contribute to increased bone loss during the older years[27]. It is important to note that exercise, proper rest, and stress reduction all have a positive influence on growth hormone levels in both boys and girls during puberty[100,101].

2.12.*2 Nutrition*

There is a growing body of evidence indicating that nutrition during growth plays an integral part in achieving a strong healthy skeleton. We are all familiar with the importance of calcium. The skeleton serves as the primary reservoir for calcium. It is an integral part for bone

formation and the size of that reservoir is dependent on the balance between dietary intake and excretory loss[7]. The maintenance and repair of skeletal tissue as well as tissue deposition and the cells responsible for resorption and formation are nutritionally dependent[7]. Phosphorus (also predominantly stored in the skeleton) has been shown to be important for bone cell activity and bone formation[7]. Formation of the bone matrix also involves the interaction between collagen and other nutrients such as protein, vitamins C, D, K and the minerals manganese, copper, and zinc[7,27].

2.12.*2.*1 Calcium

Calcium is the most common mineral in the human body and it is the primary component of bone mineral. The skeleton serves as a storage site for excess calcium as well as a supply mechanism in times of shortage[27]. There is strong support for the relationship between calcium and increased bone mass during growth[7]. However, there is some controversy over that amount of calcium that is required during growth and whether the intake during childhood affects fracture risk in later life[27]. It is speculated that the amount of bone accrued during growth is somewhat dependent on the amount of calcium in the diet, and in the incidence of calcium deficiency may decrease the ability to achieve peak bone mass[7]. During childhood and adolescence, it is estimated that approximately 150 mg of dietary calcium must be retained per day to meet the demands of growth, with much of the demand occurring during the “peak” pubertal stage[102,103].

Results from the majority of studies report a positive effect of increased calcium intake during the growth years[10]. Clinical trials conducted by Matkovic et al (1990) and Bonjour et al (1997) and others have indicated that calcium supplementation does indeed increase bone gain during childhood[104,105]. However, all of these studies were relatively short in duration (1-3 years) and didn’t account for the remodeling transient[7]. Further, the difference between the study and control groups diminished following calcium withdrawal[7]. This change could be explained by the body’s “re-organization” or altered remodeling processes to accommodate the decrease in calcium available. Nevertheless, studies did confirm there is in an increase in bone

with adequate dietary and calcium supplementation. This positive change carries with it, an augmentation in bone strength.

2.12.*2*.2 Phosphorus

Phosphorus (in the form of phosphate) is also a major component of bone mass, thus it must be present in adequate quantities for important for proper bone cell activity and bone formation. For most healthy Americans, phosphorus levels are adequate, and it is unlikely it affects peak bone mass accrual. However, there is debate whether some are getting too much phosphorus via excessive cola drink consumption. Some speculate that excessive phosphorus intake can have a detrimental effect on calcium's abilities[7]. In laboratory animals, a high phosphate to calcium ratio has been implicated in boss loss, and there are non-conclusive reports of children and low bone density and increased fracture rates that present a history of excessive intake of phosphate via phosphoric acid-containing soft drinks[106]. However, further research is needed.

2.12.*2*.3 Vitamin D

Vitamin D plays an important role in calcium absorption. It is necessary for calcium transport and stimulates absorption by initiating the synthesis of calcium-binding proteins within the intestine[7]. This role has been long recognized, thus leading to dietary supplementation (e.g. milk). Americans do not typically lack vitamin D (obtaining much of it from sunlight and/or diet), however a lack of this vitamin can lead to severe deficiencies in calcium absorption, resulting in poor bone accrual and in some extreme conditions, diseases such as rickets[7].

2.12.*3 Gender & Age: Bone*

There are definite gender-based differences in bone structure between non-active males and females. Some of these differences may explain the discrepancy in fracture rates between men and women during competition and in later life. In addition, they further reinforce

the importance of peak bone mass, especially for females. Early in life there are few differences in bone size, mass, and structure between boys and girls. However, during puberty both longitudinal growth and bone mass increases for boys at a greater rate than girls on average[27]. By early adulthood, males typically possess greater total aBMD as well as wider appendicular skeletons (e.g. tibia and femur) and thicker cortices[27]. Typically, men are able to tolerate greater loads than women due to the differences in these bone dimensions[40]. Men and women generally have similar vertebral heights and vertebral trabecular volume density, however men have larger vertebral cross-sections, which accounts for a greater bone strength in that area[40]. Men also benefit from greater periosteal apposition with aging, thus increasing bone width and yielding a strength advantage[27].

In children, distinct differences in bone are not quite clear. Since growth happens at different times for each individual, it is more difficult to "pinpoint" actual numbers. It is known that girls tend to stop growing earlier and typically do not achieve the same heights (e.g. bone length) as boys[107]. In terms of bone size, boys also tend to have greater cortical bone size at the tibial diaphysis (approximately 10% more) compared to girls[107]. In contrast, peak bone mass is achieved at a greater speed in girls than boys, but at a lesser magnitude[27,107]. In relation to both cortical and trabecular densities, results are still somewhat uncertain. However, there is some indication that through the growth stage(s), girls tend to have higher cortical bone density but lower overall trabecular thickness and volume[107].

2.12.*3.*1 Bone Loss & Fragility

Bone fragility is typically greater in women than in men. This is due to the fact that women lose more bone overall during aging, with much of it due to the lack of production of sex hormones brought about by menopause[27,40]. This affects all bone but particularly the cortices of long bones, increasing porosity and increased endosteal resorption[27,40]. At trabecular sites, the rate of loss is similar between males and females. The differences between the sexes is that males lose trabecular density through trabecular thinning, influenced by the reduction of formation by the BMU, whereas women lose it by trabecular perforation or increased resorption within the BMU[40]. In the following sections, this thesis will present

important technologies and their capabilities, strengths, and weaknesses in relation to assessing bone health, bone structure, and bone strength.

2.13 Non-Invasive Measurement Modalities of Bone: Dual Energy X-ray Absorptiometry (DXA) & Peripheral Quantitative Computed Tomography (pQCT)

Currently, dual energy X-ray absorptiometry (DXA) is the most commonly used non-invasive measurement modality to assess bone status in both clinical and research settings. This device uses two contrasting X-ray beams to yield a two dimensional representation of the skeleton. DXA calculates the attenuation values of photons that pass from the X-ray tube through the measurement site of interest and yields outcomes including bone mineral content (BMC) and areal bone mineral density (aBMD) as well as lean muscle (LM) and fat mass (FM) and percent body fat. Peripheral quantitative computed tomography (pQCT) is a recently developed bone measurement modality that provides a three-dimensional representation of a particular site of interest. It scans a single tomographic "slice" and measures the attenuation of radiation as it passes from the source to the site and yields outcome measures including BMC, BMD, bone and muscle cross-sectional area, and estimates of bone strength. Peripheral QCT also has the ability to differentiate between types of bone and may be more sensitive to changes in bone due to physical activity.

2.13*.1 Measurement Capabilities of Non-invasive Modalities*

Bone strength is determined by its material properties and structural characteristics. Ideally, to effectively estimate bone strength, both components, but also the stresses within bone would need to be assessed. At the present time, only a few of the relevant characteristics are available.

2.13*.1.*1 Material & Tissue Level Properties

Unfortunately, the assessments of material properties related to bone strength are not currently available with non-invasive techniques. It is only by invasive modalities, such as the

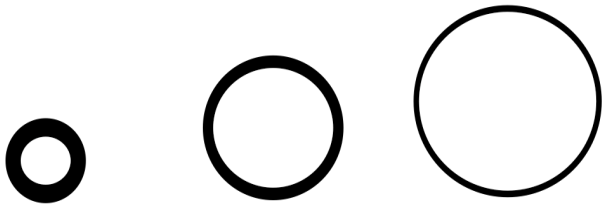
use of bone biopsies, relative mineral ash weights, and mechanical testing can the degree of bone mineralization (at the material level) and failure points be reliably assessed[55]. Recently, it has been suggested that it might be possible to ascertain an "estimate" of material mineral density with pQCT, however a true measurement of mineralization (the extent to which organic bone matrix has been filled with mineral) is unlikely, due to the inability (by both DXA and pQCT) to effectively distinguish bone matrix from actual mineral[55].

In a previous section it was mentioned that, at the tissue level, bone can be divided into trabecular and cortical bone compartments. Differentiating between the two compartments is helpful in examining certain metabolic effects (e.g. bone modeling) that influence bone adaptation and development. Between the two measuring devices, only pQCT has the abilities to separate the compartments and assess both cortical and trabecular vBMD. Mathematically, these densities are computed by dividing the mass of each compartment (separately to yield individual vBMD's or together to get the total) by the volume of the compartment(s)[59]. Tissue vBMD's are expressed in mg/cm^3, and represent the amount of bone mineral averaged over the volume of the compartments[55].

2.13.*1.*2 Structural/Geometric Properties

Structural properties are greatly influenced by the forces imposed on bone in their loading environment. Thus, it is necessary to assess the geometric effects or structural properties and how they influence strength. Several properties are currently available through the use of non-invasive technologies. The measures of BMC, areal (by DXA), and volumetric (by pQCT) bone densities have been used clinically as reasonable predictors of bone strength and fracture risk. Although it is a significant measure, it is important to note that bone mineral density (either areal or volumetric) is not a mechanical strength property, thus using density alone may lead to inaccurate assessment of actual bone strength[55]. The figure below is a representation of 3 bone cross-sections with constant section moduli (Z). Notice as the periosteal surface expands (from example 1-3), the section modulus or bending strength remains the same, but there are significant reductions in aBMD and/or vBMD. This is because as bone surface expands (via periosteal apposition and endosteal resorption) the bone surface

is distributed further away from the center of mass of the cross-section. Therefore, as the diameter increases, less mass or material is needed to keep the section modulus or bending strength constant[55]. Hence, in some cases (especially in regards to children and adolescents), densities (alone) may be of limited value when assessing overall bone strength.



	1	2	3
aBMD (DXA)	1.00	0.53	**0.36**
vBMD (pQCT)	1.00	0.46	**0.27**
Z	**1.00**	**1.00**	**1.00**
CSA or BMC	1.00	0.66	**0.53**

Figure 2-8: Representation of 3 bone cross-sections with expanding periosteal diameter and constant section modulus (Z). Changes in the distribution of bone mass greatly influence the bone bending strength. Changes are not evident using conventional measures on areal bone mineral density (aBMD) via dual energy x-ray absorptiometry (DXA). Adapted from Petit[55].

It has been suggested that other variables, such as bone cross-sectional area (CSA) and section modulus (indicator of long bone bending strength) may be more appropriate when evaluating bone strength (this especially applies to the measurement of children)[55]. Recent technologies and mathematical algorithms have allowed for the measure and calculation of these more relevant bone strength variables. Both DXA and pQCT have the ability to measure (in some cases mathematical calculations are required) Z, CSA, and CSMI. However, DXA is somewhat limited in its ability to clearly and reliably measure most geometric variables. This is primarily due to fact that DXA wasn't originally designed to specifically measure geometry. Consequently, images oftentimes do not provide the clarity to make accurate assessments. However, recently developed DXA-based software may provide better means of measuring geometry. In contrast, pQCT has been shown to be very effective in the measurement of cross-sectional dimensions. In addition to the section modulus and cross-sectional moment of inertia, pQCT software provides outcome measures including total cross-sectional area and

apparent densities (pQCT does not have the ability to measure the degree of mineralization, thus the apparent density is a volume that includes porous spaces) of the total bone slice[55,59]. In addition, it has the ability to separate trabecular and cortical compartments (volumes and areas of each), and provide measures of periosteal and endosteal circumferences, cortical thickness, and other "area" outcomes such as *total bone area* (ToA) (which is the sum of the trabecular and cortical areas)[55,59]. Peripheral QCT can also provide estimates of true bone density and several strength measurements including moments of inertia (I_{max} and I_{min}) and the strength-strain index (SSI)[57]. SSI combines CSMI and the tissue level BMD (trabecular + cortical), and is used to predict bone-breaking strength[55].

2.13.*1*.3 *Measurement of Muscle*

As discussed previously, muscle may be an important variable when examining bone development due to physical activity. Both DXA and pQCT have the ability to measure muscle but in different ways. Dual energy X-ray absorptiometry has the ability through X-ray attenuation to effectively distinguish between and separate hard (bone) and soft tissues (lean and fat mass) yielding total body mass (kg), total body percent fat (%), total body fat (g), and total body lean tissue (g)[108]. With the ability to generate 3-dmensional images via its single tomographic slice measurement technique, unlike DXA, pQCT has the ability to yield muscle cross-sectional area (MCSA) of the site of interest. MCSA is important because is can be used alone as a surrogate or with a combination of other measures (e.g. grip strength) for estimating potential or actual muscle force acting on the bone site[61,67,109].

2.13.*2 Strengths and Limitations of DXA & pQCT*

DXA provides a reasonable overall picture of bone status. The measurement of BMD has been very important in the clinical management of osteoporosis, primarily because the reduction of bone density is closely related to fracture risk later in life[25]. It also has the ability to measure BMC and fat and lean body mass under low radiation dose conditions[27]. However, it does possess significant limitations. For instance, DXA has low spatial resolution, which

limits its ability to measure such components as bone geometry effectively. Assessments are based on the analysis of the shadow image of bone, which also make it difficult to examine mineral density irrespective of bone size and shape[57]. DXA is also inherently planar in nature. It is only capable of providing a two-dimensional estimation of a three dimensional object. Consequently, it fails to properly measure the architecture (e.g. the inability to differentiate between cortical and trabecular bone) of bone properties that have been suggested (along with mass) to influence fracture rates[26-28]. Further, DXA only provides measures of how much bone is present and therefore neglects the distribution of bone or its structural properties, thus reinforcing the notion that these measures (alone) should not be used to estimate strength[28]. DXA is also unable to account for changes that occur during growth (bone size and geometry) as well as those induced by mechanical loading[28]. As a result, it may over-and-under estimate BMC and aBMD depending on the stature of the individual[27].

In contrast, pQCT does have the ability to assess changes due to growth and mechanical loading. It also is also capable of measuring and analyzing cross-sectional dimensions (bone and muscle), and independently evaluating bone size, shape, and mineral density (apparent densities)[55,57]. Further, it is able to separate and assess volumetric densities of the different cortical and trabecular compartments and provides estimates of bone strength, which ultimately is the bottom line[26,57]. However, pQCT also possesses a few limitations. First, since pQCT is a rather novel modality, there are no standardized analyses and data acquisition protocols (e.g. placement of the reference line), which makes it difficult to compare results across studies[55]. Second, unlike other more advanced devices, pQCT doesn't have the ability to measure micro-cracks, which would be particularly beneficial for older and athletic populations[55]. Finally, due to the design of some of the devices, it may be difficult to measure the clinically important proximal femur and lumbar spine regions.

In skeletal research two common models are used to assess the influence of mechanical loading on skeletal adaptations. In the following sections, this thesis will present both animal and human models and discuss important findings in both models in relation to physical loading and and bone health.

2.14 Models of Loading: Animal and Human

The basic structure of the skeleton is genetically determined. However, its final strength (combination of structural and material properties) and bone mass are influenced by other factors, specifically mechanical loading. Numerous types of exercise and physical activity have been studied to determine which type or types of mechanical loading are the most osteogenic. Thus far, it has been shown that mechanical loading regimens must be dynamic in nature and include appropriate levels of all of the strain-related variables (e.g. rate, frequency, rest).

A unique feature of bone is that it adapts (depositing new bone) its structure in places where mechanical demands are the greatest, thus increasing bone strength without a substantial increase in bone mass[28,44]. To reiterate from a previous section, mechanical stimuli improve strength by altering bone in three ways:

- Mechanical loading strengthens long bone by causing the apposition of new bone tissue on the periosteum, thus increasing the second moment of inertia (or resistance to bending) and/or torsional loading.
- Mechanical loading causes trabecular bone to become anistropic (aligned in the direction of the greatest stress), thus increasing the load-carrying capacity and structural efficiency, without increasing its mass.
- Mechanical loading improves cortical bone strength by influencing collagen alignment. For instance, in areas of tensile and compressive forces, fibers are aligned along the long bone axis and transverse the long bone axis respectively[44].

The positive effect of mechanical loading on BMD and bone strength is well documented in human and animal models. Evidence from epidemiological, clinical, and experimental exercise studies indicate that physical activity can be an effective intervention for increasing and maintaining bone mass and strength as well as decreasing the rate of bone loss, which may in turn reduce the risk of fracture[110,111]. Data from both adult and animal studies show that those who are more physically active have higher areal bone mineral density (aBMD), and a lower risk of osteoporotic fractures[112].

2.14.*1 Animal Models*

Animals (particularly rodents) have been the primary models in bone research. A value(s) of this model is that it provides a variety of mechanical testing modalities, and the ability to examine (upon sacrifice) the effects of mechanical loadings more in depth than with the use of *in vivo* studies. Numerous exercise protocols have been used in the study of animals including treadmill walking and running, jumping, climbing, swimming, and various forms of electrical stimulation and mechanical testing via non-invasive axial loading and bending tests. Overall, results have indicated that the exercise or mechanical loading groups have significantly more bone mineral that non-exercised controls. Studies on rats using treadmill protocols have shown that running increases mineral apposition and bone formation rates in the proximal and distal tibial metaphyses[113]. In addition, similar running experiments have demonstrated increases in cortical and cancellous bone mass of the tibia and a retardant effect on bone loss at the same site (by eliciting bone formation and decreasing bone resorption) in young, adult, and female ovariectomized rats[114]. In jumping experiments, Umemura et al (1997), Westerland et al (1998), and Kodama et al (2000) all showed significant increases in femoral cortical bone strength, trabecular bone mass of the proximal tibia, and tibial bone strength in most, if not all, exercise groups versus non-exercising controls[12,115,116]. Further, Mori et al (2003) demonstrated that voluntary climbing exercise caused increases in periosteal bone formation, bone cross-sectional area, and second moment of inertia of the mid-femur as well as increases in trabecular and cortical bone volume of both the total femur and proximal tibia[117]. Finally, protocols, involving mechanical bending and axial-loading techniques have indicated that strain promotes periosteal formation and expansion at all diaphyseal sites within the tibia as well as increases in trabecular volume in the same bone segment[118].

Table 2-1: Mechanical loading outcomes in animals.

Author	Study Design	Significant Outcome(s)	Conclusion(s)
Robling[84]	Sprague-Dawley Female rats. Loading Groups: 360 load cycles/day, 3x/week for 16-weeks. Group 1 (LG1)-all 360 cycles were given un-interrupted (360x1). Group 2 (LG2)-4 bouts of 90 cycles, with 3-hours of recovery between bouts	BMC, aBMD, vBMD, CSA was greater in loading groups vs. controls. LG2 had 70% greater BMC than LG1. LG2 had 60% greater aBMD than LG1.	Long-term exercise protocols that target bone health might result in greater returns in bone mass and structural properties if the daily exercise is partitioned in shorter, frequent bouts.
Umemura[12]	Female Fischer 344 rats: 5 days/week for 8-weeks. Group 1: 5 jumps/day. Group 2: 10 jumps/day. Group 3: 20 jumps/day. Group 4: 40 jumps/day. Group 5: 100 jumps/day. Control Group	Jumping groups exhibited stronger bones vs. controls. -Bone values were greater in Group 5 vs. all groups. -Strength of the tibia and femur of Group 1 was greater vs. control. -Periosteal bone formation was greater in all loading groups vs. controls.	As little as five jumps per day significantly increased overall bone density. Dynamic jumping exercise(s) are shown to have beneficial effects on bone density and strength.
Cullen[58]	Retired female Sprague-Dawley breeder rats. External force of 25N or 30N or 0, 40, 120, 400 cycles at 2Hz for 3days/wk for 3wks.	Tibiae loaded at 25N at 400 cycles showed 2.8-fold greater periosteal bone formation rate vs. controls. Tibiae loaded at 30N and 120 or 400 cyc had 8- to 10-fold greater periosteal formation rate, 2- to 3-fold greater formation surface, and 1-fold greater endocortical formation surface than controls.	As applied load or strain magnitude decreased, the number of cycles required for activation of formation increased. At constant frequency, the number of cycles required to activate formation is dependent on strain, and as number increases, the bone response increases.
Warden[28]	Female virgin Sprague-Dawley rats. Side-by-side differences between a loaded (right) and unloaded limb (left). Loadings: 360 cycle/day, 3days/week, for 5 consecutive weeks.	BMC and aBMD for the entire ulna and BMC for the ulna mid-shaft were greater in the loaded arm. Second moment of area (I_{min}) was 86.9% larger in loaded vs. unloaded ulnas, and loaded ulnas were more resistant to fatigue testing.	Mechanical loading increases both BMC and aBMD. Mechanical loading increases overall bone strength and reduces *fracture risk*.

