Evaluating the influence of muscle function and physical activity on bone strength in endurance trained individuals

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Abstract

Background: Endurance athletes are at an increased risk of bone loss and bone stress injury (BSI) due to high-volume training and elevated nutritional demand. Risk factors associated with low bone mineral density (BMD) and BSIs in endurance sport include poor muscle size and strength and high training volume. However, we have a limited understanding of which muscle- and activity-related factors explain the most variability in true estimates of bone strength and volumetric BMD (vBMD) and area in endurance athletes measured by advanced bone imaging technology.

Purpose: This thesis evaluated the relationships between muscle size and strength, accelerometer-measured MVPA, and bone strength, vBMD, and area in young endurance-trained individuals.

Methods: We recruited healthy men and women aged 18-35 years (body mass index (BMI) $\leq$30 kg/m^2 with no known medical conditions/medication use affecting bone metabolism) that participate on a competitive endurance sports team and/or perform weight-bearing endurance exercise (i.e., running) $\geq$180 minutes/week in the past 6 months for one-time measures. Trabecular and cortical vBMD and area, and stress-strain index (SSI) at the proximal and distal tibia and calf muscle CSA were determined using peripheral quantitative computed tomography. aBMD at the proximal femur and lumbar spine were measured by dual-energy X-ray absorptiometry. Handgrip and knee extensor isometric muscle strength were assessed using validated dynamometer protocols. Maximal aerobic capacity (VO_2max) was measured during a progressive treadmill test to volitional exhaustion. MVPA levels were determined using accelerometers and the International Physical Activity Questionnaire. Self-reported lifetime

bone-specific physical activity (types and frequency) was measured using the Bone-specific Physical Activity Questionnaire. Pearson/Spearman correlations between each of muscle and physical activity and bone parameters were performed with and without adjustment for sex. Independent t-tests compared these outcomes in participants stratified by sex.

Results: Eighteen participants were included (66% male, mean±SD age 26±4 years, BMI 22.1±2.4g/m^2, percent body fat 18.2±6%, and VO_2max 56.5±9.2 mL/kg/min). Fifty-three percent of participants engaged in >5 hours/week of weight-bearing endurance training, with 60% running ≥40 kilometers/week. Male participants had a higher trabecular vBMD (p=0.027) and area (p=0.016), cortical areas at 38% and 66% sites (p=0.006 and 0.002, respectively), SSI at 38% and 66% sites (p=0.004 and p=0.003, respectively), and larger calf muscle CSA compared to the females (p=0.044). Male participants had a higher total body (p=0.020), femoral neck (p=0.017), and total hip aBMD (p=0.019) compared to the females. Male participants also had higher handgrip and knee extensor muscle strength compared to females (p<0.05). Moderate-to-strong positive correlations were found between muscle CSA and trabecular area (r=0.583, p=0.014), cortical area at the 38% site (r=0.737, p<0.001), SSI at the 66% site (r=0.667, p=0.003), femoral neck aBMD (r=0.505, p=0.038), and total hip aBMD (r=0.516, p=0.034). When adjusted for sex, muscle CSA remained significantly correlated with cortical area and SSI at the 66% site (r=0.602, p=0.014 and r=0.519, p=0.039, respectively). Knee extensor strength also remained significantly correlated with cortical area at the 38% site (r=0.523, p=0.037) after adjusting for sex.

Conclusions: Lower limb muscle CSA and strength were important determinants of tibial bone strength in endurance athletes regardless of sex. This exploratory research provides new

knowledge on bone adaptations to anabolic stimuli (exercise/loading, muscle forces). These results will guide a bone-targeted training program to improve musculoskeletal health and prevent BSIs in male and female endurance athletes.

Résumé

Contexte: Les athlètes d'endurance présentent un risque accru de perte osseuse et de lésions dues au stress osseux (BSO) en raison d'un entraînement de grande ampleur et d'une alimentation inadéquate. Les facteurs de risque associés à une faible densité minérale osseuse (DMO) et aux lésions dues au stress osseux dans les sports d'endurance comprennent une taille et une force musculaire insuffisantes et un volume d'entraînement élevé. Cependant, nous avons une compréhension limitée des facteurs liés aux muscles et à l'activité qui expliquent la plus grande variabilité dans les estimations réelles de la force osseuse et de la DMO volumétrique (DMOv) et de la surface mesurée par une technologie avancée d'imagerie osseuse chez les athlètes d'endurance.