2.14.*2 Humans: Sports Participation and Bone Development in Adults*

The majority of available data has been from research conducted on adult athletes. It is well established that sporting activities are known to elicit greater peak bone mass at loaded sites[9]. Adult athletes tend to have greater aBMD and bone mineral content (BMC) (e.g. lumbar spine and femoral neck)[9,119] as well as higher cortical bone mineral density (BMD) compared to those who are less active[120]. More importantly, those sports that feature high-impact (e.g. volleyball), "odd"-impact (e.g. soccer), and high magnitude (e.g. weightlifting) loadings have been shown to be more osteogenic than those lacking such loading characteristics[9]. Specifically, athletes who compete in these sports, exhibited substantially greater bone mass at loaded sites, had larger diaphysis, thicker cortices, and denser trabecular bone than those in less skeletally demanding sports (e.g. swimming). Consequently, the loading-induced additional bone mass significantly increased bone strength and reduced fracture risk[9].

Specific to this research, studies examining both male and female hockey players show significantly greater BMD at all sites including the total body, lumbar spine, pelvis, humerus, femur, proximal tibia, tibia diaphysis, femoral neck, Ward's triangle, and trochanter compared to healthy non-active controls[119,121]. In addition, all athletes had greater muscle mass and muscle strength and significantly higher lean body mass compared to the controls[119,121]. Similar results were reported in both male and female soccer players in which athletes exhibited greater overall bone structures, significantly higher whole body, spine, hip, leg and calcaneal BMD as well as greater whole-body and lumbar spine BMC's, and femoral neck BMC compared to controls[22,122]. However, in swimmers, despite large muscle force and movement, several studies have indicated no significant differences in bone structures compared to controls[13]

Table 2-2: Mechanical loading outcomes in adult humans

Author	Study Design	Outcome(s)	Conclusion(s)
Nikander[9]	N=255 adult female: 5 Loading groups: High Impact (volleyball and hurdling). Odd Impact (squash, soccer, speed-skating, step aerobics). High-Magnitude (Weightlifters). Low-Impact (orienteering and cross-country skiing). Non-Impact (swimming and cycling).	High-impact, odd-impact loading groups exhibited greater overall aBMD, CASE, and bone strength (Z) to all other groups. aBMD was greater in all athletic groups (except swimming and cycling) vs. controls. High impact loading was more strongly associated with aBMD (vs. repetitive loadings). High-impact and odd-impact were more positively associated vs. repetitive low-impact	Athletic activities that involve high and/or odd-impacts are associated with greater bone densities and bone structure compared to those lacking sufficient impact.
Sandstrom[119]	N=28, Female ice hockey players and controls. Investigate BMD at different sites in female hockey players as well as study the relationship between BMD, muscle strength, and body composition parameters.	Hockey players had significantly higher BMD: total body, lumbar spine, femoral neck, Ward's triangle, and trochanter Hockey players had significantly greater lower extremity leg strength than controls.	Both bone mineral density and muscle strength was significantly greater in athletes vs. controls. Ice hockey appears to be a beneficial sport for both muscle and skeletal development.
Taafe[123]	N=106 young adult women (18-29 yrs.) Compared gymnasts, runners, swimmers and controls.	Gymnasts displayed greater overall total body BMD compared to runners, swimmers, and controls. Femoral neck BMD of gymnasts was higher vs. swimmers and controls.	High-impact (gymnastics) sports yielded greater overall skeletal benefits compared to repetitive impact (running) and high-muscle force repetitive unloading (swimming).
Ducher[124]	N=20, men and women tennis players (right handed). Assess bone geometry and volumetric bone mineral density in young adults who started playing tennis prior to puberty, and the role of muscle forces in the bone response by investigating the muscle-bone relationship.	Muscle volume, grip strength, BMC, total bone volume, and sub-cortical volume were greater at the dominant radius vs. non-dominant. Grip strength was closely linked to muscle volume and all bone parameters except vBMD.	Along with the influence of mechanical loading, it appears that muscle size and strength is a factor in the development and maintenance of bone.
Nikander[13]	113 adult female athletes: Loading group: high-impact, odd-impact, repetitive-non-impact, and controls. Assess the influence of loading type and muscle performance on skeletal development and maintenance of adult female athletes.	Bone mass was significantly greater in all athletic groups compared to controls at loaded sites. Athletes (excepts swimmers) had larger diaphyses, thicker cortices, and denser trabeculae.	Loading significantly influenced not only bone mass, but also appeared to build mechanically strong and appropriate bone structures.

2.14.*3 Children and Sports and Physical Activity*

Compared with adults, much less research has been conducted on children and adolescents, which is somewhat puzzling given that peak bone mass is achieved by the end of the second decade of life. However, what does exist is promising. Given the osteogenic effects of certain types of mechanical loading and childhood providing the single most opportune time to increase mass, material properties, and structural strength[125], it seems that a logical modality to reduce osteoporosis and osteoporotic fractures later in life is via athletic participation. Several cross-sectional studies of young athletes participating in moderate-to-high impact weight bearing sports exhibited greater overall aBMD at loaded sites compared to less active children[126,127]. Liu et al (2003) found that young female athletes, participating in high impact sports (e.g. volleyball and gymnastics), have greater aBMD at most skeletal sites compared to less active females as well as those who compete in "active" non-loading sports (e.g. swimming), which have the ability to elicit great numbers of musculoskeletal movements, but lack the essential ground or surface impacts[25]. More recent findings report large differences in bone properties and bone strength in young athletes versus less active controls. Ward et al (2005) found both male and female gymnasts had greater total and cortical area and greater long bone bending and torsional strength (SSI: stress-strain index) versus less active controls[28]. In elite female adolescent distance runners, both Duncan (2002) and Greene (2005) reported greater mid-femur and distal tibia cortical area, cross-sectional moment of inertia (bending and torsional strength of bone), and bone strength index (resistance to bending) compared to swimmers, cyclists, and less active controls[10,129]. Similar findings were reported by Bellew and Gehrig (2006) and Nilsson et al (2009) indicating that participating (at younger ages) in sports that put higher strains on the skeleton exhibited greater positive bone changes in adolescent females and conferred positive effects on bone geometry in young men even after sport cessation[130,131].

Table 2-3: Mechanical loading outcomes in adolescent humans.

Author	Study Design	Outcome(s)	Conclusion(s)
Bellew[130]	N=64, adolescent and young female athletes from soccer, swimming, and weightlifting. Purpose was to assess the effect of sports on varying skeletal loading on bone density in young female athletes.	BMD was significantly greater in the soccer group compared to both weightlifting and swimming. Compared to adult norms, soccer players exhibited greater whole body BMD results while swimmers were significantly less.	At younger ages, participation is weight bearing high impact sports, especially those with dynamic movements, appear to convey beneficial musculoskeletal effects in females.
Sone[120]	N = 37, adolescent males Basketball, volleyball, soccer, baseball, rugby, tennis. Evaluated the characteristics of tibial bones (dominant vs. non-dominant) in young male athletes and controls using pQCT, with a particular focus on the effect of mechanical stress on volumetric density of cortical bone.	Total BMD, cortical and trabecular BMD, total diaphysial BMC, periosteal CSA, polar moment of inertia, total diaphysial BMD cortical BMD Were all sig. greater in athletes vs. controls.	Compared to controls, those in the athlete groups exhibited greater overall bone structure. During the growing years, it appears (as in adults) that weight bearing impact-type exercises are most beneficial.
Duncan[10]	N=50, adolescent female cyclists, runners, triathletes, non-athletic controls. Purpose: Compare long-bone (femur) total and cortical volumetric BMD, geometry, biomechanical, and estimated bone-strength characteristics of elite teenage female athletes from sports with different mechanical-loading patterns.	All sport groups had larger lean tissue mass than controls. Runners had higher aBMD than cyclists. -Runners had highest values for CSMI and BSI vs. all groups except triathletes.	Similar to other reports, weight bearing exercise appears to augment both bone and lean tissue development.
Heinonen[133]	N=139, adolescent girls. Purpose was to examine the effects of high-impact exercise in growing girls (9-month intervention). Exercise Group: N=64 (25 pre-menarchial, 39 postmenarchail). Control Group: N=62 (33 premenarachial, 29 postmenarchial)	In the premenarchail girls, BMC increased more in the trainees vs. controls in the lumbar spine and femoral neck After 9mos of training, the training-induced BMC changes in the femoral neck and in the trochanter of the premenarchial girls differed sig. from those in the postmenarchial girls.	Results support the concept that the pre-menarchial period of puberty is a critical time for additional bone mineral acquisition. Findings support previous cross-sectional studies, which suggested that bones respond more favorably to mechanical loading before or at menarche.

2.14.*4 Recreational Activities*

There is even less data available on the effectiveness of normal recreational or leisure activity on bone material and structural strength of bone in children. What does exist suggests that there is a good association between the amounts of physical activity during childhood and bone health in later years. Bailey et al (1999) reported that children with the highest physical activity levels accrued more bone and had greater femoral neck and total body bone mineral content than those who were less active during childhood and adolescence. Further, it has been suggested that individuals who are consistently active during childhood and adolescence enter adulthood with healthier stronger skeletons[55].

2.14.*5 Physical Activity Interventions*

Since exercise has been shown to be an effective tool to increase bone structure, many have sought to implement various types of interventions in different populations to determine their efficacy. Several have focused on weight and/or resistance training, while others have examined more high impact/high intensity interventions. In premenopausal women, those interventions that included high impact activities and strenuous weightlifting exhibited the greatest gains in BMD at loaded sites[134-136] compared to less strenuous protocols[137]. In post-menopausal women, interventions ranged from vigorous walking to jogging, static to dynamic strength training as well as low to high-impact exercises. In the lumbar spine and the hip, those exercises that included high-impact running and/or jogging and climbing stairs showed the greatest benefits[27]. Far fewer studies have been conducted on men, however results are/were similar to those in pre-and-post-menopausal women. Those interventions that included high-impact and strength training regimens were the most effective[27]. In fact, Kujala et al (2000) reported that men who participated in high-impact vigorous sporting activities were as much as 60% less likely to suffer a hip fracture compared to those who didn't[138].

In recent years, elementary schools have become an ideal setting to implement a number of school-based physical activity interventional programs. Most have involved high-impact activities including skipping, jumping, hopping, running as well as strength training. Thus far, results have been very promising, pointing to activities that feature moderate impact and high strain rates with unusual movements (with inserted rest between bouts) appear to be the most osteogenic[80].

Although the exact exercise prescription (frequency, magnitude, duration, etc.) is not quite understood, it can be concluded that exercise has a crucial role in the healthy growth and development of the young skeleton as well as the maintenance and reduced fracture rates in older individuals.

2.14.*6 Detraining*

Although it is well established that physical activity, recreation and sport positively affect bone structure, many questions are still unanswered as to the "staying effects" of exercise early in life into later adulthood. In other words, are the induced benefits (aBMD and bone structure) retained following cessation of exercise or retirement? Several studies conducted in both animals and humans have explored this issue and report somewhat different results. Iwamota et al (2000) subjected young rats to 12-weeks of training and reported showed positive changes in bone structure (bone weight, bone volume, bone cross-sectional area, and cortical area). However, following 4-weeks of detraining these same rats showed marked losses in these areas[139]. Another study conducted by Jarvinen et al. [140], reported similar results in rats at the femoral neck following a training/detraining regimen. However, a recent study from Warden et al. [28] showed that exercise during early adolescence improved overall bone structure and that these enhancements were maintained into later life.

In humans, results are also somewhat ambiguous. Studies using biochemical markers of bone report that decreased activity leads to an increase in bone resorption, citing only 2-weeks of detraining in active soccer players lead to an increase and decrease in bone resorption and bone formation markers respectively[141,142]. In addition, one study followed 23 middle-aged runners (ages 55-77 years) for 5-years and reported 3-fold losses in spine aBMD for those who stopped running versus those who didn't[143]. In contrast, a cross-sectional study involving tennis players showed no changes in BMC in those who had reduced their training volume[17].

Results must be viewed with some caution. As indicated above, at this point, due to conflicting results, it is unknown whether bone gains during the early part of life are sustained following cessation of exercise and/or retirement. However, it must be noted that fractures (all types and fragility) are far fewer in former athletes after participation in high-level exercise compared to non-athletes[132]. This suggests that physical activity (e.g. sports participation) early in life may have long-term benefits in relation to falls and fracture risk.

CHAPTER 3 METHODS AND MATERIALS

In the following sections, this thesis will detail the methods and materials used throughout the data collection process. It will present the participants, data collection methods for each of the measured variables as well as the statistical analyses used to determine outcomes. Following this chapter, it will discuss the research findings in the form of two manuscripts to be submitted for potential publication.

3.1 Participants

A total 160 elite collegiate (18-25 years) ice hockey (male = 19, female = 21), swimming (male = 13, female = 17), soccer (female = 15), and running (male = 19, female = 22) athletes and non-active controls (male = 15, female = 19) were used in the study. Athlete participants were recruited from NCAA Division I and III colleges and universities as well as competitive sporting clubs and associations. The University of Minnesota's Institutional Review Board (HSC#: 0511M77197) and the General Clinical Research Center (Protocol #1133) approved all study procedures and personnel, and each participant gave his or her written informed consent prior to any data collection.

3.2 Inclusion and Exclusion Criteria

Athletes were selected on the basis of their current sport participation status. They were required to be currently competing and have at least 5 years of "competitive" playing experience in that sport. Competitive was defined as having college or university varsity athletic and/or club playing experience. All athletes were required to have at least 3+ hours of practice and/or game situations per week during the respective season. Controls were required to be healthy, but participating in less than 2.5 hours of physical activity per week. All potential participants were excluded if they currently have/had an eating disorder, known chronic health problem, taking medication known to influence bone density and/or bone metabolism, or pregnant.

3.3 Classification of Loading Modality

Sports and control groups were placed in separate categories for both lower and upper extremities. For the lower extremities, overall classification for the sporting groups included either weight bearing (with categories of impact or non-impact) or non-weight bearing. Upper extremities

were categorized as loading or non-loading. Controls were classified as *non-impact, non-loading* for both extremities. See Table 3-1 for Classification of Loading Modality.

3.3.*1 Lower Extremities*

Soccer was defined as a *weight-bearing impact* sport based on its intense nature that includes rapid accelerating and decelerating movements with a high magnitude of non-conventional movements of the body and hips. *Ice Hockey* was classified in the *weight-bearing impact-2* group. Similar to soccer, it includes rapid accelerating and decelerating movements with a high magnitude of non-conventional movements of the body and hips, however lacks the exact same type of ground reaction forces. *Swimming* is an endurance-type sport that has the ability to elicit great numbers of similar musculoskeletal movements. However, it does lack essential ground or surface impacts and thus classified as *non-impact loading.*

3.3.*2 Upper Extremities*

Given the upper extremities are seldom used, *Soccer* athletes were placed in a *Non-impact loading* group. *Ice Hockey* athletes were placed in the *High-magnitude loading* group due to the sport's physical nature (e.g. intentional body contact) and carrying a hockey stick (which is used for shooting, passing and other aspects of the game) places varying amounts of tensile, compression, and shear stress on the hands and forearms. Although swimmers perform a great number of repetitive upper extremity movements, the majority of the forces experienced are due to the drag of the water and not actual impacts. Therefore, *Swimming* was again classified as the *Repetitive non-impact loading* modality.

Table 3-1: Classification of Loading Modality

Group	Upper Extremities	Lower Extremities
Soccer	*Non-impact loading*	*Weight-bearing impact loading*
Ice Hockey	*High-magnitude loading*	*Weight-bearing-2 impact loading*
Swimming	*Repetitive non-impact loading*	*Repetitive non-impact loading*
Control	*Non-impact, non-loading*	*Non-impact, non-loading*

3.4 Procedures

Participants were required to visit one-time for approximately 1.5 hours. Upon consent, basic anthropometry (height and weight), muscle performance, bone characteristics, and general health and level of physical activity and maturity status were assessed. All procedures were conducted at the University of Minnesota's Laboratory of Musculoskeletal Health (LMH) and General Clinical Research Center (GCRC), and performed by IRB approved and Minnesota State certified limited X-ray technicians and nurses. See Appendix B for data collection procedures.

3.4.*1 Anthropometry*

Total body weight in kilograms was obtained (to the nearest 0.1 kg) using an electronic scale accurate to 200kg (Tanita BWB 800: Tokyo, Japan)**.** Standing height was obtained using a standard wall stadiometer (Accustat™ Genentech: San Francisco, CA) to the nearest 0.1 centimeter. Tibial length was measured to the nearest millimeter from the tibial plateau to the medial malleolus using an anthropometric ruler. Radial length was obtained, using the same measurement device, measuring from the ulnar head to the styloid process. Body mass index (BMI) was calculated as body weight (kg) divided by height (m) squared. Total body lean and fat mass and percent body fat were taken from dual energy X-ray absorptiometry (GE Lunar Prodigy: Software v. 6.7, Madison, WI) total body scans.

3.4.*2 Muscle Performance*

Lower extremity strength was estimated using the Vertec® vertical jump tester (Questek Corp: Northridge, CA) according to established testing procedures[144]. Prior to testing, participants were instructed to first walk on a treadmill to ensure proper warm-up. Participants were then instructed to perform three maximal stationary vertical jumps and the highest jumping height obtained was used for further calculations. Estimated lower extremity power (Watts) was calculated using the Lewis Formula[145] and nomogram (r=.88, SD = 603 W)[146] and later computed into Watts per kilogram of body weight.

$$\text{Vertical Power (W)} = 61.9 \times \text{Jump Height (cm)} + 36 \times \text{Body Mass (kg)} + 1822$$

Below are validity scores for the vertical jump in relation to four power generating activities: 40-yard sprint = .62[147]; Wingate Anaerobic Cycling Test, r = .70 and .74 for older persons and younger boys[148]; force-plate power jump, r = .83[149]; force platform jump (jump height), r = .92[150]. Test-retest reliability for college students = .99[151] and test-retest reliabilities for children ranged from .90-.97[152].

Forearm grip strength was calculated in kilograms with the use of a digital hand dynamometer (Smedley III T-18; Creative Health Products: Ann Arbor, MI) in both arms. The greatest score of three trials was used to report the final results. Combined grip strength was calculated by adding the greatest score from both the right and left scores. Grip strength was also converted to Newtons for the left and right as well as combined (N) using:

Example: Newtons (N) = 9.8066 x Total Grip Strength (kg)

When standard procedures are followed, accuracy of handgrip strength was highest in the three-trial method (r = .90), with inter-rater reliability, r = .97[153].

3.4.*3 Bone Characteristics*

Single axial (2.5 mm thick) tomographic slices at both the distal tibia and shaft and distal radius and shaft were scanned using peripheral quantitative computed tomography (pQCT; XCT 3000, Stratec Mediznintechnik GmbH: Pforzbein, Germany) according to standardized procedures. For the *lower extremities,* the 66, 50, and 4 percent sites of the estimated length of the tibia proximal to the distal endplate were included. *Upper extremity* measurements included the 4% site of the estimated length of the radius proximal to the distal endplate and the 50% site of the radial shaft. For shaft regions (50%, 66%) of the radius and tibia, *total bone area* (mm^2), *cortical bone area* (mm^2), *cortical density* (mg/cm^3), *cortical thickness (mm), section modulus* (mm^3), and *polar strength strain index* (mm^3) were determined**.** *Muscle cross-sectional area* (mm^2) was also calculated at the 66% site. At the distal sites (4%) *total bone density (*mg/cm^3), *total bone area* (mm^2)*, trabecular density* (mg/cm^3), and *bone strength index* (mg/mm^4) were determined. Areal bone mineral density (aBMD) was measured for the total body, femoral neck, hip, and lumbar spine (L1-L4) via dual energy X-ray absorptiometry and standard operating procedures (GE Lunar

Prodigy: Software v. 6.7, Madison, WI). Quality assurance for all DXA-related information was performed bi-weekly and the coefficient of variation (CV) for repeated measures was 0.136.

3.4.*4 General Health and Physical Activity Questionnaire*

All participants completed a standard health and physical activity questionnaire assessing family histories of disease, past fracture and/or hospitalization history, past/current medication use, current health condition, past/current dietary habits, and past/current dietary supplement use (e.g. multivitamin use). For females, a history of oral contraceptive use, age of first menstrual cycle, and average days between menstrual periods was also obtained. Athletes were also required to report their sport-related injuries (bone, muscle, and tendon), the number of years of playing experience, number of days and hours participating in their sport per week as well as other sport-specific training (e.g. weight-lifting). See Appendix A.

3.4.*5 Statistical Analyses*

All analyses were performed using SPSS version 12.0 (Chicago, Il). Means and standard deviations (SD) are presented as descriptive statistics. Outliers were inspected visually via scatterplot and any cases lying definitively outside the centroid were removed. One-way analysis of variance (ANOVA) was used to examine differences between groups in age, anthropometry, muscle performance, training history, and age of menarche (females only). Between-group differences in DXA-derived variables were evaluated via analysis of covariance (ANCOVA) using age, height, and body weight as covariates. Differences in pQCT-derived bone characteristics were analyzed by ANCOVA, using age, limb length, and body weight as covariates. These factors are typically controlled for in studies of bone outcomes. Sidak correction as used in post hoc tests of ANOVA and ANCOVA. The Sidak was utilized due to its ability to be more sensitive to detecting actual differences between groups in which sample size inequalities exist. Statistical significance was set at $p < 0.05$.

CHAPTER 4 RESEARCH RESULTS

Chapter 4 is a presentation of the results in the form of two manuscripts that were developed from the study design and results generated. The chapter is broken into two sections: (1) Comparison of Bone strength Based on Sport in Elite Adult Female Athletes and (2) Comparison of Bone Strength Between Males and Females Within the Same Sport. Each manuscript is preceded by a general overview, which includes the rationale, the objective(s), the hypothesis, and its contribution to the literature. All unadjusted data from each study can be found in Appendix C (manuscript 1) and Appendix D (manuscript 2).

Manuscript One: Bone mineral density and bone strength based on sport in elite female athletes

Rationale: Osteoporosis affects 1 in every 2 women. It is a condition characterized by low bone mass and bone structural deterioration, which leads to a decrease in bone strength and an increase in fracture susceptibility. Physical activity is a critical element for building a strong skeletal structure and offsetting bone fragility in later life. Thus, there is interest in identifying activities that are osteogenic. Some sports are capable of increasing bone mineral mass and strengthening bone structure. However, the majority of the results were generated from dual energy X-ray absorptiometry (DXA) and interpretation, without structural and strength information, is somewhat ambiguous. With the use of peripheral quantitative computed tomography (pQCT) we have the ability to assess bone's structure and better explore the contribution of different types of physical activity on skeletal strength.

Objectives: To investigate differences in bone mineral density (BMD) and bone strength in elite female soccer players, ice hockey players, swimmers, and controls.

Hypothesis: Athletes participating in weight-bearing sports will be exhibit greater areal bone mineral densities and bone strength than those in repetitive/non-impact sports and control groups.

Secondary Hypothesis: Bones adapt their structure and strength to accommodate mechanical influences. Hence, strength increases will be evident in sites of greatest loading.

Contribution to the Literature*:* The purpose of this cross-sectional study was to add to both the mainly DXA-based information and expand on the current pQCT data in relation to the influence of mechanical loading-types and osteogenesis.

Bone Mineral Density and Bone Strength Based on Sport in Elite Female Athletes

ABSTRACT

Purpose: To investigate differences in bone mineral density (BMD) and bone strength in elite female soccer players, ice hockey players, swimmers, and controls.
Methods and Materials: A total of 53 elite collegiate-aged (18-25 years) female soccer (n = 15), ice hockey (n = 21), and swimming (n = 17) athletes and 19 non-active controls were included. Areal BMD of the total body, hip, and spine were assessed by dual energy X-ray absorptiometry (DXA). Estimated bone strength (section modulus, polar strength strain index), bone geometry (total bone area, cortical area) and bone volumetric density (vBMD) of the dominant tibia and radius were examined using peripheral quantitative computed tomography (pQCT, Stratec XCT 3000).
Results: Soccer and ice hockey were associated with the highest adjusted total body (soccer players had 6.7% and 8.2% higher values compared to swimmers and controls; ice hockey players had 10.1% and 11.5% higher values compared to swimmers and controls) and lumbar spine (both soccer and hockey groups had 9.6% and 8% higher values compared to the swimming and control groups) aBMD. At the distal tibia, the soccer group demonstrated significantly greater trabecular vBMD and bone strength index compared to all other groups. At the distal radius, ice hockey players were associated with greater bone strength index, (25.2% and 23.7% greater) compared to swimmers and controls. There were no differences between the soccer and ice hockey groups in any of the tibia measurements.
Conclusion: Hockey and soccer athletes exhibited greater bone strength, areal and volumetric bone densities at loaded sites compared to both swimmers and controls. These findings suggest that soccer and ice hockey are beneficial activities to enhance bone mass and structure in females. However, future studies should focus on non-weight bearing sports and look at sport-specific skeletal sites.

Key Words: bone mineral density, bone structure, loading, female athletes, sports.

INTRODUCTION

Mechanical loading is a crucial element for building a strong skeletal structure and offsetting bone fragility in later life [1]. Thus, there is interest in identifying activities that are osteogenic. As mechanical strain generated from loading constitutes the stimulus for bone functional adaptation, it has traditionally been postulated that high-impact sports that generate large ground reaction forces are the most osteogenic. This theory has been supported by several studies demonstrating that impact[2,3] and weight-bearing sports such as gymnastics [4,5], volleyball[6], and soccer [7,8] are associated with a greater areal bone mineral density (aBMD) and bone mineral content (BMC) than non-weight bearing sports, such as cycling[9] and swimming [10,11]. However, forces applied to bone are primarily the result of muscle contractions [12], and by extension, sports that involve muscle contraction with little or no impact (ground reaction) forces, should theoretically also be osteogenic at sites of greatest muscle pull.

Previous studies of sports involving little or no weight bearing, though associated with repetitive muscle contraction such as cycling and swimming, have utilized the measurement

technique, dual energy X-ray absorptiometry (DXA). Although DXA is an important modality for assessing potential fracture risk, the interpretation of bone structure and strength, using bone mineral content (BMC, g) and areal bone mineral density (aBMD, g/cm^2) alone, may be somewhat ambiguous [10,13]. Due to its planar nature, DXA is only capable of providing a two-dimensional estimation of a three dimensional object. Consequently, it is unable to provide important information about the structure (e.g. contribution of cortical bone) [14], which ultimately determines mechanical ability.[13]

With the use of peripheral quantitative computed tomography (pQCT), we are able to examine bone structure. Unlike DXA, pQCT has the ability to separate and assess volumetric density (vBMD, mg/mm^3) of the different cortical and trabecular compartments and provides measures of bone cross-sectional geometry. With volumetric BMD and bone cross-sectional area, we are able to derive estimates of bone strength. It also provides muscle size or muscle cross-sectional area (CSA) at the region of interest, and in conjunction with DXA-derived lean body mass, we are able to assess bone strength relative to surrogates of the loads applied to bone via muscle force [15,16].

The purpose of this cross-sectional study is to explore differences in bone mass, geometry and bone mechanical strength in collegiate female swimmers, soccer and hockey players. Our primary research question is whether a sport such as swimming, that involves very little or no impact, is as effective in developing strong bone structures as other weight bearing impact-loading sports.

METHODS AND MATERIALS

Study Participants

A total of 53 elite collegiate (18-25 years) female soccer (n = 15), ice hockey (n = 21), and swimming (n = 17) athletes and 19 non-active controls were included in the study. Athlete participants were recruited from NCAA Division I and III colleges and universities as well as competitive sporting clubs and associations. The study protocol was approved by the University of Minnesota's Institutional Review Board, and each participant gave her written informed consent prior to participation in the study.

Questionnaire

Sport-specific training days per week, training hours per day, number of years competing, and additional training hours per week were documented via recall questionnaire format. The form also included background information, family history, sport-specific and other related injuries, general health status, menstrual status and age of first menarche, medication use, use of oral contraceptives, alcohol and other beverage consumption (e.g. tea/coffee) as well as cigarette and dietary supplement use (Appendix A).

Anthropometry

Total body weight in kilograms was obtained (to the nearest 0.1 kg) using an electronic scale accurate to 200kg (Tanita BWB 800: Tokyo, Japan)**.** Standing height was obtained using a standard wall stadiometer (Accustat™ Genentech: San Francisco, CA) to the nearest 0.1 centimeter. Tibial length was measured to the nearest millimeter from the tibial plateau to the medial malleolus using an anthropometric ruler. Radial length was obtained, using the same measurement device, measuring from the radial head to the styloid process. Body mass index (BMI) was calculated as body weight (kg) divided by height (m) squared. Total body lean and fat mass and percent body fat were taken from dual energy X-ray absorptiometry (GE Lunar Prodigy: Software v. 6.7, Madison, WI) total body scans. Quality assurance was performed daily and the coefficient of variation (CV) for repeated measures was 0.136.

Muscle Performance

Lower extremity strength was estimated using the Vertec® vertical jump tester (Questek Corp: Northridge, CA) according to established testing procedures[17]. Prior to testing, participants were allowed an adequate warm-up via treadmill. Each participant performed three maximal stationary vertical jumps and the highest jumping height obtained was recorded. Estimated jumping power was then calculated in Watts (W) and Watts per kilogram of body weight (W/kg) using the Lewis Formula[18] and nomogram (r=.88, SD = 603 W)[19].

Forearm grip strength was calculated in kilograms with the use of a digital hand dynamometer (Smedley III T-18; Creative Health Products: Ann Arbor, MI) for both arms. Each participant performed three trials (alternating hands) per arm and the greatest score of the three

trials was used to report the final results. Combined grip strength was calculated by adding the greatest score from both the right and left scores and then converted to Newtons (N = kg x 9.8066).

Bone/Muscle Characteristics

Single axial (2.5 mm thick) tomographic slices at both the distal tibia and shaft and distal radius and shaft were scanned using peripheral quantitative computed tomography (pQCT; XCT 3000, Stratec Mediznintechnik GmbH: Pforzbein, Germany) according to standardized procedures. For the *lower extremities,* the 66, 50, and 4 percent sites of the estimated length of the tibia proximal to the distal endplate were included. *Upper extremity* measurements included the 4% site of the estimated length of the radius proximal to the distal endplate and the 50% of the radial shaft. For shaft regions (50%, 66%) of the radius and tibia, *total bone area* (mm^2), *cortical bone area* (mm^2), *cortical density* (mg/cm^3), *section modulus* (mm^3), and *polar strength strain index* (mm^3) were determined**.** *Muscle cross-sectional area* (mm^2) was also calculated at the 66% site*.* At the distal sites (4%) *total bone density (*mg/cm^3), *total bone area* (mm^2)*, trabecular density* (mg/cm^3), and *bone strength index* (mg/mm^4) were determined.

Areal bone mineral density (aBMD) was measured for the total body, femoral neck, hip, and lumbar spine (L1-L4) via dual energy X-ray absorptiometry and standard operating procedures (GE Lunar Prodigy: Fairfield, CT, USA).

Statistical Analysis

Means and standard deviations (SD) are presented as descriptive statistics. SPSS statistical software, version 12.0, was used for all analyses. Outliers were inspected visually via scatterplot and any cases lying definitively outside the centroid were removed. A linear regression model was also utilized to determine any unusual relationships between variables. One-way analysis of variance (ANOVA) was used to examine differences between groups in age, anthropometry, muscle performance, training history, and age of menarche. Between-group differences in DXA-derived variables were evaluated via analysis of covariance (ANCOVA) using age, height, and body weight as covariates. Differences in pQCT-derived bone characteristics were analyzed by ANCOVA, using age, limb length, and body weight as covariates. Choice of these covariates for both DXA and pQCT are consistent with current research practices. Sidak correction was used in post hoc tests of ANOVA and ANCOVA. The Sidak was utilized due to its ability to be

more sensitive in detecting actual differences between groups in which sample size inequalities exist. Statistical significance was set at $p < 0.05$.

RESULTS

Descriptive characteristics are shown in Table 1. Participants ranged in age from 18-25 years and were of normal weight and height with BMI ranging from 22.5-25.4. Groups were well matched with no significant differences between groups in age, height, age at menarche, training sessions, sport-specific training hours per week, and other training hours per week ($p < 0.05$ for all). However, swimmers reported less overall years of competing compared to soccer and ice hockey players (4 years on average). All athlete groups reported additional types of training outside their respective sport, which included weightlifting and plyometrics.

Anthropometry and Muscle Performance

Ice hockey players were heavier (+7.0-8.4 kg) than all sport and control groups. There were no differences in total body lean and fat masses between groups, however swimming had significantly less percent body fat compared to controls. Muscle strength results are summarized in Table 1. Vertical jump power (W) was significantly greater in both soccer (+6.7%, 477.8 W, $p < 0.05$) and ice hockey (+9.2%, 672.2 W, $p < 0.05$) groups compared to controls, but not swimmers. Combined grip strength (N) was significantly greater in ice hockey players compared to all other groups (+104.8 -164.8 N).

Areal Bone Mineral Density

Upon adjustment for age, height, and body weight, both soccer (6.7%, 8.2%) and ice hockey (10.1%, 11.5%) players had significantly higher ($p < 0.05$) total body BMD compared to swimmers and controls. In addition, both soccer and ice hockey groups were associated with greater lumbar spine BMD (9.6%, 9.6%). No differences were evident at the femoral neck or total hip between groups. Adjusted values and mean percentage differences in aBMD between athletes and controls can be found in Tables 2 and 3.

Bone Characteristics at the Lower and Upper Extremities

Adjusted means with confidence intervals (95%) and mean percentage differences between athletes and controls in the bone variables are summarized in Tables 4 and 5. At the distal tibia, the soccer group demonstrated significantly greater trabecular vBMD (12.6%, 14.8%, and 14.0%) and bone strength (15.1%, 26.6% and 26.2%) compared to all other groups (ice hockey, swimming, and control**)** (Figures 1 and 2). In addition, soccer players had significantly higher total area (8.5% and 8.8%), cortical area (11.3% and 13.1**%),** and section modulus (13.8% and 14.6%) at the tibial mid-shaft compared to both swimmers and controls (Figure 3). Furthermore, the soccer group had greater cortical area (10.6% and 13.3%), section modulus (14.9% and 14.8%), and strength-strain index (14.4% and 13.5%) at the tibial shaft (66%) compared to the swimming and control groups. Interestingly, there were no differences between the soccer and ice hockey groups in any of the tibial shaft measurements. At the radial shaft, results were similar among groups for the majority of the measured variables. However, ice hockey players (+12%) did exhibit greater cortical area compared to controls. Furthermore, hockey players, had a 25.2% and 23.7% greater BSI compared to swimmers and controls, respectively, at the distal radius (Figure 4).

DISCUSSION

The main findings of this study include a significantly greater difference in tibial bone strength in female soccer and hockey players compared to controls as well as greater bone strength at the distal radius in female ice hockey players compared to swimmers and controls. No significant bone strength differences were observed in female swimmers compared to controls. Furthermore, we found that total body, lumbar spine, and femoral neck aBMD were greater in soccer and ice hockey players compared to both swimmers and controls. However, again, no significant differences were observed between swimmers and controls in the same regions (0%, 1%, and 5.7%). These data are consistent with other studies that have demonstrated that weight bearing sports such as soccer [8,10,11,20], and ice hockey [21-22] are associated with higher aBMD compared to a non-weight bearing sport such as swimming[10-11,23-24].

With the use of pQCT, we were able to determine bone structure and a true volumetric density, which allowed us to identify the structural and densitometric underpinnings of strength differences in various athletic populations. Bone functional adaptation is site-specific [25], and

therefore, skeletal adaptations should be sport-specific. At the distal tibia, the soccer group had higher trabecular density (12.6%, 14.8%, and 14.0%) compared to the ice hockey, swimming, and control groups. At the distal radius, we found a 23.7% difference in bone strength at the distal radius in ice hockey players compared to controls, 25.2% difference compared to swimmers, and 11.6% difference compared to soccer players. These finding may be explained by the adaptation of the region to accommodate the impact and influence of muscle forces generated from running, jumping, and kicking and/or carrying and using the hockey stick during practice and competition. Our results parallel earlier findings[11,26] using other sport models (weightlifters, hurdlers, and volleyball players) in which bone strength was greater at loaded sites, despite little or no differences in total vBMD and total area. Thus, reinforcing the notion that bone structure (e.g. trabecular vBMD and bone strength) evolves via interaction with the local mechanical (e.g. magnitude of the loading) demands on the affected skeletal site[1,11].

As mentioned, the bone strength of swimmers was not greater compared to any of the other groups at the tibia or radius. These findings are congruent with DXA-based studies that have demonstrated a lack of difference in aBMD between swimmers and controls or even a lower aBMD in swimmers compared to the same group[10,11,22,27]. It is proposed that these findings represent the consequences of swimming as a non-weight bearing sport. Nevertheless, swimming does generate muscle forces, and as muscle forces are the primary loads on bone, swimming should theoretically be osteogenic. It is plausible that skeletal sites experiencing the greater forces during swimming (i.e. humerus, scapula, ect.) may provide us with different outcomes, and thus, these sites should be measured in future studies before swimming is ruled out as an osteogenic athletic event. Another potential explanation for the null findings in swimmers in the present study is that swimmers reported less overall years of competing compared to soccer and ice hockey players (approx. 4 years).

The current findings of this study suggest that ice hockey and soccer are osteogenic. However, a limitation of the current study is that it is cross-sectional, and therefore, does not confer causality. It is possible that athletes with greater tibial and/or radial bone strength self-select to play sports such as hockey and soccer. It is also recognized that elite athletes typically have a history of sports participation in childhood, and thus, may have had a better opportunity to build stronger skeletons compared to their less-active counterparts. Unfortunately, the direct influence of those experiences cannot be measured. Another limitation that could potentially affect the results

between athletic groups is training frequency, intensity and the influence out of sport training (weightlifting, plyometrics, etc.). There is evidence that weightlifting[6,27] is an osteogenic activity, especially at the radius. However, all athlete groups had similar training frequencies, sport-specific training hours as well as other non-sport specific training hours. It must be noted that swimmers did have slightly greater non-sport specific training hours per week compared to the other athletic groups, however they had approximately 4 fewer years competing.