Objectif: Cette thèse a évalué les relations entre la taille et la force musculaire, l'activité physique modérée à vigoureuse (MPVA) mesurée par accéléromètre, et la force osseuse, la DMOv et la surface osseuse chez de jeunes individus entraînés en endurance.

Méthodes: Nous avons recruté des hommes et des femmes en bonne santé, âgés de 18 à 35 ans (indice de masse corporelle (IMC) <30 kg/m^2, sans condition médicale connue ni prise de médicaments affectant le métabolisme osseux), faisant partie d'une équipe de sport d'endurance de compétition et/ou pratiquant des exercices d'endurance avec mise en charge (c'est-à-dire la course) >180 min/semaine au cours des 6 derniers mois, pour des mesures uniques. La DMOv totale, trabéculaire et corticale et sa surface, ainsi que l'indice de stress-déformation (ISD) au niveau du tibia et la surface de section transversale (SST) du muscle du mollet ont été déterminés à l'aide d'une tomographie quantitative périphérique. L'absorptiométrie biénergétique à rayons- X a permis de mesurer la surface de la DMO au niveau du fémur proximal et de la colonne

lombaire. La force musculaire isométrique de la poignée de main et de l'extenseur du genou a été évaluée à l'aide de protocoles validés sur dynamomètre. La capacité aérobie maximale a été mesurée au cours d'un test progressif sur tapis roulant jusqu'à épuisement volontaire. Les niveaux de MPVA ont été déterminés à l'aide d'accéléromètres et du questionnaire international sur l'activité physique. L'activité physique spécifique à l'os (type et fréquence) a été mesurée à l'aide du questionnaire sur l'activité physique spécifique à l'os. Des corrélations Pearson/Spearman entre chacun des paramètres musculaires et d'activité physique et les paramètres osseux ont été effectuées avec et sans ajustement pour le sexe. Des tests t indépendants ont comparé ces résultats chez les participants stratifiés par sexe.

Résultats: Dix-huit participants ont été inclus (66% d'hommes, âge moyen±ET 26±4 ans, IMC 22,1±2,4g/m^2, pourcentage de graisse corporelle 18,2±6%, et VO_2 max 56,5±9,2 mL/kg/min). Cinquante-trois pour cent des participants pratiquaient >5 heures/semaine d'entraînement d'endurance en charge, dont 60 % couraient ≥40 km/semaine. Les hommes présentaient un DMOv (p=0,027) et une surface (p=0,016) trabéculaires plus élevés, des surfaces corticales aux sites de 38% et 66% (p=0,006 et 0,002, respectivement), un ISD aux sites de 38% et 66% (p=0,004 et p=0,003, respectivement) et une plus grande SST des muscles du mollet par rapport aux femmes (p=0,044). Les hommes présentaient un indice d'adiposité total du corps (p=0,020), du col du fémur (p=0,017) et de la hanche (mg/cm^2) (p=0,019) supérieur à celui des femmes. Les hommes présentaient également une plus grande force musculaire de la poignée et de l'extenseur du genou que les femmes (p<0,05). Des corrélations positives modérées à fortes ont été trouvées entre la SST musculaire et la surface trabéculaire (r=0,583, p=0,014), la surface corticale au site 38% (r=0,737, p<0,001), le SSI (r=0,667, p=0,003), DMOs du col fémoral (r=0,505, p=0,038), et l'aBMD total de la hanche (r=0,516, p=0,034). Après ajustement pour le

sexe, la SST musculaire est restée significativement corrélée à la surface corticale (r=0,63, p=0,009) et au ISD (r=0,519, p=0,039). La force de l'extenseur du genou est également restée significativement corrélée à la zone corticale au niveau du site 38% (r=0,523, p=0,037) après ajustement pour le sexe.

Conclusions: Cette recherche exploratoire apporte de nouvelles connaissances sur les adaptations osseuses aux stimuli anaboliques (exercice/charge, forces musculaires). La SST et la force des muscles des membres inférieurs étaient des déterminants importants de la force osseuse tibiale chez les athlètes d'endurance, indépendamment du sexe. Ces résultats guideront un programme d'entraînement ciblant les os afin d'améliorer la santé musculo-squelettique et de prévenir les BSO chez les athlètes d'endurance masculins et féminins.

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Contribution of Authors

Jennifer Levee: Completed the data acquisition, analysis, and interpretation of data, and wrote the original draft.

Jenna C. Gibbs: Supervision, conception, design, analysis, and interpretation of data, and revised work for important intellect content.