In summary, we found that weight bearing sports are associated with greater volumetric bone mineral density and bone strength at loaded sites compared to non-weight bearing sports and controls. These findings suggest that weight bearing sports such as hockey and soccer are beneficial activities to enhance bone mass and strength in females. However, future studies should focus on non-weight bearing sports and look at sport-specific skeletal sites.

Table #1 Descriptive Characteristics of Athlete and Control Groups

	Soccer (A)	Ice Hockey (B)	Swimming (C)	Control (D)
N	15	21	17	19
Age	20.3 (1.8)	20.1 (1.2)	20.8 (2.6)	20.7 (2.0)
Height (cm)	165.3 (7.9)	168.1 (3.9)	169.4 (4.9)	168.4 (6.4)
Weight (kg)	62.7 (6.9)	71.1 (5.2)[a,c,d]	64.1 (8.1)	63.9 (11.6)
BMI	23.2	25.4	22.5	22.6
Age at menarche	13.5 (0.8)	12.7 (1.5)	12.8 (2.0)	12.8 (1.3)
Training sessions per week	5.3 (1.2)	6.0 (0.4)	5.4 (1.3)	NA*
Sport specific training (hours/day)	2.7 (0.6)	2.9 (0.7)	3.0 (1.1)	NA*
Other training (hours/week)	2.8 (1.6)	3.1 (1.9)	3.4 (1.4)	NA*
Years competing	14.2 (2.3)[c]	14.1 (2.3)[c]	10.5 (4.4)	NA*
Vertical jump height, cm	48.7 (7.7)[d]	46.6 (6.8)[d]	45.2 (6.3)	40.3 (6.3)
Estimated vertical jump power, W	7096.3 (593.5)[d]	7290.7 (424.6)[d]	6926.7 (490.3)	6618.5 (476.5)
Estimated vertical jump power per body weight, kg/W	114.1 (11.0)[b]	96.5 (24.7)	109.2 (11.4)	106 (15.6)
Combined grip strength, N*	655.6 (94.3)	760.4 (65.2)[a,c,d]	595.6 (177.6)	602.5 (105.5)

Values are expressed as means (SD). The mean difference is significant at the .05 level. *NA, not available.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Table #2: *Adjusted* Mean (CI) Values of DXA for Athlete and Control Groups

	Soccer (A)	Ice Hockey (B)	Swimming (C)	Control (D)
Total Body BMD (g•cm^{-2})	1.27 (1.24 to 1.30)[c,d]	1.28 (1.25 to 1.30)[c,d]	1.17 (1.14 to 1.20)	1.17 (1.15 to 1.20)
Total Body BMC (g)	3271.72 (3072.46 to 3470.98)[c,d]	2967.81 (2793.18 to 3142.44)	2697.36 (2514.02 to 2880.70)	2760.38 (2589.64 to 2931.13)
Lumbar Spine BMD (g•cm^{-2})	1.36 (1.30 to 1.42)[d]	1.36 (1.31 to 1.42)[d]	1.25 (1.20 to 1.31)	1.23 (1.18 to 1.29)
Femoral Neck BMD (g•cm^{-2})	1.22 (1.11 to 1.32)	1.22 (1.13 to 1.31)	1.05 (.96 to 1.15)	1.11 (1.02 to 1.19)
Total Hip BMD (g•cm^{-2})	1.14 (1.02 to 1.27)	1.18 (1.07 to 1.30)	1.07 (.95 to 1.19)	1.11 (1.00 to 1.22)

Values adjusted for age, height, and body weight and expressed as means (95% CI). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Table #3: Age, Height, and Body Weight Adjusted *Mean Percentage Differences* in Areal Bone Mineral Densities Between Athlete and the Control Group

	Soccer (A)	Ice Hockey (B)	Swimming (C)
Total Body BMD (g•cm^{-2})	7.9[c,d]	8.6[c,d]	0.0
Total Body BMC (g)	15.6[c,d]	7.0	-2.3
Lumbar Spine BMD (g•cm^{-2})	9.6 [d]	9.6[d]	1.6
Femoral Neck BMD (g•cm^{-2})	9.0	9.0	-5.7
Total Hip BMD (g•cm^{-2})	2.6	5.9	-3.74

Values adjusted for age, height, and body weight and expressed as mean percentages. The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Table #4: *Adjusted* Mean (CI) Values for the Tibia and Radius via pQCT for Athlete and Control Groups

	Soccer (A)	Ice Hockey (B)	Swimming (C)	Control (D)
Lower Extremity				
Distal tibia 4%				
Total density, mg/cm^3	379.05 (361.26 to 396.84)	337.15 (320.61 to 353.68)	318.92 (302.55 to 335.28)	331.13 (315.79 to 346.46)
Total area, mm^2	933.47 (864.93 to 1002.00)	988.71 (925.00 to 1052.42)	949.63 (886.58 to 1012.68)	890.34 (831.27 to 949.41)
Trabecular density, mg/cm^3	310.35 (297.60 to 323.10)[b,c,d]	271.29 (259.44 to 283.14)	264.38 (252.65 to 276.10)	266.84 (255.85 to 277.83)
Bone strength index, mg/mm^4	13259.82 (12407.74 to 14183.90)[b,c,d]	11250.12 (10398.56 to 12101.67)[c]	9729.41 (8891.00 to 1040567.81)	9772.72 (9014.37 to 10531.07)
Tibial midshaft 50%				
Total area, mm^2	460.53 (441.32 to 479.75)[c,d]	427.49 (409.62 to 445.35)	421.32 (403.64 to 438.99)	419.79 (403.22 to 436.35)
Cortical area, mm^2	340.20 (324.23 to 356.16)[c,d]	318.16 (303.32 to 333.00)	301.87 (287.18 to 316.55)	295.79 (282.03 to 309.55)
Cortical density, mg/cm^3	1154.96 (1144.91 to 1165.02)	1165.56 (1156.25 to 1174.87)	1160.68 (1151.43 to 1169.92)	1162.91 (1153.94 to 1171.87)
Section modulus, mm^2	2139.68 (2007.54 to 2271.82)[c,d]	1923.74 (1800.91 to 2046.57)	1844.09 (1722.53 to 1965.65)	1826.36 (1712.47 to 1940.25)
SSIp, mm^3	2086.34 (1963.04 to 2209.64)	1878.92 (1764.31 to 1993.54)	1808.00 (1694.57 to 1921.43)	1823.15 (1716.88 to 1929.42)
Tibial shaft 66%				
Total area, mm^2	583.09 (554.73 to 611.45)[c]	548.01 (521.65 to 574.37)	530.65 (504.56 to 556.73)	542.28 (517.84 to 566.72)
Cortical area, mm^2	328.39 (314.34 to 342.44)[c,d]	308.49 (295.43 to 321.55)	293.46 (280.54 to 306.39)	284.56 (272.45 to 296.67)
Cortical density, mg/cm^3	1131.74 (1122.97 to 1141.51)	1136.23 (1128.11 to 1144.35)	1138.42 (1130.35 to 1146.48)	1139.56 (1131.75 to 1147.38)
Section modulus, mm^2	2721.37 (2568.51 to 2874.24)[c,d]	2436.78 (2294.68 to 2578.88)	2314.55 (2173.93 to 2455.18)	2319.74 (2187.99 to 2451.49)
SSIp, mm^3	2674.26 (2516.84 to 2831.68)[c,d]	2392.77 (2246.44 to 2539.10)	2288.98 (2144.17 to 2433.80)	2314.02 (2178.35 to 2449.69)
MCSA, mm^2	7551.12 (7033.56 to 8068.68)	7000.03 (6518.94 to 7481.13)	7708.28 (7232.16 to 8184.40)	7200.87 (6754.80 to 7646.94)
Upper Extremity				
Distal radius 4%				
Total density, mg/cm^3	371.07 (333.75 to 408.38)	398.51 (364.14 to 432.89)	345.78 (312.49 to 379.06)	352.79 (320.59 to 385.00)
Total area, mm^2	295.39 (255.70 to 335.08)	291.61 (255.19 to 328.03)	302.12 (266.99 to 337.25)	299.01 (265.86 to 332.17)
Trabecular density, mg/cm^3	260.84 (240.04 to 281.63)[d]	235.35 (216.27 to 254.43)	224.68 (206.27 to 243.08)	218.87 (201.50 to 236.24)
Bone strength index, mg/mm^4	4089.50 (3542.23 to 4636.77)	4627.88 (4125.73 to 5130.02)[c,d]	3460.28 (2975.88 to 3944.68)	3531.70 (3074.53 to 3988.87)
Radial shaft 50%				
Total area, mm^2	113.46 (106.04 to 120.88)	112.82 (106.01 to 119.63)	111.12 (104.55 to 117.69)	104.11 (97.91 to 110.31)
Cortical area, mm^2	88.23 (83.11 to 93.35)	91.95 (87.25 to 96.65)[d]	86.21 (81.68 to 90.75)	80.89 (76.61 to 85.16)
Cortical density, mg/cm^3	1179.35 (1167.74 to 1190.96)	1181.44 (1170.30 to 1192.58)	1182.18 (1170.30 to 1194.05)	1184.24 (1174.05 to 1194.43)
Section modulus, mm^2	251.96 (228.76 to 275.17)	254.42 (233.13 to 275.71)	247.77 (227.24 to 268.31)	218.55 (199.17 to 237.94)
SSIp, mm^3	255.50 (231.83 to 279.17)	255.60 (233.89 to 277.32)	250.21 (229.27 to 271.16)	223.95 (204.18 to 243.72)

Values adjusted for age, limb length, and body weight and expressed as means (95% CI). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Table #5: Age, Limb Length, and Body Weight Adjusted *Mean Percentage Differences* in Bone Variables Between Athlete and the Control Group

	Soccer (A)	Ice Hockey (B)	Swimming (C)
Lower Extremity			
Distal tibia 4%			
Total density, mg/cm^3	12.6	1.8	-3.8
Total area, mm^2	4.6	10.0	6.2
Trabecular density, mg/cm^3	14.0^{b,c,d}	1.6	-0.9
Bone strength index, mg/mm^4	26.2^{b,c,d}	13.1^{c}	-0.4
Tibial midshaft 50%			
Total area, mm^2	8.9^{c,d}	1.8	0.4
Cortical area, mm^2	13.1^{c,d}	7.0	2.0
Cortical density, mg/cm^3	-0.7	0.2	-0.2
Section modulus, mm^2	14.6^{c,d}	5.1	1.0
SSIp, mm^3	12.6	3.0	-0.8
Tibial shaft 66%			
Total area, mm^2	7.0^{c}	1.1	-2.2
Cortical area, mm^2	13.4^{c,d}	7.8	3.0
Cortical density, mg/cm^3	-0.7	-0.3	-0.1
Section modulus, mm^2	14.8^{c,d}	4.8	-0.2
SSIp, mm^3	13.5^{c,d}	3.3	-1.1
MCSA, mm^2	4.6	-2.9	6.6
Upper Extremity			
Distal radius 4%			
Total density, mg/cm^3	4.9	11.5	-2.0
Total area, mm^2	-1.2	-2.5	1.0
Trabecular density, mg/cm^3	16.1^{d}	7.0	2.6
Bone strength index, mg/mm^4	13.6	23.7^{c,d}	-2.1
Radial shaft 50%			
Total area, mm^2	8.2	7.7	6.3
Cortical area, mm^2	8.3	12.0^{d}	6.2
Cortical density, mg/cm^3	-0.4	-0.2	-0.1
Section modulus, mm^2	13.3	14.1	11.8
SSIp, mm^3	12.4	12.4	10.5

Values adjusted for age, limb length, and body weight and expressed as mean percentages. The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D

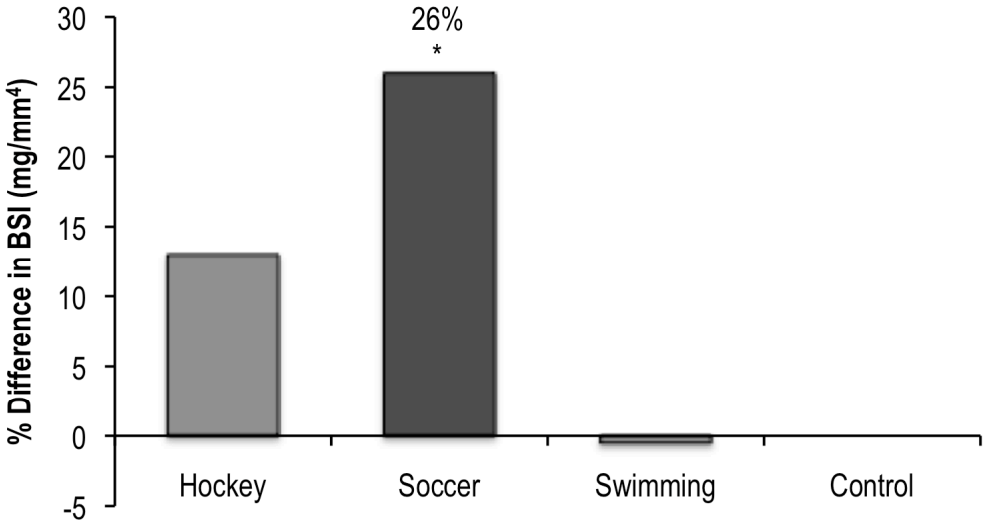


Figure 1: The age, limb length, and body weight adjusted mean percent difference in bone strength index at the distal tibia between athletic and control groups. *Significantly different from all other groups, $p < 0.05$.

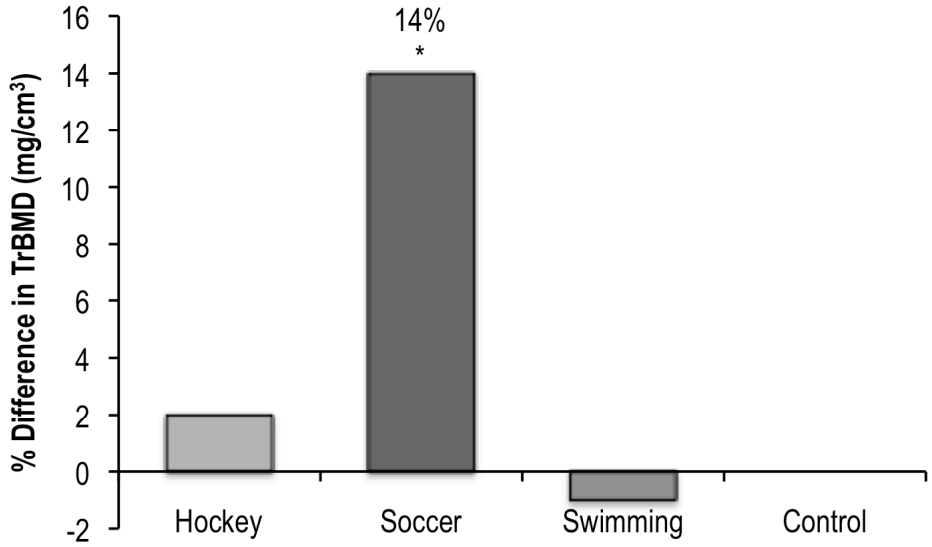


Figure 2: The age, limb length, and body weight adjusted mean percent difference in trabecular BMD at the distal tibia between athletic and control groups. *Significantly different from all other groups, $p < 0.05$.

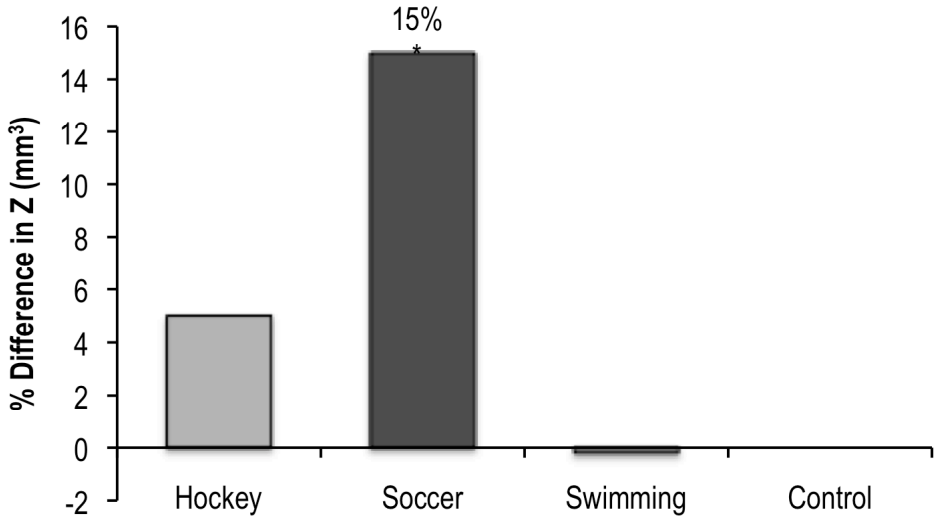


Figure 3: The age, limb length, and body weight adjusted mean percent difference in section modulus (Z) at the tibial mid-shaft between athletic and control groups. *Significantly different from swimming and controls, $p < 0.05$.

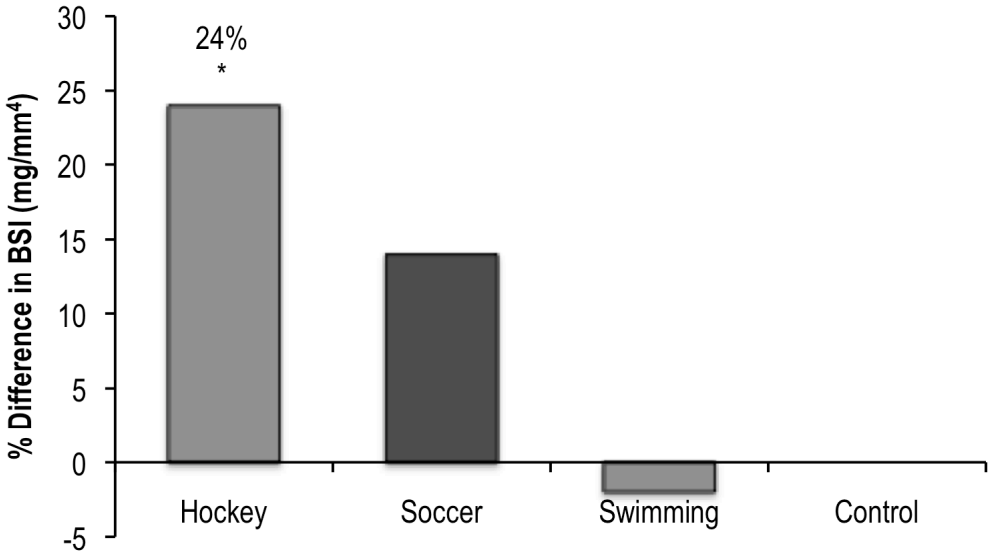


Figure 4: The age, limb length, and body weight adjusted mean percent difference in bone strength index (BSI) at the distal radius between athletic and control groups. *Significantly different from swimming and controls, $p < 0.05$.