Ada Sevinc: Completed the data acquisition and analysis.

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List of Abbreviations

aBMD = areal bone mineral density
BMC= bone mineral content
BMI= body mass index
BSI= bone stress injury
CSA= cross-sectional area
DXA= dual x-ray absorptiometry
EA= energy availability
IGF-1= insulin growth factor- 1
LEA= low energy availability
MVPA= moderate-to-vigorous physical activity
PA= physical activity
pQCT= peripheral quantitative computed tomography
SSI= stress strain index
vBMD= volumetric bone mineral density

CHAPTER 1. INTRODUCTION

Endurance athletes are at an increased risk of bone loss and bone stress injury (BSI) due to high-volume training and inadequate nutrition (Schnackenburg et al. 2011). A BSI occurs when bone is unable to withstand chronic repetitive mechanical loading, causing structural fatigue and localized pain and tenderness (Warden et al. 2014). Strenuous training patterns and elevated energetic demand consistent with endurance sport have been shown to accelerate bone remodelling, leading to greater bone resorption and the accumulation of microdamage within the bone (Bennell et al. 1999; Warden et al. 2014). The most common area for a BSI in long-distance runners is at the lower limbs, including the tibia, femur, fibula, tarsals, and metatarsals (Bennell et al. 1999; Warden et al. 2014). Previous studies suggest that 33%-66% of long-distance runners have a history of BSI (Crossley et al. 1999; Kelsey et al. 2007) and 5%-21% of track and field athletes develop at least one BSI over a 1-year training period (Bennell et al. 2004). Risk factors associated with BSIs in young endurance athletes include low areal bone mineral density (aBMD), poor muscle strength and size, and high training volume/mileage (Bennell et al. 1999; Warden et al. 2014). However, we have a limited understanding of which muscle- and activity-related factors explain the most variability in true estimates of bone strength in endurance athletes measured by advanced bone imaging technology (such as peripheral quantitative computed tomography – pQCT).

Substantial evidence supports the positive effects of muscle function on bone strength in healthy, young individuals and athletes (DiGirolamo et al. 2013; Frost, 1987; Popp et al. 2009; Schipilow et al. 2013). The mechanical and metabolic processes in which the muscle can influence bone indicate a functional muscle-bone relationship, suggesting the importance of targeting muscle size and strength to maintain optimal bone strength. Repetitive muscle-

generated loading has been shown to up-regulate bone formation in collegiate athletes (Young et al. 2014). Muscle-derived growth factors can also stimulate bone formation independent of muscle contraction, further demonstrating evidence of a muscle-bone interaction (Hamrick, 2011). Female runners with a history of stress fracture demonstrate significantly lower cortical area, bone strength, and muscle cross-sectional area (CSA) compared to their non-fractured counterparts (Popp et al. 2009). In a large, prospective study of military recruits, fractured participants had lower fitness levels, muscle CSA, and bone strength than the non-fractured group, regardless of sex (Beck et al. 2000). Thus, a larger muscle CSA may contribute to favourable bone strength adaptations in highly active individuals (Beck et al. 2000). Despite this evidence, few studies have investigated the muscle-bone relationship in endurance athletes (Popp et al. 2009), and even less is known about the sex-specific differences.

Although exercise is proven to be beneficial to bone, certain exercise modalities may confer different effects on bone strength. Repetitive skeletal loading (i.e., running) can cause excessive bone deformation and microdamage accumulation, subsequently leading to musculoskeletal decline and injury, such as BSIs, soft tissue injuries, and fractures. Endurance athletes consistently tend to have lower aBMD than athletes participating in high-impact (e.g., gymnastics, volleyball) or odd-impact loading sports (e.g., soccer, racquet sports) (Nikander et al. 2004; Rantalainen et al. 2010, 2011). Physical activity that includes high muscle forces and high-magnitude, low-repetition loading (i.e., resistance training) and high-impact exercise (e.g., jumping) are considered most effective at inducing changes in bone properties (Rantalainen et al. 2011). Self-reported physical activity methods (i.e., questionnaires, logs) have been widely used to assess the influence of physical activity on bone outcomes, however, these subjective measures often lead to recall bias and inaccuracies. The use of accelerometers provides an

objective evaluation of movement accelerations, which can improve our understanding of the influence of weight-bearing physical activity on bone strength in endurance athletes.