Manuscript Two: Bone Strength in Collegiate Female and Male Athletes

Rationale: Osteoporosis is a condition characterized by low bone mass and micro-architectural deterioration which leads to a decrease in bone strength and an increase in fracture susceptibility. It is estimated that 30-50% of women and 15-30% of men will suffer an osteoporotic fracture in their lifetime. One potential explanation for the discrepancy in fracture rates between males and females is the difference in bone mass and strength. However, as physical loading is an important modifier of bone strength, it remains unclear if gender differences in bone strength remain when active individuals are studied. It is well established that certain sport activities have the ability to generate beneficial types of mechanical loadings resulting in increased bone mass and strength at loaded sites. This model, therefore offers a unique opportunity to examine if gender differences do exist outside of sedentary populations.

Objectives: The purpose of this cross-sectional study was to compare bone strength and geometry in female and male collegiate athletes.

Hypothesis: Once adjusted for relevant variables, gender differences in athletes who participate in the same sport will attenuate.

Contribution to the Literature*:* To date, it is unknown if gender differences do exist in relation to bone strength amongst active individuals. Thus, this study seeks to contribute to furthering the knowledge in this area.

Title: Bone Volumetric Density, Bone Geometry, and Bone Strength in Elite Female and Male Athletes

ABSTRACT

Purpose: To compare bone volumetric density, bone geometry and strength in female and male collegiate athletes
Materials and Methods: A total of 111 elite collegiate-aged female and male ice hockey (19 male, 21 female), swimming (13 male, 17 female), and running (19 male, 22 female) athletes and 34 non-active controls (15 male, 19 female) were included in the study. Peripheral quantitative computed tomography (pQCT, Stratec XCT 3000) was used to examine bone structures, including total bone area, cortical density, section modulus, muscle cross-sectional area, and bone strength index, of the tibia in elite male and female athletes. We used analysis of covariance and adjusted for tibia length, weight and muscle cross-sectional area to compare bone outcomes between groups.
Results: After adjustment for body size, weight and muscle size, bone strength remained significantly higher (14-18%, $p < 0.05$) in males who participated in weight-bearing sports (ice hockey and running) compared to their female counterparts. Differences in bone strength were due mainly to a greater total bone area (+9.5-17.7%), with no differences in bone vBMD. Interestingly, after adjusting for body size and weight, there were no significant differences in bone outcomes among collegiate male and female swimmers.
Conclusion: In the weight-bearing sports, male athletes exhibited greater volumetric bone densities (vBMD) and bone strength measures in the tibia compared to their female counterparts. These findings suggest that there are gender differences in bone structure even among active individuals. However, future research should follow athletes over time directly assessing hormonal and nutritional levels to determine their influences and measure additional sport-specific loading sites.

Key Words: bone mineral density, bone strength, gender-differences, athletes.

INTRODUCTION

Osteoporosis is a condition characterized by low bone mass and micro-architectural deterioration which leads to a decrease in bone strength and an increase in fracture susceptibility. It is estimated that 30-50% of women and 15-30% of men will suffer an osteoporotic fracture in their lifetime [1]. One potential explanation for the discrepancy in fracture rates between males and females is the difference in bone mass and strength.

Several studies have demonstrated gender differences in bone mass and bone strength in healthy, sedentary populations. Particularly, males tend to possess greater total aBMD[2,3], thicker cortices[4], greater periosteal diameters[5], greater total bone area[5,6] as well as greater muscle mass[7]. Although males and females generally have similar vertebral heights and vertebral trabecular volume density, men have larger vertebral cross-sections[8]. It is suggested that these structural differences confer strength advantages and reduced fracture rates for males[8].

However, as physical loading is an important modifier of bone strength[Frost 2003], it remains unclear if gender differences in bone strength remain when active individuals are studied. It is well established that certain sport activities have the ability to generate beneficial types of mechanical loadings resulting in increased bone mass[10-12] and strength[13,14] at loaded sites. This model, therefore offers a unique opportunity to examine if gender differences do exist outside of sedentary populations.

Previous research examining gender differences in bone have utilized sedentary individuals or cadaver ash weights of bone as well as the measurement technique, dual energy X-ray absorptiometry (DXA). Although DXA is an important modality for assessing potential fracture risk, the interpretation of bone structure and strength, using bone mass (BMC) and areal bone mineral density (aBMD) alone, may be somewhat ambiguous [15,16]. DXA outcomes do not provide enough information about bone geometry and are confounded by bone and body size[17]. Furthermore, it is unable to provide important details about the structure (e.g. contribution of cortical bone) [18], which ultimately determines mechanical ability[16]. With the use of peripheral quantitative computed tomography (pQCT), we are able to examine bone structure in greater detail. Peripheral QCT has the ability to separate and assess volumetric densities (vBMD) of the different cortical and trabecular compartments and provides measures of bone cross-sectional geometry and strength. Use of this modality may provide a more accurate depiction of structural differences between active males and females.

The purpose of this cross-sectional study was to compare bone strength and geometry in female and male collegiate athletes.

METHODS AND MATERIALS

Participants

A total of 111 elite collegiate-aged female and male ice hockey (19 male, 21 female), swimming (13 male, 17 female), and running (19 male, 22 female) athletes and 24 non-active controls (15 male, 19 female) were included in the study. Athlete participants were recruited from NCAA Division I and III colleges and universities as well as competitive sporting clubs and associations from in and around the Twin Cities Metro Area. Controls were volunteers, recruited from the same colleges and universities. The study protocol was approved by the

University of Minnesota's Institutional Review Board, and each participant gave his or her written informed consent prior to the measurements.

Questionnaire

Sport-specific training days per week, training hours per day, number of years competing, and additional training hours per week were documented via recall questionnaire format. The form also included family history of illness, sport-specific and other related injuries, general health status, menstrual status and age of first menarche, medication use, use of oral contraceptives, alcohol and other beverage consumption (e.g. tea/coffee) as well as cigarette and dietary supplement use (Appendix A).

Anthropometry

Total body weight in kilograms was obtained (to the nearest 0.1 kg) using an electronic scale accurate to 200kg (Tanita BWB 800: Tokyo, Japan)**.** Standing height was obtained using a standard wall stadiometer (Accustat™ Genentech: San Francisco, CA) to the nearest 0.1 centimeter. Body mass index (BMI) was calculated as body weight (kg) divided by height (m) squared. Tibial length was measured to the nearest millimeter from the tibial plateau to the medial malleolus using an anthropometric ruler.

Bone/Muscle Characteristics

Single axial (2.5 mm thick) tomographic slices at 3 sites on tibia and shaft were scanned using peripheral quantitative computed tomography (pQCT; XCT 3000, Stratec Mediznintechnik GmbH: Pforzbein, Germany) according to standardized procedures. Sites measured were 66, 50, and 4 percent sites of the estimated length of the tibia proximal to the distal endplate. For shaft regions (50%, 66%) of the tibia, *total bone area* (mm^2), *cortical bone area* (mm^2)*, section modulus* (mm^3), and *polar strength strain index* (SSI; mm^3) were determined**.** *Muscle cross-sectional area* (mm^2) was also calculated at the 66% site*.* At the distal sites (4%) *total bone density (*mg/cm^3), *total bone area* (mm^2)*, trabecular density* (mg/cm^3), and *bone strength index* (BSI; mg/mm^4) were determined.

Statistical Analyses

All analyses were performed using SPSS version 12.0 (Chicago, Il). Means and standard deviations (SD) are presented as descriptive statistics. Outliers were inspected visually via scatterplot and any cases lying definitively outside (>3SD) the centroid were removed (one participant was removed due to a history of an eating disorder). We used analysis of variance (ANOVA) to examine differences between males and females in age, anthropometry, and training history. Differences in pQCT-derived bone characteristics were analyzed by ANCOVA, using age, limb length, and body weight as covariates. These factors are typically controlled for in studies of bone outcomes. Sidak correction as used in post hoc tests of ANOVA and ANCOVA. The Sidak was utilized due to its ability to be more sensitive to detecting actual differences between groups in which sample size inequalities exist. Statistical significance was set at $p < 0.05$.

RESULTS

Separate group characteristics for males and females are shown in Tables 1 and 2. Males and females ranged in age from 18-25 years and were of normal weight and height with BMI ranging from 20.6-26.1 kg/m^2. The ice hockey group was well matched in age, BMI, training sessions, sport-specific training hours per week, and other training hours per week ($p < 0.05$ for all). However, as expected, males were heavier (+16.1 kg) and taller (14.4 cm) and reported 2 more years of competition ($p < 0.05$). Similar to ice hockey, male and female swimmers did not differ in relation to age, BMI, daily and weekly training variables as well as years competing ($p < 0.05$). However, males weighed more (+16.2 kg) and were taller (+14.3 cm) than females. Running athletes were similar in age and BMI, however males were heavier (+13.2 kg), taller (+11.4 cm), and reported running more miles per week (+7.1) compared to females ($p < 0.05$).

Unadjusted models

Prior to adjusting for body weight or muscle size, there were significant gender differences in all bone outcomes including vBMD (+2-9%), bone strength (+29-31%), and bone geometry (both total and cortical areas) at all sites in hockey players, runners and swimmers.

Models adjusting for body weight or muscle CSA and tibia length reduced differences between groups. We present below bone outcomes adjusted for body weight and tibia length for each group.

Ice Hockey Players

Adjusted means with confidence intervals (95%) and mean percentage differences between male and female athletes in the bone variables are summarized in Tables 3 and 4. Graphical representations of mean percentage differences in section modulus (Z) and BSI for all athletic groups are shown in Figures 1 and 2. At the 66% site, males had greater ($p < 0.05$ for all) total bone and cortical areas (12% and 6.8%) as well as section modulus (14.9%) and strength strain index (14.8%). Furthermore, at the tibial mid-shaft, females had significantly lower total area (-8.4%), section modulus (-10.7%), and SSI (-10.3%). At the distal tibia, males had higher total areas (17.7%) and BSI (18.5%). Although not statistically significant, females had greater total bone density at all sites and higher cortical density and cortical thickness at both shaft sites.

Swimmers

At all 3 tibia sites, males and females were not significantly different in all bone variables, except males were 12.9% higher in total area at the distal site. Females did have greater total density at all sites as well as higher trabecular density at the distal site and greater cortical density at both shaft sites. These differences, however, were not statistically significant.

Runners

At the 66% site males had higher total area (9.5%), cortical area (14.9%), section modulus (18.4%), and SSI (16.6%) values compared to females. Similar results were reported at the tibial mid-shaft in which males had greater total area (11.8%), cortical area (14.4%) as well as section modulus (19.9%) and strength strain index (17.8%). At the distal site, females had lower total area (-14.5%), trabecular density (-11.2%), and bone strength index (-25.1%).

DISCUSSION

The main findings of our study indicate a significantly greater difference in tibial bone strength in active males compared to active females. With the exception of swimmers, male athletes had a higher section modulus, strength strain index, and bone strength compared to their female counterparts. Furthermore, males exhibited consistently greater total bone and cortical areas.

With the use of pQCT, we were able to assess bone structure and a true volumetric density, which allowed us to better identify the structural differences of males and females in these athletic populations. In the weight-bearing sports (ice hockey and running), males had greater bone strength estimates (15-18%) at the tibial mid-shaft compared to their female counterparts. Aside from 13% greater distal total area, males and females did not significantly differ in any of the bone measurements in the swimming group.

The current findings of this study suggest that there are gender differences in bone structure and strength even amongst active individuals. Much of this advantage in males may be attributed to greater body size (height and weight) and the greater amount of lean muscle mass. Several studies show the direct relationship between bone mass and lean tissue [19-21]. Physical loading is a crucial element for building a strong skeletal structure[9], and since forces applied to bone are primarily the result of muscle contractions[22], theoretically, the bigger and stronger individual would place greater mechanical stress on the bone site. In our data, although gender differences decreased after adjusting for size and weight or muscle mass, there were still significant differences in bone strength between male and female hockey players and runners. Interestingly, no significant differences in bone strength were evident in our group between female and male swimmers after adjusting for body weight or muscle size despite male swimmers being taller, heavier, and having greater muscle area at the tibial site.

Differences in bone strength in our athletes were due mainly to differences in bone geometry (total area) with no difference in volumetric BMD. As well, the magnitude of the differences decreased after adjusting for body weight or muscle CSA. These data help explain conflicting findings in DXA studies and illustrate the importance of adjusting for body size and muscle force when comparing individuals of different size.

Our findings suggest that bone strength was higher in male athletes due mainly to greater total area, with smaller differences in cortical area (suggesting females had more bone

on the endocortical surface) and no difference in vBMD. These data are consistent with evidence from animal studies that suggest estrogen inhibits periosteal expansion in response to mechanical loading and stimulates endocortical contraction in women[23]. Importantly, we did not measure hormonal levels directly and there is a clear continuum of estrogens and andorgens in both males and females[24-26]. Future studies that assess hormone levels directly and follow athletes over time are needed to further explore these theories.

The athlete model used in this study offered us a unique opportunity to measure bone strength in very elite athletes. The participants were well matched, mostly Division I athletes, representing diverse mechanical loading conditions. However, it is important to consider the limitations of this study. It is clear that its cross-sectional design does not confer causality. Longitudinal data are needed that directly measure hormonal and nutritional levels and follow athletes over time. In addition, we only measured the tibia in this study and it is possible that other bone regions may show different outcomes. For instance in swimming, skeletal sites such as the humerus and scapula experience higher forces than the tibia and would be important to assess in future studies. Finally, we were not able to assess genetic, hormonal, or nutritional factors directly in this study so can only speculate on the potential influence. Future studies are needed to explore what factors may explain differences in bone strength between male and female athletes.

In summary, we found male athletes exhibited greater bone structure and strength measures of the tibia compared to their female athlete counterparts. These findings suggest that gender differences in bone structure even among active individuals do exist. This may help explain gender differences in fracture rates in older life.

Table #1 Descriptive Characteristics of Male Adult Athlete and Control Groups

	Ice Hockey (A1)	Swimming (B1)	Running (C1)
N	19	13	19
Age	20.7 (1.5)	20.0 (1.0)	21.5 (1.7)
Height	182.7 (7.0)a_2	183.5 (7.3) b_2	180.7 (7.1) c_2
Weight	87.6 (7.4) a_2	80.0 (7.2) b_2	69.6 (5.9) c_2
BMI	26.1	23.6	21.2
Sport-specific training sessions (days/week)	6 (.32)	6 (0.0)	NA*
Sport-specific training (hours/day)	2.9 (.55)	3.5 (.58)	NA*
Other training (hours/week)	3 (.89)	3.1 (1.1)	NA*
Miles per week (Runners only)	NA*	NA*	55.6 (14.5) c_2
Years competing	16.24 (2.14) a_2	10.9 (2.2)	NA*

Values are expressed as means (SD). The mean difference is significant at the .05 level.

a_2 = sig. different from A2, b_2 = sig. different from B2, c_2 = sig different from C2, d_2 = sig different from D2 ($p<.05$).

*NA, not available.

Table #2 Descriptive Characteristics of Female Adult Athlete and Control Groups

	Ice Hockey (A2)	Swimming (B2)	Running (C2)
N	21	17	22
Age	20.1 (1.2)	20.8 (2.6)	20.3 (1.9)
Height	168.3 (3.9)	169.2 (4.9)	167.5 (6.3)
Weight	71.5 (5.2)	63.8 (8.1)	58.2 (7.0)
BMI	25.4	22.5	20.6
Age at menarche	12.7 (1.5)	12.8 (2.0)	13.9 (2.1)
Sport-specific training sessions (days/week)	6.0 (0.4)	5.4 (1.3)	NA*
Sport-specific training (hours/week)	2.9 (0.7)	3.0 (1.1)	NA*
Other training (hours/week)	3.1 (1.9)	3.4 (1.4)	NA*
Miles per week (Runners only)	NA*	NA*	48.5 (11.01)
Years competing	14.1 (2.3)	10.5 (4.4)	NA*

*NA, not available.

Table 3: Mean percentage differences in bone variables females and males of the same sport

	Ice Hockey (A)	Swimming (B)	Running (C)
Lower Extremity			
Distal tibia 4%			
Total density, mg/cm^3	1.8	7.3	-8.3
Total area, mm^2	-17.7*	-12.9*	-14.5*
Trabecular density, mg/cm^3	-3.1	4.6	-11.2*
Bone strength index, mg/mm^4	-18.5*	-0.5	-25.1*
Tibial midshaft 50%			
Total density, mg/cm^3	3.5	4.6	-0.8
Total area, mm^2	-8.4*	-5.9	-11.8*
Cortical area, mm^2	-5.6	-2.6	-14.4*
Section modulus, mm^2	-10.7*	-5.0	-19.9*
SSIp, mm^3	-10.3*	-5.1	-17.8*
Tibial shaft 66%			
Total density, mg/cm^3	5.0	6.0	-2.3
Total area, mm^2	-12.0*	-6.4	-9.5*
Cortical area, mm^2	-6.8*	-4.2	-14.9*
Section modulus, mm^2	-14.9*	-6.9	-18.4*
SSIp, mm^3	-14.8*	-6.4	-16.6*
Muscle cross-sectional area, mm^2	-3.0	-1.9	-11.7*

Values adjusted for age, limb length, and body weight and expressed as mean percentages. The mean difference is significant at the .05 level.
*Denotes significant differences between females and males. Calculated as [females – males].

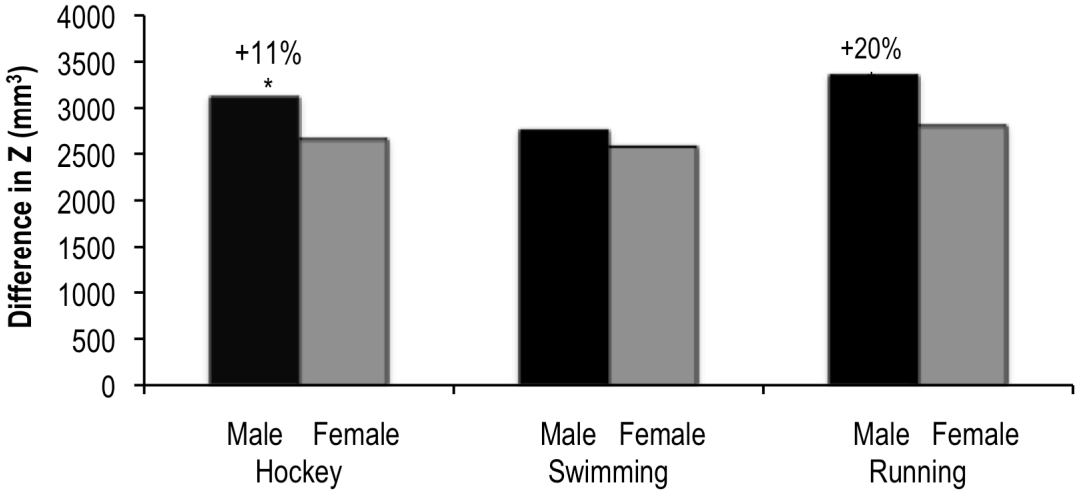


Figure 1: Age, tibia length, and weight adjusted difference in section modulus (Z) at the tibial mid-shaft between male and female athletes. Results are expressed in absolute terms. *Denotes significance. Significance reported as percent differences, $p < 0.05$.

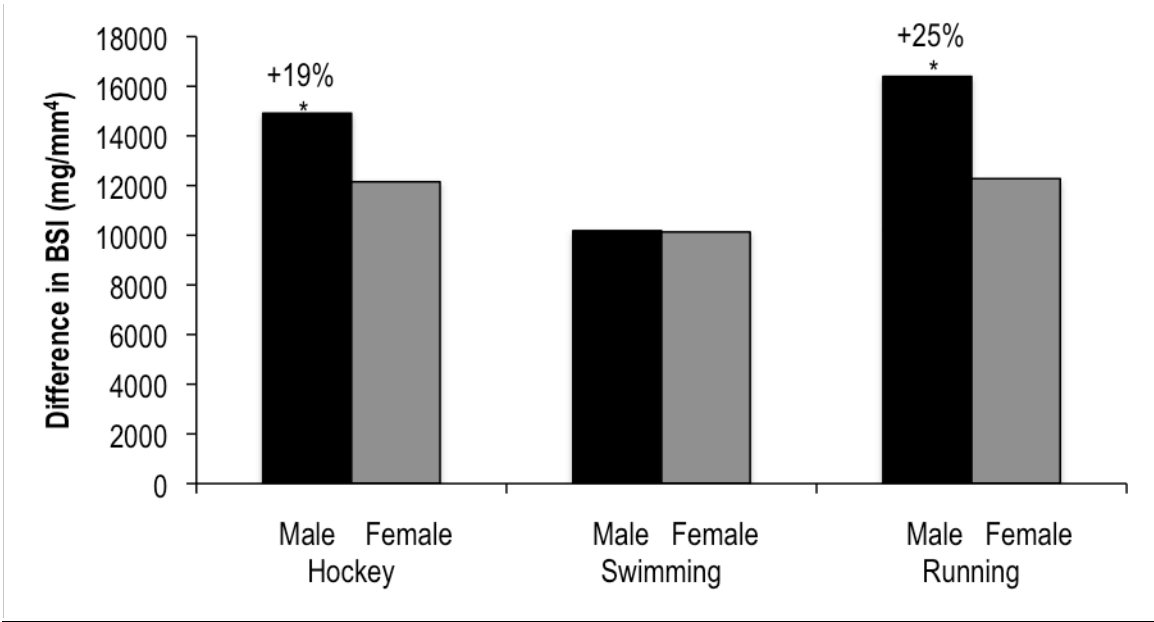


Figure 2: Age, tibia length, and weight adjusted difference in bone strength index (BSI) at the distal tibia between male and female athletes. Results are expressed in absolute terms. *Denotes significance. Significance reported as percent differences, $p < 0.05$.

CHAPTER 5 THESIS SUMMARY

Osteoporosis is a condition characterized by low bone mass and micro-architectural deterioration which leads to a decrease in bone strength and an increase in fracture susceptibility. It is estimated that 1 in 2 of women and 1 in 4 of men will suffer an osteoporotic fracture in their lifetime. High peak bone mass is associated with reduced risk of osteoporotic-related fractures later in life. Typically, strategies to combat the disease have focused on older populations, and most interventions have been pharmacological in nature, which can be expensive and oftentimes can contain negative physical side effects.

Mechanical loading, primarily through physical exercise, is a viable preventative tool and may be the most cost-effective method to counteract low bone mass. It is well established that loadings, specifically inherent in many sports, increases both bone mineral mass and bone strength. However, the majority of previous research has utilized the measurement technique, dual energy X-ray absorptiometry (DXA). Although DXA is an important modality for assessing potential fracture risk in older populations, the interpretation of bone structure and strength, using bone mineral content (BMC, g) and areal bone mineral density (aBMD, g/cm^2) alone does not give us enough information. With the use of peripheral quantitative computed tomography (pQCT), we were able to examine bone structure and strength; vital components when examining fracture risk and changes due to physical activity. The purpose of this research was to measure bone structure and strength in selected sport activities in order to explore if some sports are more beneficial for overall skeletal health compared to others. Utilizing more thorough technology, we were able to explore the benefits of different types of physical activity on bone structure and strength in young male and female adult athletes.

The findings suggest that weight-bearing sports (specifically ice hockey, soccer, and running) are beneficial activities to enhance bone mass and bone strength. In addition, it appears that there are clear differences between males and females in relation to overall skeletal structure and bone strength. These latter findings may help explain gender differences in fracture rates in later life.

However, it is important to note that these studies came from a cross-sectional design. Thus, it does not confer causality. Future studies should focus on non-weight bearing sports and look at sport-specific skeletal sites as well as follow athletes over time directly assessing hormonal and nutritional levels and their influence on skeletal development. In addition, given the importance

of childhood, it will be vital to focus on the influence of childhood athletics on peak bone mass and later adulthood fracture rates.

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APPENDICES

Appendix A: Health and Physical Activity Questionnaire

Appendix B: Testing Procedures

Appendix C: Unadjusted Information for Manuscript 1

Appendix D: Unadjusted Information for Manuscript 2

Appendix A: Health and Physical Activity Questionnaire

Evaluation of Musculoskeletal Health in Young and Collegiate Hockey and Soccer Players and Swimmers
[HSC#0511M77197;GCRC#1133]

We would like to know some things about your health and your experience playing sports/physical activity. The following is a questionnaire designed to assess your overall health. Please answer each question the best you can. If you need help you may ask your parent(s) or guardian and/or one of the study investigators.

IDENTIFICATION

1. **ID Number**_____ **Control**_____ **Athlete**_____

2. **Date of Birth: Month**_____**Day**_____**Year**_____

3. **Age:** _____

4. **Sex: Male Female (circle one)**

HEALTH HISTORY

1. **Are you or your child currently taking any medications?** _____ **Yes** _____ **No**
   If yes, what medication(s) is your child taking? _____
   _____
   _____

   What are these medication(s) for? _____
   _____
   _____

2. **Is there a history of osteoporosis in your family?** _____ **Yes** _____ **No**
   If yes: indicate who was affected
   _____ **mother** _____ **father**
   _____ **maternal grandmother** _____ **paternal grandmother**
   _____ **maternal grandfather** _____ **paternal grandfather**

3. **Is there a history of wrist, hip, or spine fractures in your family?** _____ **Yes** _____ **No**
   If yes: indicate who was affected
   _____ **mother** _____ **father**
   _____ **maternal grandmother** _____ **paternal grandmother**
   _____ **maternal grandfather** _____ **paternal grandfather**

4. **Is there a history of any other bone disease in your family?** _____ **Yes** _____ **No**
   If yes: please indicate the family member(s) affected
   1. _____
   2. _____

   What is the name of the condition(s) affecting this family member?
   1. _____
   2. _____

5. Have you ever been hospitalized, confined to bed or had a limb immobilized (i.e., arm in a cast)?_____ Yes _____ No If yes: list condition, approximate date and time involved (Example: wrist fracture, summer, 1990 10 weeks)

Reason	Date	Time Involved

6. Have you ever been treated for any of the following conditions?

	YES	NO
Allergies		
Scoliosis		
Diabetes		
Asthma		
Anemia		
Eating disorder		
Other conditions:		
(please list)		

7. Reproductive History (females only)

7a. Have you ever used birth control pills or oral contraceptives? _Yes _____No

If Yes, at what age did you start (approximately)? _____ years of age

For approximately how long did you use birth control pills? ____ _____years _ months

7b. How old were you when you had your first menstrual period? _____ years old

7c. Did you have regular periods once they began? _____Yes _____No

7d. On average, how often do you have menstrual periods? (check one)

_____20 days or less
_____21-25 days
_____26-30 days
_____31-36 days
_____37 days or more
_____do not know

LIFESTYLE/NUTRITION DATA

1. Have you ever smoked (circle one)? Yes No (if no, go to question 3.4)

2. Do you still smoke (check one)? ____Yes, daily _____Yes, occasionally _____No, not at all

3. When you are/were smoking, how many cigarettes do/did you usually smoke per day?

About _____ cigarettes per day

4. How often do you drink some kind of alcoholic beverage (check one)?

_____Daily or almost every day
_____3 or 4 times a week
_____Once or twice a week
_____Once or twice a month
_____Less than once a month
_____NEVER
_____Don't know

5. How many cups of coffee do you/did you usually have during the time periods indicated?

	Childhood	Young Adulthood
Never	_____	_____
Sometimes	_____	_____
1 to 2 cups per day	_____	_____
3 cups or more per day	_____	_____

6. How many cups of tea do you/did you usually have during the time periods indicated?

	Childhood	Young Adulthood
Never	_____	_____
Sometimes	_____	_____
1 to 2 cups per day	_____	_____
3 cups or more per day	_____	_____

7. How many cans/bottles of cola do you/did you usually have during the time periods indicated?

	Childhood	Young Adulthood
Never	_____	_____
Sometimes	_____	_____
1 to 2 cups per day	_____	_____
3 cups or more per day	_____	_____

8. Do you drink milk? Yes _____ If yes: How often and how much? _____

_____

9. Do you eat a special diet? _____Yes _____No

If Yes, please circle one vegetarian no dairy (lactose intolerant)
low sodium low cholesterol
other (please specify): _____

10. Do you take a calcium supplement? _____Yes _____No

If Yes, how many times a day do you take it? _____ times/day

What is the name of the supplement? _____

11. Do you take a multivitamin supplement? _____Yes _____No

If Yes, how many times a day do you take it? _____ times/day

What is the name of the supplement? _____

ATHLETES ONLY

SPORT RELATED INJURY HISTORY:

1. **Have you ever experienced a sports related injury? Yes_____ No_____**
   If yes, what type and when
   _____
   _____
2. **Have you ever fractured a bone due to sports participation? If yes, what type and when**

_____
_____

3. **Have you ever had any tendon related injuries? If yes, what type and when (e,g, separated shoulder, dislocation, etc.).** _____
_____

SPORT PARTICIPATION/TRAINING HISTORY:

1. **How long have you been playing your particular sport? _____ years.**
2. **How often do you participate in your sport?**

   Days per week_____

   Hours per day_____
3. **What type of training do you do to play your sports? (e.g. weightlifting, running, plyometrics--- jumping, skipping, hopping).**
   _____
   _____
   _____
   _____

4. How many hours do you train (other than your sport) per week? _____

THANK YOU

Appendix B: Laboratory Testing Procedures

Anthropometry, Muscle Strength, Bone Characteristics

All participants were instructed to wear light, "athletic-type" clothing and shoes for the duration of the study. For height, weight, and sitting height, participants were measured without shoes. Prior to all muscle strength tests, participants were able to perform a walking warm-up (less than 3 mph) on a standard treadmill for approximately 5-10 minutes.

1. Athropometry: Standing height and Body Weight

Standing Height: Wall Stadiometer

Procedures:

1. Participant (with shoes off) was instructed to stand facing away from the wall stadiometer with heels, scapulae, and buttocks in contact with the wall.
2. The participant was instructed to stand as tall as possible.
3. Taking a deep, the measure unit was put into place on top of the participant's head so that the unit and the wall device were at a right angle.
4. Height was recorded to the nearest tenth of a centimeter (0.1 cm).

Testing Equipment: Accustat™ Genentech, San Francisco, CA.

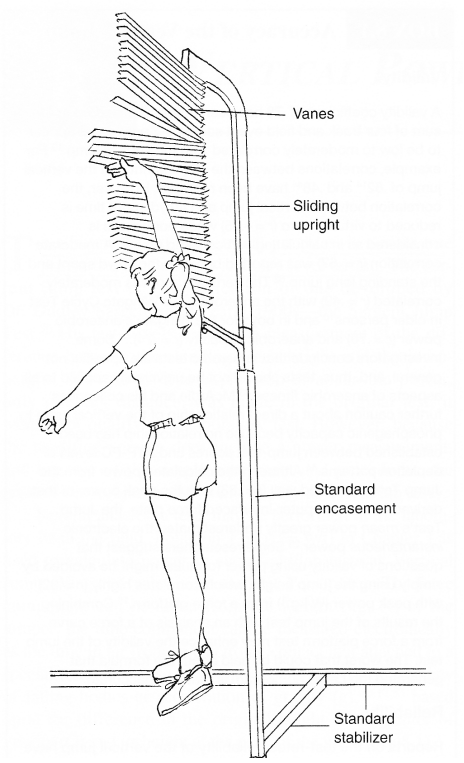
Weight (kg): Certified Electronic Scale
Procedures:

1. Important: Scale was "zeroed" prior to every measurement.
2. Participant was instructed to step up on the scale, standing still and placing equal weight in each foot.
3. Procedures were performed twice to ensure accuracy.
4. Weight was measured in both kilograms and pounds.

Testing Equipment: Tanita BWB 800 Digital Scale, Tokyo, Japan.

2. Muscle Performance: Vertical Jump Power and Forearm Grip Strength

Vertical Jump Power (W):



Procedures:

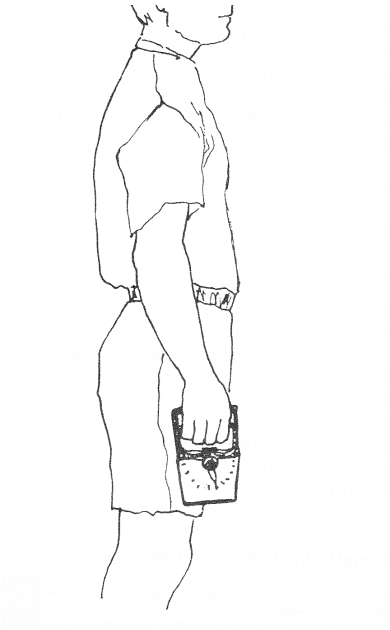
1. Participant was instructed to stand with his or her side to the unit (feet and hips up against the unit).
2. Participant was then instructed to reach (as high as possible with the dominant arm.
3. The peak height reached was referred to as "zero" height.
4. Standing under the jumping device, feet shoulder width apart; the participant was instructed to jump as high as possible, touching the highest vane possible.
5. The best of three trials was recorded.
6. Important: No steps or shuffling of feet were allowed.

Power Equation: Estimated lower extremity power (Watts) was then calculated using the Lewis Formula (1974) and nomogram (r=.88, SD = 603 W; Harman et. al, 1991).

Equation: Vertical Power (W) = 61.9 x Jump Height (cm) + 36 x Body Mass (kg) + 1822

Testing Equipment: Vertec® Vertical Jump Tester (Questek Corporation, Northridge, CA).

Forearm Grip Strength (kg):



Procedures:

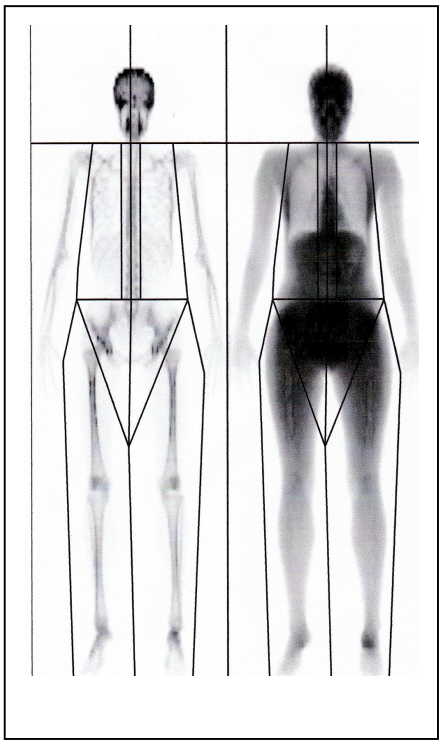
1. Participant was in the standing position for all trials.
2. Grip size was adjusted to so that the third digit (middle finger) was at approximately a right angle.
3. The participant's forearm was placed at an 180˚ angle. See left.
4. The participant was readied, instructed to take a deep breath and squeeze as hard as possible until told to relax.
5. Number of Trials: 3 per arm separated by 30s of rest.
6. Greatest of the three trials (for each hand) was recorded in kilograms.
7. Total was calculated by adding both the right and left together.
8. Strength was also converted to Newtons by:

   Newtons = Grip Strength (kilograms) x 9.8066

Testing Equipment: Smedely III T-18 Hand Dynamometer, Creative Health Products, Ann Arbor.

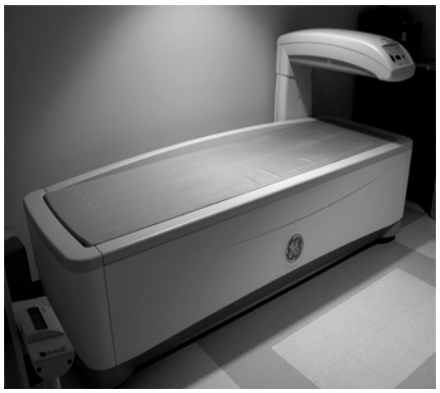
3. Bone Characteristics: Dual Energy X-ray Absorptiometry (DXA) and Peripheral Quantitative Computed Tomography (pQCT).

Dual Energy X-ray Absorptiometry (DXA):



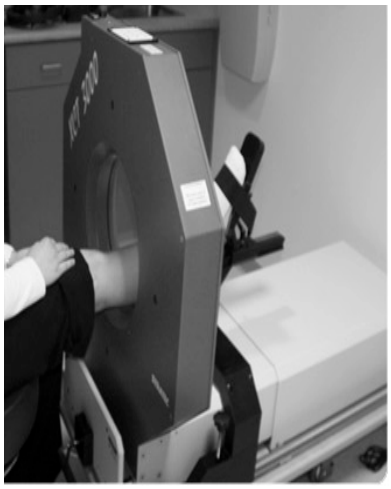
Procedures: Full Body

1. All participants were required to wear athletic-type clothing, free from any metal (e.g. zippers).
2. All females (who have begun menstruation were required to take a precautionary urinary pregnancy test).
3. Participants were then positioned on the padded DXA table, centering the spine on the *mid-line* (see below).
4. Once positioned, the participant was instructed to close his/her eyes for the duration of the test.
5. The scanner is a two-piece device, consisting of an X-ray generator is located below the patient, under the table and an imaging device, or detector positioned above.
6. Once started, the imaging detector or arm passes over the participants from head to foot.
7. Exam time: 8-10 minutes.
8. Other images taken: dual hip and lumbar spine.



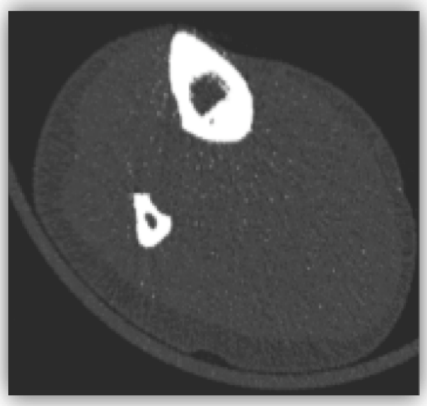
Testing Equipment: GE Lunar Prodigy, Madison WI).

Peripheral Quantitative Computed Tomography (pQCT):



Procedures:

1. Prior to testing each participant, radial and tibial lengths (of the dominant limbs) were measured to the nearest millimeter.
2. For the tibial scan, the participant is positioned into scanning device with leg extended through the gantry device and secured.
3. The pQCT performs the pre-determined number of scans
4. The pQCT scans are obtained via a rotating mechanism that performs series of 15 total scans. One series = 1 topographic slice.
5. Similar procedures were used for the radial scans.



Example of Tibial scan (66%)

Testing Equipment: XCT 3000, Stratec Mediznintechnik GmbH, Pforzbein, Germany.

Appendix C: Manuscript One
Unadjusted and Adjusted Results

Unadjusted Descriptive Characteristics of Athlete and Control Groups

	Soccer (A)	Ice Hockey (B)	Swimming (C)	Control (D)
N	15	21	17	19
Age	20.3 (1.8)	20.1 (1.2)	20.8 (2.6)	20.7 (2.0)
Height (cm)	165.3 (7.9)	168.1 (3.9)	169.4 (4.9)	168.4 (6.4)
Weight (kg)	62.7 (6.9)	71.1 (5.2)[a,c,d]	64.1 (8.1)	63.9 (11.6)
BMI	23.2	25.4	22.5	22.6
Age at menarche	13.5 (0.8)	12.7 (1.5)	12.8 (2.0)	12.8 (1.3)
Training sessions per week	5.3 (1.2)	6.0 (0.4)	5.4 (1.3)	NA*
Sport specific training (hours/day)	2.7 (0.6)	2.9 (0.7)	3.0 (1.1)	NA*
Other training (hours/week)	2.8 (1.6)	3.1 (1.9)	3.4 (1.4)	NA*
Years competing	14.2 (2.3)[c]	14.1 (2.3)[c]	10.5 (4.4)	NA*
Vertical jump height, cm	48.7 (7.7)[d]	46.6 (6.8)[d]	45.2 (6.3)	40.3 (6.3)
Estimated vertical jump power, W	7096.3 (593.5)[d]	7290.7 (424.6)[d]	6926.7 (490.3)	6618.5 (476.5)
Estimated vertical jump power per body weight, kg/W	114.1 (11.0)[b]	96.5 (24.7)	109.2 (11.4)	106 (15.6)
Combined grip strength, N*	655.6 (94.3)	760.4 (65.2)[a,c,d]	595.6 (177.6)	602.5 (105.5)

Values are expressed as means (SD). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D ($p<.05$).
*NA, not available.

Absolute Mean (SD) Values of DXA for Athlete and Control Groups

	Soccer (A)	Ice Hockey (B)	Swimming (C)	Control (D)
% Fat	26.7 (5.6)[d]	29.7 (5.9)	25.3 (6.1)[d]	33.0 (9.4)
Total Fat Tissue (g)	22227.73 (14698.72)	19706.33 (6398.58)	15784.47 (5050.96)	21128.68 (9446.45)
Total Lean Tissue (g)	39083.53 (12281.83)	43266.01 (14035.56)	45652 (5218.07)	40092.84 (3492.53)
Total Body BMD ($g \cdot cm^{-2}$)	1.25 (.06)[c,d]	1.30 (.06)[c,d]	1.16 (.08)	1.17 (.08)
Total Body BMC (g)	3105.48 (718.48)[c]	3127.26 (321.26)[c,d]	2664.32 (366.63)	2706.9 (462.48)
Lumbar Spine BMD ($g \cdot cm^{-2}$)	1.34 (.09)[d]	1.39 (0.14)[c,d]	1.25 (.14)	1.23 (0.11)
Lumbar Spine BMC (g)	75.13 (12.87)	80.08 (11.63)[c,d]	69.52 (9.5)	66.36 (11.24)
Lumbar Spine Area (cm^{-2})	55.99 (7.64)	57.64 (4.78)	55.55 (3.51)	53.79 (4.97)
Femoral Neck BMD ($g \cdot cm^{-2}$)	1.20 (.08)	1.24 (.31)[c]	1.05 (.13)	1.10 (.10)
Femoral Neck BMC (g)	5.39 (.73)	6.11 (1.53)[c,d]	5.0 (.63)	5.14 (0.60)
Femoral Neck Area (cm^{-2})	4.67 (.29)	4.7 (1.13)	4.78 (.24)	4.69 (.40)
Total Hip BMD ($g \cdot cm^{-2}$)	1.13 (.32)	1.22 (.31)	1.05 (.13)	1.10 (.09)
Total Hip BMC (g)	34.93 (10.48)	39.69 (10.22)	32.45 (4.82)	33.25 (3.79)
Total Hip Area (cm^{-2})	28.64 (8.20)	31.08 (7.45)	30.76 (1.75)	30.34 (1.99)

Values are expressed as means (SD). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Absolute Mean (SD) Values for the Tibia and Radius via pQCT for Athlete and Referent Groups

	Soccer	Ice Hockey	Swimming	Control
Lower Extremity				
Distal tibia 4%				
Total area, mm^2	913.39 (123.44)	1013.57 (150.18)[d]	935.57 (140.49)	893.41 (99.28)
Trabecular density, mg/cm^3	307.68 (20.69)[b,c,d]	279.53 (28.49)	262.99 (24.25)	262.17 (30.70)
Bone strength index, mg/mm^4	12728.80 (1932.86)[c,d]	12336.77 (2265.35)[c,d]	9371.16 (2258.83)	9462.75 (2431.62)
Tibial midshaft 50%				
Total area, mm^2	445.6 (55.86)	452.21 (39.99)	415.13 (60.84)	419.65 (52.86)
Cortical area, mm^2	327.96 (41.47)	337.56 (31.22)[c,d]	294.0 (42.92)	294.47 (41.18)
Cortical density, mg/cm^3	1155.07 (18.65)	1160.96 (17.38)	1161.84 (14.61)	1166.15 (25.34)
Section modulus, mm^2	2036.02 (367.02)	2087.01 (270.44)	1795.18 (393.97)	1822.97 (350.40)
SSIp, mm^3	1990.27 (366.42)	2032.82 (243.56)	1761.01 (371.64)	1825.79 (331.59)
Tibial shaft 66%				
Total area, mm^2	566.42 (67.33)	576.58 (56.72)	521.76 (71.78)	542.84 (84.76)
Cortical area, mm^2	320.09 (34.85)[c,d]	324.17 (30.69)[c,d]	287.60 (35.23)	284.03 (34.05)
Cortical density, mg/cm^3	1132.23 (15.15)	1131.47 (18.02)	1140.63 (13.38)	1140.89 (21.73)
Section modulus, mm^2	2626.41 (450.12)	2628.87 (312.87)[c]	2255.98 (414.39)	2317.65 (450.68)
SSIp, mm^3	2579.65 (468.0)	2576.03 (307.51)	2228.44 (419.10)	2317.09 (447.58)
Muscle cross sectional area, mm^2	7501.93 (619.07)	7355.53 (1004.87)	7585.24 (1200.97)	6951.76 (1235.68)
Upper Extremity				
Distal radius 4%				
Total area, mm^2	286.13 (51.66)	312.27 (78.60)	295.05 (75.59)	292.59 (86.35)
Trabecular density, mg/cm^3	257.72 (48.10)[d]	242.09 (32.68)	222.52 (27.46)	216.70 (42.69)
Bone strength index, mg/mm^4	3920.46 (1244.26)	4829.15 (1041.42)[c,d]	3436.91 (833.07)	3486 (1069.76)
Radial shaft 50%				
Total area, mm^2	109.24 (17.98)	119.27 (11.42)[d]	110.24 (17.0)	102.65 (18.25)
Cortical area, mm^2	85.78 (13.17)	95.59 (8.54)[c,d]	85.79 (11.47)	80.16 (10.10)
Cortical density, mg/cm^3	1179.67 (17.28)	1177.15 (23.63)	1185.09 (22.63)	1184.62 (26.38)
Section modulus, mm^2	237.32 (56.34)	273.21 (39.61)[d]	245.23 (53.10)	214.70 (50.33)
SSIp, mm^3	241.04 (58.71)	274.72 (38.89)[d]	247.86 (55.46)	220.16 (51.56)

Values are expressed as means (SD). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Adjusted Mean (CI) Values of DXA for Athlete and Control Groups: Model 1

	Soccer (A)	Ice Hockey (B)	Swimming (C)	Control (D)
Total Body BMD (g•cm^{-2})	1.26 (1.23 to 1.30)^{c,d}	1.30 (1.27 to 1.33)^{c,d}	1.16 (1.13 to 1.19)	1.16 (1.14 to 1.19)
Total Body BMC (g)	3229.56 (3000.37 to 3458.75)^{c,d}	3135.29 (2950.64 to 3319.94)^{c,d}	2604.64 (2397.74 to 2811.54)	2689.30 (2495.16 to 2883.45)
Lumbar Spine BMD (g•cm^{-2})	1.35 (1.29 to 1.42)^{d}	1.39 (1.34 to 1.44)^{c,d}	1.24 (1.18 to 1.30)	1.22 (1.17 to 1.28)
Lumbar Spine BMC (g)	77.58 (72.59 to 82.58)^{c,d}	80.24 (76.22 to 84.26)^{c,d}	67.81 (63.30 to 72.31)	65.91 (61.68 to 70.14)
Lumbar Spine Area (cm^{-2})	57.23 (55.18 to 59.29)	57.65 (56.0 to 59.31)^{d}	54.70 (52.84 to 56.56)	53.61 (51.87 to 55.35)
Femoral Neck BMD (g•cm^{-2})	1.21 (1.11 to 1.32)	1.24 (1.16 to 1.32)^{c}	1.04 (.95 to 1.13)	1.10 (1.01 to 1.18)
Femoral Neck BMC (g)	5.48 (4.94 to 6.02)	6.09 (5.66 to 6.23)^{c,d}	4.97 (4.48 to 5.45)	5.14 (4.69 to 5.60)
Femoral Neck Area (cm^{-2})	4.71 (4.34 to 5.07)	4.69 (4.39 to 4.98)	4.78 (4.45 to 5.11)	4.70 (4.39 to 5.01)
Total Hip BMD (g•cm^{-2})	1.14 (1.01 to 1.27)	1.22 (1.12 to 1.32)	1.05 (.94 to 1.17)	1.09 (.98 to 1.20)
Total Hip BMC (g)	35.55 (31.21 to 39.89)	39.78 (36.28 to 43.28)^{c}	32.11 (28.19 to 36.03)	33.12 (29.44 to 36.80)
Total Hip Area (cm^{-2})	28.67 (25.54 to 31.80)	31.10 (28.58 to 33.63)	30.70 (27.87 to 33.53)	30.32 (27.66 to 32.97)

Values adjusted for age and height and are expressed as means (95% CI). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Adjusted Mean (CI) Values for the Tibia and Radius via pQCT for Athlete and Control Groups: Model 1

	Soccer (A)	Ice Hockey (B)	Swimming (C)	Control (D)
Lower Extremity				
Distal tibia 4%				
Total area, mm^2	930.34 (862.22 to 998.46)	999.18 (939.61 to 1058.75)[d]	947.11 (884.38 to 1009.85)	884.84 (827.03 to 942.66)
Trabecular density, mg/cm^3	307.52 (292.91 to 322.12)[c,d]	280.79 (268.02 to 293.56)	262.09 (248.64 to 275.54)	261.86 (249.46 to 274.25)
Bone strength index, mg/mm^4	12911.45 (11714.53 to 14108.38)[c,d]	12353.00 (11306.34 to 13399.66)[c,d]	9374.67 (8272.37 to 10476.98)	9309.83 (8293.99 to 10325.66)
Tibial midshaft 50%				
Total area, mm^2	455.19 (431.64 to 478.74)[c]	445.39 (424.79 to 465.98)	417.01 (395.32 to 438.69)	410.39 (390.41 to 430.38)
Cortical area, mm^2	336.0 (316.79 to 355.20)[c,d]	332.23 (315.44 to 349.02)[c,d]	298.48 (280.79 to 316.16)	288.40 (272.11 to 304.70)
Cortical density, mg/cm^3	1155.90 (1145.42 to 1166.38)	1161.41 (1152.23 to 1170.57)	1161.75 (1152.13 to 1171.37)	1165.35 (1156.19 to 1174.51)
Section modulus, mm^2	2103.82 (1943.21 to 2264.43)[d]	2043.93 (1903.48 to 2184.38)[d]	1815.15 (1667.24 to 1963.06)	1763.29 (1626.98 to 1899.60)
SSIp, mm^3	2054.01 (1905.85 to 2202.17)[c,d]	1987.29 (1857.73 to 2116.85)	1781.90 (1645.45 to 1918.35)	1766.29 (1640.54 to 1892.03)
Tibial shaft 66%				
Total area, mm^2	576.79 (544.33 to 609.26)	569.11 (540.72 to 597.50)	525.57 (495.67 to 555.46)	531.21 (503.66 to 558.76)
Cortical area, mm^2	325.18 (308.98 to 341.38)[c,d]	319.23 (305.06 to 333.40)[c,d]	290.88 (275.96 to 305.80)	278.93 (267.18 to 292.68)
Cortical density, mg/cm^3	1132.59 (1123.40 to 1141.77)	1132.48 (1124.46 to 1140.49)	1139.39 (1130.97 to 1147.81)	1141.78 (1133.76 to 1149.80)
Section modulus, mm^2	2680.58 (2495.83 to 2865.33)[c,d]	2573.50 (2411.95 to 2735.06)[d]	2281.63 (2111.48 to 2451.77)	2248.00 (2091.20 to 2404.80)
SSIp, mm^3	2635.53 (2450.10 to 2820.96)[c,d]	2522.58 (2360.42 to 2684.73)	2257.72 (2086.95 to 2428.49)	2245.91 (2088.53 to 2403.28)
MSCA, mm^2	7436.02 (6843.22 to 8028.81)	7385.83 (6867.45 to 7904.20)	7615.37 (7069.44 to 8161.31)	6998.43 (6495.32 to 7501.54)
Upper Extremity				
Distal radius 4%				
Total area, mm^2	289.04 (247.24 to 330.84)	308.55 (271.95 to 345.15)	295.90 (258.95 to 332.84)	293.20 (258.33 to 328.08)
Trabecular density, mg/cm^3	258.36 (237.07 to 279.64)	241.97 (223.33 to 260.60)	222.25 (203.43 to 241.06)	216.60 (198.84 to 234.36)
Bone strength index, mg/mm^4	4023.0 (3461.93 to 4584.07)	4805.42 (4314.17 to 5296.67)	3395.11 (2899.17 to 3891.05)	3470.82 (3002.68 to 3938.95)
Radial shaft 50%				
Total area, mm^2	111.74 (103.41 to 120.07)	117.41 (110.11 to 124.70)	109.44 (102.07 to 116.80)	102.53 (95.58 to 109.49)
Cortical area, mm^2	87.32 (81.85 to 92.79)	94.38 (89.59 to 99.18)	85.32 (80.48 to 90.16)	80.05 (75.48 to 84.62)
Cortical density, mg/cm^3	1180.95 (1169.37 to 1192.53)	1178.47 (1167.82 to 1189.12)	1182.72 (1170.72 to 1189.12)	1185.01 (1175.13 to 1195.57)
Section modulus, mm^2	247.37 (222.14 to 272.60)	266.68 (244.59 to 288.77)	243.27 (220.97 to 265.58)	214.35 (193.30 to 235.40)
SSIp, mm^3	250.94 (225.32 to 276.55)	267.78 (245.35 to 290.21)	245.74 (223.10 to 268.38)	219.77 (198.40 to 241.14)

Values are adjusted for age and limb length and expressed as means (95% CI). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Adjusted Mean (CI) Values of DXA for Athlete and Control Groups: Model 2

	Soccer (A)	Ice Hockey (B)	Swimming (C)	Control (D)
Total Body BMD (g•cm^{-2})	1.27 (1.24 to 1.30)^{c,d}	1.28 (1.25 to 1.30)^{c,d}	1.17 (1.14 to 1.20)	1.17 (1.15 to 1.20)
Total Body BMC (g)	3271.72 (3072.46 to 3470.98)^{c,d}	2967.81 (2793.18 to 3142.44)	2697.36 (2514.02 to 2880.70)	2760.38 (2589.64 to 2931.13)
Lumbar Spine BMD (g•cm^{-2})	1.36 (1.30 to 1.42)^{d}	1.36 (1.31 to 1.42)^{d}	1.25 (1.20 to 1.31)	1.23 (1.18 to 1.29)
Lumbar Spine BMC (g)	78.24 (73.54 to 82.94)^{c,d}	77.64 (73.52 to 81.76)^{c}	69.25 (64.92 to 73.58)	67.01 (62.99 to 71.04)
Lumbar Spine Area (cm^{-2})	57.47 (55.50 to 59.44)	56.72 (54.99 to 58.44)	55.22 (53.41 to 57.03)	54.01 (52.32 to 55.70)
Femoral Neck BMD (g•cm^{-2})	1.22 (1.11 to 1.32)	1.22 (1.13 to 1.31)	1.05 (.96 to 1.15)	1.11 (1.02 to 1.19)
Femoral Neck BMC (g)	5.52 (4.98 to 6.05)	5.94 (5.47 to 6.41)	5.05 (4.56 to 5.54)	5.21 (4.75 to 5.67)
Femoral Neck Area (cm^{-2})	4.72 (4.36 to 5.09)	4.62 (4.30 to 4.94)	4.82 (4.48 to 5.15)	4.72 (4.41 to 5.04)
Total Hip BMD (g•cm^{-2})	1.14 (1.02 to 1.27)	1.18 (1.07 to 1.30)	1.07 (.95 to 1.19)	1.11 (1.00 to 1.22)
Total Hip BMC (g)	35.92 (31.65 to 40.19)	38.31 (34.57 to 42.06)	32.92 (28.99 to 36.85)	33.74 (30.08 to 37.40)
Total Hip Area (cm^{-2})	28.85 (25.71 to 31.98)	30.40 (27.66 to 33.15)	31.09 (28.21 to 33.97)	30.61 (27.93 to 33.30)

Values adjusted for age, height, and body weight and expressed as means (95% CI). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Adjusted Mean (CI) Values for the Tibia and Radius via pQCT for Athlete and Control Groups: Model 2

	Soccer	Ice Hockey	Swimming	Control
Lower Extremity				
Distal tibia 4%				
Total area, mm^2	933.47 (864.93 to 1002.00)	988.71 (925.00 to 1052.42)	949.63 (886.58 to 1012.68)	890.34 (831.27 to 949.41)
Trabecular density, mg/cm^3	310.35 (297.60 to 323.10)[b,c,d]	271.29 (259.44 to 283.14)	264.38 (252.65 to 276.10)	266.84 (255.85 to 277.83)
Bone strength index, mg/mm^4	13224.47 (12339.46 to 14109.47)[b,c,d]	11303.89 (10481.24 to 12126.55)[c]	9627.32 (8813.17 to 10441.46)	9860.33 (9097.57 to 10623.09)
Tibial midshaft 50%				
Total area, mm^2	460.53 (441.32 to 479.75)[c,d]	427.49 (409.62 to 445.35)	421.32 (403.64 to 438.99)	419.79 (403.22 to 436.35)
Cortical area, mm^2	340.20 (324.23 to 356.16)[c,d]	318.16 (303.32 to 333.00)	301.87 (287.18 to 316.55)	295.79 (282.03 to 309.55)
Cortical density, mg/cm^3	1154.96 (1144.91 to 1165.02)	1165.56 (1156.25 to 1174.87)	1160.68 (1151.43 to 1169.92)	1162.91 (1153.94 to 1171.87)
Section modulus, mm^2	2139.68 (2007.54 to 2271.82)[c,d]	1923.74 (1800.91 to 2046.57)	1844.09 (1722.53 to 1965.65)	1826.36 (1712.47 to 1940.25)
SSIp, mm^3	2086.34 (1963.04 to 2209.64)	1878.92 (1764.31 to 1993.54)	1808.00 (1694.57 to 1921.43)	1823.15 (1716.88 to 1929.42)
Tibial shaft 66%				
Total area, mm^2	583.09 (554.73 to 611.45)[c]	548.01 (521.65 to 574.37)	530.65 (504.56 to 556.73)	542.28 (517.84 to 566.72)
Cortical area, mm^2	328.39 (314.34 to 342.44)[c,d]	308.49 (295.43 to 321.55)	293.46 (280.54 to 306.39)	284.56 (272.45 to 296.67)
Cortical density, mg/cm^3	1131.74 (1122.97 to 1141.51)	1136.23 (1128.11 to 1144.35)	1138.42 (1130.35 to 1146.48)	1139.56 (1131.75 to 1147.38)
Section modulus, mm^2	2721.37 (2568.51 to 2874.24)[c,d]	2436.78 (2294.68 to 2578.88)	2314.55 (2173.93 to 2455.18)	2319.74 (2187.99 to 2451.49)
SSIp, mm^3	2674.26 (2516.84 to 2831.68)[c,d]	2392.77 (2246.44 to 2539.10)	2288.98 (2144.17 to 2433.80)	2314.02 (2178.35 to 2449.69)
MCSA, mm^2	7551.12 (7033.56 to 8068.68)	7000.03 (6518.94 to 7481.13)	7708.28 (7232.16 to 8184.40)	7200.87 (6754.80 to 7646.94)
Upper Extremity				
Distal radius 4%				
Total area, mm^2	295.39 (255.70 to 335.08)	291.61 (255.19 to 328.03)	302.12 (266.99 to 337.25)	299.01 (265.86 to 332.17)
Trabecular density, mg/cm^3	260.84 (240.04 to 281.63)[d]	235.35 (216.27 to 254.43)	224.68 (206.27 to 243.08)	218.87 (201.50 to 236.24)
Bone strength index, mg/mm^4	4089.50 (3542.23 to 4636.77)	4627.88 (4125.73 to 5130.02)[c,d]	3460.28 (2975.88 to 3944.68)	3531.70 (3074.53 to 3988.87)
Radial shaft 50%				
Total area, mm^2	113.46 (106.04 to 120.88)	112.82 (106.01 to 119.63)	111.12 (104.55 to 117.69)	104.11 (97.91 to 110.31)
Cortical area, mm^2	88.23 (83.11 to 93.35)	91.95 (87.25 to 96.65)[d]	86.21 (81.68 to 90.75)	80.89 (76.61 to 85.16)
Cortical density, mg/cm^3	1179.35 (1167.74 to 1190.96)	1181.44 (1170.30 to 1192.58)	1182.18 (1170.30 to 1194.05)	1184.24 (1174.05 to 1194.43)
Section modulus, mm^2	251.96 (228.76 to 275.17)	254.42 (233.13 to 275.71)	247.77 (227.24 to 268.31)	218.55 (199.17 to 237.94)
SSIp, mm^3	255.50 (231.83 to 279.17)	255.60 (233.89 to 277.32)	250.21 (229.27 to 271.16)	223.95 (204.18 to 243.72)

Values adjusted for age, limb length, and body weight and expressed as means (95% CI). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Adjusted Mean (CI) Values of DXA for Athlete and Control Groups

	Soccer (A)	Ice Hockey (B)	Swimming (C)	Control (D)
Total Body BMD (g•cm^{-2})	1.27 (1.23 to 1.30)^{c,d}	1.30 (1.27 to 1.33)^{c,d}	1.15 (1.12 to 1.18)	1.17 (1.14 to 1.20)
Total Body BMC (g)	3217.52 (2988.42 to 3446.62)^{c,d}	3144.44 (2959.94 to 3328.95)^{c,d}	2621.75 (2413.85 to 2829.65)	2672.75 (2477.56 to 2867.95)
Lumbar Spine BMD (g•cm^{-2})	1.36 (1.30 to 1.42)^{c,d}	1.39 (1.33 to 1.44)^{c,d}	1.23 (1.17 to 1.29)	1.23 (1.17 to 1.28)
Lumbar Spine BMC (g)	78.01 (73.12 to 82.90)^{c,d}	79.92 (75.98 to 83.86)^{c,d}	67.21 (62.77 to 71.65)	66.49 (62.32 to 70.66)
Lumbar Spine Area (cm^{-2})	57.36 (55.32 to 59.41)	57.56 (55.91 to 59.20)^{d}	54.52 (52.66 to 56.37)	53.79 (52.05 to 55.53)
Femoral Neck BMD (g•cm^{-2})	1.22 (1.11 to 1.32)	1.24 (1.15 to 1.32)^{c}	1.03 (.94 to 1.13)	1.10 (1.01 to 1.19)
Femoral Neck BMC (g)	5.49 (4.95 to 6.03)	6.08 (5.65 to 6.52)^{c,d}	4.95 (4.46 to 5.44)	5.16 (4.70 to 5.62)
Femoral Neck Area (cm^{-2})	4.70 (4.33 to 5.07)	4.69 (4.39 to 4.99)	4.79 (4.45 to 5.12)	4.69 (4.37 to 5.00)
Total Hip BMD (g•cm^{-2})	1.14 (1.01 to 1.27)	1.22 (1.11 to 1.32)	1.05 (.93 to 1.17)	1.10 (.99 to 1.21)
Total Hip BMC (g)	35.69 (31.31 to 40.06)	39.67 (36.15 to 43.19)^{c}	31.91 (27.94 to 35.88)	33.31 (29.59 to 37.04)
Total Hip Area (cm^{-2})	28.63 (25.46 to 31.80)	31.13 (28.58 to 33.69)	30.76 (27.88 to 33.64)	30.26 (27.56 to 32.96)

Values adjusted for age, height, and lean mass and expressed as means (95% CI). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Adjusted Mean (CI) Values for the **Tibia** via pQCT for Athlete and Control Groups

	Soccer	Ice Hockey	Swimming	Control
Lower Extremity				
Distal tibia 4%				
Total area, mm^2	928.40 (860.74 to 996.06)	998.27 (939.13 to 1057.40)	941.50 (878.72 to 1004.27)	891.87 (833.63 to 950.11)
Trabecular density, mg/cm^3	306.76 (292.81 to 320.71)^{b,c,d}	280.44 (286.24 to 292.63)	259.91 (246.97 to 272.85)	264.59 (252.58 to 276.59)
Bone strength index, mg/mm^4	12831.20 (11734.40 to 13927.99)^{c,d}	12315.26 (11356.73 to 13273.79)^{c,d}	9142.48 (8124.94 to 10160.03)	9600.25 (8656.12 to 10544.37)
Tibial midshaft 50%				
Total area, mm^2	453.65 (431.97 to 475.33)^{c}	444.66 (425.72 to 463.61)	412.54 (392.42 to 432.65)	415.98 (397.32 to 434.65)
Cortical area, mm^2	334.83 (316.91 to 325.75)^{c,d}	331.68 (316.02 to 347.34)^{c,d}	259.09 (278.47 to 311.72)	292.64 (277.21 to 308.06)
Cortical density, mg/cm^3	1154.91 (1144.16 to 1165.66)	1161.56 (1152.43 to 1170.69)	1162.43 (1152.76 to 1170.69)	1164.50 (1155.25 to 1173.75)
Section modulus, mm^2	2093.74 (1944.64 to 2242.83)^{c,d}	2039.19 (1908.88 to 2169.49)	1785.98 (1647.65 to 1924.30)	1799.78 (1671.43 to 1928.12)
SSIp, mm^3	2044.59 (1907.36 to 2181.82)^{c}	1982.86 (1862.93 to 2102.79)	1754.66 (1627.34 to 1881.97)	1800.37 (1682.24 to 1918.50)
Tibial shaft 66%				
Total area, mm^2	574.94 (544.33 to 605.55)	568.24 (541.49 to 594.99)	520.20 (491.80 to 548.59)	537.92 (511.58 to 564.27)
Cortical area, mm^2	324.18 (309.10 to 339.26)^{c,d}	318.76 (305.58 to 331.94)^{c,d}	278.98 (273.99 to 301.97)	282.55 (269.57 to 295.53)
Cortical density, mg/cm^3	1133.07 (1123.50 to 1142.65)	1132.59 (1124.46 to 1140.72)	1139.63 (1131.03 to 1148.24)	1141.67 (1133.43 to 1149.90)
Section modulus, mm^2	2667.78 (2499.72 to 2835.84)^{c,d}	2567.48 (2420.61 to 2714.36)^{c}	2244.59 (2088.67 to 2400.51)	2294.33 (2149.66 to 2438.99)
SSIp, mm^3	2623.03 (2453.31 to 2792.74)^{c,d}	2516.70 (2368.38 to 2665.02)	2221.54 (2064.10 to 2378.99)	2291.16 (2145.07 to 2437.24)

Values are adjusted for age, limb length, and muscle cross-sectional area and are expressed as means (95% CI). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Appendix D: Manuscript Two
Unadjusted and Adjusted Results

Absolute Mean (SD) Values for the Tibia via pQCT for Male Athlete and Referent Groups

	Ice Hockey (A)	Swimming (B)	Running (C)	Referents (D)
Lower Extremity				
Distal tibia 4%				
Total density, mg/cm^3	359.98 (39.19)[b]	310.29 (27.75)	369.23 (39.74)[b]	367.15 (31.55)[b]
Total area, mm^2	1324.48 (232.09)	1178.42 (183.30)	1180.95 (150.67)	1227.06 (154.45)
Trabecular density, mg/cm^3	292.98 (28.26)[b]	256.12 (17.76)	315.33 (33.80)[b]	303.89 (32.02)[b]
Bone strength index, mg/mm^4	16971.11 (2962.32)[b]	11262.62 (1630.04)	15952.96 (2284.41)[b]	16525.38 (2862.94)[b]
Tibial midshaft 50%				
Total density, mg/cm^3	868.75 (48.92)	832.69 (36.35)	893.84 (41.35)[b]	860.52 (49.52)
Total area, mm^2	570.15 (67.93)[b,d]	516.18 (50.55)	548.84 (48.44)	557.69 (64.21)
Cortical area, mm^2	408.21 (39.25)[b]	355.62 (34.19)	409.36 (31.83)[b]	398.15 (52.86)
Cortical density, mg/cm^3	1140.15 (21.75)	1144.60 (15.42)	1145.13 (19.65)	1151.63 (18.63)
Cortical thickness, mm	6.32 (.44)[b]	5.68 (.43)	6.57 (.43)[b]	6.21 (.68)
Section modulus, mm^2	2851.94 (473.63)[b]	2402.13 (309.72)	2970.51 (395.25)[b]	3001.32 (501.89)
SSIp, mm^3	2758.34 (465.97)[b]	2344.19 (301.04)	2706.28 (364.60)	2777.20 (469.49)
Tibial shaft 66%				
Total density, mg/cm^3	665.62 (69.47)	654.78 (45.85)	724.51 (57.05)[a,b]	685.55 (67.7)
Total area, mm^2	765.52 (112.65)[b,c,d]	669.64 (92.55)	689.27 (64.94)	654.14 (88.0)
Cortical area, mm^2	394.01 (31.64)[b]	349.33 (46.93)	406.17 (34.05)[b,d]	359.51 (56.16)
Cortical density, mg/cm^3	1114.44 (18.75)	1122.12 (16.25)	1118.69 (18.48)	1130.26 (15.85)
Cortical thickness, mm	4.79 (.48)	4.51 (.49)	5.35 (.54)[a,b,d]	4.76 (.67)
Section modulus, mm^2	3776.42 (585.99)[b]	3143.04 (511.67)	3933.63 (475.25)[b]	3512.57 (763.19)
SSIp, mm^3	3696.48 (571.72)[b]	3074.13 (530.77)	3468.84 (408.91)	3186.16 (622.59)
Muscle cross sectional area, mm^2	8411.60 (1054.48)	8712.04 (820.95)	8546.40 (779.45)	8518.90 (1275.12)

Values are expressed as means (SD). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Absolute Mean (SD) Values for the Tibia via pQCT for Female Athlete and Referent Groups

	Ice Hockey (A)	Swimming (B)	Running (C)	Referents (D)
Lower Extremity				
Distal tibia 4%				
Total density, mg/cm^3	349.80 (38.35)	317.04 (43.92)	335.26 (38.87)	324.38 (44.09)
Total area, mm^2	1013.57 (150.18)[d]	935.57 (140.49)	941.43 (102.44)	893.41 (99.28)
Trabecular density, mg/cm^3	279.53 (28.49)	262.99 (24.25)	279.04 (31.90)	262.17 (30.70)
Bone strength index, mg/mm^4	12336.77 (2265.35)[b,d]	9371.16 (2258.83)	10581.25 (2068.76)	9462.75 (2431.62)
Tibial midshaft 50%				
Total density, mg/cm^3	910.36 (66.18)	872.38 (60.90)	894.09 (50.44)	862.50 (44.03)
Total area, mm^2	452.21 (40.0)	415.13 (60.84)	422.78 (42.21)	419.65 (52.86)
Cortical area, mm^2	337.56 (31.22)[b,d]	293.99 (42.92)	309.19 (33.38)	294.47 (41.18)
Cortical density, mg/cm^3	1160.96 (17.38)	1162.30 (14.26)	1162.04 (17.53)	1165.97 (26.02)
Cortical thickness, mm	6.0 (.62)[b,d]	5.31 (.58)	5.60 (.51)	5.25 (.53)
Section modulus, mm^2	2087.01 (270.44)[b]	1795.17 (393.97)	1976.55 (271.41)	1822.97 (350.40)
SSIp, mm^3	2032.82 (243.82)[b]	1761.01 (371.64)	1832.75 (247.37)	1825.79 (331.59)
Tibial shaft 66%				
Total density, mg/cm^3	716.44 (71.56)	705.15 (50.97)	713.66 (50.30)	675.43 (59.93)
Total area, mm^2	576.58 (56.72)	521.76 (71.78)	540.54 (51.10)	542.84 (84.76)
Cortical area, mm^2	324.17 (30.69)[b,d]	287.60 (35.23)	306.81 (31.34)	284.03 (34.05)
Cortical density, mg/cm^3	1131.47 (18.02)	1140.4 (12.99)	1131.01 (19.23)	1142.16 (22.29)
Cortical thickness, mm	4.62 (.59)[d]	4.27 (.40)	4.51 (.44)	4.10 (.46)
Section modulus, mm^2	2628.87 (312.87)[b]	2255.98 (414.39)	2682.75 (361.87)[b,d]	2317.65 (450.68)
SSIp, mm^3	2576.03 (307.51)[b]	2228.44 (419.10)	2376.86 (308.07)	2317.09 (447.58)
Muscle cross sectional area, mm^2	7355.53 (1004.87)	7585.24 (1223.12)	7057.12 (609.19)	6951.76 (1235.68)

Values are expressed as means (SD). The mean difference is significant at the .05 level.
a = sig. different from A, b = sig. different from B, c = sig different from C, d = sig different from D.

Absolute Mean Difference Values for Females and Males Within the Same Group

	Ice Hockey (A)	Swimming (B)	Running (C)	Referents (D)
Lower Extremity				
Distal tibia 4%				
Total density, mg/cm^3	-10.18	6.76	-33.97*	-32.73*
Total area, mm^2	-310.91*	-242.86*	-239.53*	-316.62*
Trabecular density, mg/cm^3	-13.45	6.86	-36.30*	-33.94*
Bone strength index, mg/mm^4	-4634.33*	-1891.46*	-5371.71*	-6076.29*
Tibial midshaft 50%				
Total density, mg/cm^3	37.56*	49.52*	.24	.75
Total area, mm^2	-117.84*	-105.26*	-126.07*	-110.07*
Cortical area, mm^2	-72.18*	-60.58*	-100.18*	-83.91*
Cortical density, mg/cm^3	20.49*	19.46*	16.91*	12.67
Cortical thickness, mm	-.37*	-.31	-.97*	-.80*
Section modulus, mm^2	-766.38*	-623.92*	-993.96*	-936.49*
SSIp, mm^3	-729.64*	-599.04*	-873.53*	-752.11*
Tibial shaft 66%				
Total density, mg/cm^3	44.30*	57.55*	-10.86	-8.66
Total area, mm^2	-186.56*	-152.56*	-148.73*	-120.51*
Cortical area, mm^2	-72.08*	-60.98*	-99.36*	-99.83
Cortical density, mg/cm^3	17.56*	19.11*	12.32*	13.49*
Cortical thickness, mm	-.22	-.21	-.85*	-.78.1*
Section modulus, mm^2	-1152.61*	-905.79*	-1250.88*	-1274.51*
SSIp, mm^3	-1122.55*	-861.71*	-1091.97*	-929.13*
Muscle cross sectional area, mm^2	-1044.01*	-1078.41*	-1489.28*	-1584.85*

Values are expressed as mean differences between females and males. Differences are expressed as [females – males]. The mean difference is significant at the .05 level.

* Significant difference between males and females of the same group

Model 1: Mean Difference Values for Males vs. Females Within the Same Group (Males vs. Females)

	Ice Hockey (A)	Swimming (B)	Running (C)	Referents (D)
Lower Extremity				
Distal tibia 4%				
Total density, mg/cm^3	-8.87	5.69	-31.54*	-30.94*
Total area, mm^2	-261.79*	-191.57*	-191.16*	-285.79*
Trabecular density, mg/cm^3	-18.69	.694	-41.12*	-36.95*
Bone strength index, mg/mm^4	-4122.87*	-159.04*	-4789.12*	-5688.05*
Tibial midshaft 50%				
Total density, mg/cm^3	26.41	33.71	-8.79	-4.6
Total area, mm^2	-68.08*	-56.13*	-75.74*	-77.70*
Cortical area, mm^2	-41.77*	-32.02*	-68.75*	63.56*
Cortical density, mg/cm^3	20.77*	16.85*	18.55*	14.01*
Cortical thickness, mm	-20	-.173	-.78*	-.67*
Section modulus, mm^2	-442.01*	-309.29*	-663.28*	-723.30
SSIp, mm^3	-411.64*	-292.95*	-548.23*	-542.16
Tibial shaft 66%				
Total density, mg/cm^3	36.68	42.90	-15.21	-10.77
Total area, mm^2	-119.32*	-81.82*	-82.79*	-78.53*
Cortical area, mm^2	-43.01*	-34.54*	-68.90*	-58.30*
Cortical density, mg/cm^3	16.95*	15.82*	12.97*	14.17*
Cortical thickness, mm	-.08	-.124	-.68*	-.54*
Section modulus, mm^2	-737.26*	-481.43*	-837.54*	-1010.12*
SSIp, mm^3	-706.16*	-439.78*	-675.95*	-662.68*
Muscle cross sectional area, mm^2	-931.24*	-927.02*	-1394.04*	-1527.53*

Values adjusted for age and tibia length. Values are expressed as mean differences between females and males. Differences are expressed as [females – males].
The mean difference is significant at the .05 level.
*Denotes significant difference genders of the same group

Model 3: Mean Difference Values for Males vs. Females Within the Same Group (Males vs. Females)

	Ice Hockey (A)	Swimming (B)	Running (C)	Referents (D)
Lower Extremity				
Distal tibia 4%				
Total density, mg/cm^3	-5.35	9.2	-26.26	-25.15
Total area, mm^2	-220.20*	-150.17*	-128.91*	-217.57*
Trabecular density, mg/cm^3	-13.91	5.46	-33.97*	-29.10*
Bone strength index, mg/mm^4	-3419.25*	-825.18	-3735.82*	-4533.89*
Tibial midshaft 50%				
Total density, mg/cm^3	29.02	36.31	-4.87	-.299
Total area, mm^2	-50.33*	-38.46*	-49.17*	-48.59*
Cortical area, mm^2	-26.40*	-16.71	-45.74*	-38.35*
Cortical density, mg/cm^3	18.60*	14.68	15.29*	10.44
Cortical Thickness, mm	-.03	-.10	.54*	-.40*
Section modulus, mm^2	-305.47*	-173.37	-458.89*	-499.35
SSIp, mm^3	-287.74*	-169.61	-365.75*	-338.92
Tibial shaft 66%				
Total density, mg/cm^3	35.94	42.17	-16.31	-11.97
Total area, mm^2	-94.92*	-57.52*	-46.25*	-38.50
Cortical area, mm^2	-29.62*	-21.21	-48.85*	-36.33*
Cortical density, mg/cm^3	16.09*	14.96*	11.67	12.75
Cortical thickness, mm	.004	-.04	-.55	-.40*
Section modulus, mm^2	-545.08*	-290.13*	-549.86*	-694.89*
SSIp, mm^3	-534.09*	-268.49*	-418.38*	-380.43*

Model 3: Values adjusted for age, limb length, and muscle cross-sectional area. Values are expressed as mean differences between females and males. Differences are expressed as [females – males]. The mean difference is significant at the .05 level.

*Significant difference between males and females of the same group