1. 1 Knowledge Gaps and Rationale

We have a limited understanding of which muscle- and activity-related factors explain the most variability in pQCT measures of bone strength in endurance athletes, who are typically at a higher risk of low aBMD and BSIs than other athletic groups (Beck et al. 2000; Popp et al. 2009). The associations between self-reported physical activity and BMD are well-established, however, few studies have objectively measured physical activity to explore its relationship with true estimates of bone strength in athletic populations, with most evidence reported in children/adolescents and older adults (Ng et al. 2020; Scott et al. 2018). Further, the relationships between muscle function, physical activity, and bone strength may differ between sexes, but we do not know how these differences affect the underlying mechanisms of the muscle-bone relationship. This research will provide new knowledge on the physiological mechanisms underlying low BMD and BSI risk in both female and male endurance athletes. As well, the use of advanced imaging technology (pQCT, DXA) and accelerometers add a unique aspect to the project which will determine which muscle-and activity-related factors represent modifiable targets to improve bone strength in healthy, athletic individuals. Our findings will serve as pilot data for a future prospective study evaluating the combined and independent effects of high-intensity resistance training and high-impact training on bone strength in endurance athletes.

1.2 Objectives and Hypotheses

The primary objective of this research was to evaluate the relationships between lower-limb muscle size and strength and volumetric BMD (vBMD) in young endurance-trained males and females aged 18-35 years **(**see **Table 1.1**). Secondary objectives of this research were to

evaluate the associations between each of lower-limb muscle size and strength, moderate-to-vigorous physical activity (MVPA), and bone-specific physical activity and lower-limb bone parameters (bone strength and area, and vBMD) in young endurance-trained individuals aged 18-35 years. We hypothesized that higher muscle size and strength and physical activity (MVPA, bone-specific physical activity scores) would be associated with better bone strength variables, and that these associations would no longer be significant after adjusting for sex. **Table 1.1** summarizes the research objectives and analysis plan for the current study.

Table 1.1. Study objectives and analysis plan.

Primary Research Question	**Independent Variable**	**Dependent Variable**	**Method of Analysis**
Is there an association between lower-limb muscle size and vBMD in young endurance-trained individuals?	Calf muscle area (pQCT)	Trabecular, and cortical vBMD and area at the tibia (pQCT)	Pearson/Spearman correlations Partial correlations adjusted for sex
Secondary Research Questions	**Independent Variable**	**Dependent Variable**	**Method of Analysis**
Is there an association between lower-limb muscle size and bone strength in young endurance-trained individuals?	Calf muscle area (pQCT)	SSI at the tibia (pQCT)	Pearson/Spearman correlations Partial correlations adjusted for sex
Is there an association between muscle and bone strength in young endurance-trained individuals?	Knee extensor muscle strength (Biodex) Grip strength (handgrip strength)	Trabecular and cortical vBMD and area, and SSI at the tibia (pQCT)	Pearson/Spearman correlations Partial correlations adjusted for sex
Is there an association between accelerometer-measured moderate-to-vigorous physical activity and lower-limb bone strength in young endurance-trained individuals?	MVPA (accelerometer)	Trabecular, and cortical vBMD and area, and SSI at the tibia (pQCT)	Pearson/Spearman correlations Partial correlations adjusted for sex
Is there an association between bone-specific physical activity and lower-limb bone strength in young endurance-trained individuals?	Total, past, and current BPAQ score (BPAQ)	Trabecular, and cortical vBMD and area, and SSI at the tibia (pQCT)	Pearson/Spearman correlations Partial correlations adjusted for sex
Compare associations mentioned above stratified by sex	Sex (males versus females)	Bone and muscle parameters at the tibia (pQCT), muscle strength (Biodex, handgrip strength), physical activity (accelerometer, IPAQ, BPAQ)	Independent T-Tests

Note: vBMD=volumetric bone mineral density; pQCT=peripheral quantitative computed tomography; BSI= bone strength index; SSI= stress strain index; MVPA= moderate -to-vigorous physical activity; BPAQ= Bone-Specific Physical Activity Questionnaire.

CHAPTER 2. LITERATURE REVIEW

2.1 Burden of Osteoporotic Fractures

Osteoporosis is a metabolic bone disease that is comprised of low bone mass and the deterioration of bone macro- and microarchitecture, leading to an increased risk of fragility fractures (Dobbs et al. 1999). Fragility fractures are associated with severe consequences for health, including hospitalization (Papaioannou et al. 2001), lower quality of life (Adachi et al. 2001), declines in functional mobility, and reduced independence, social participation, and self-esteem (Adachi et al. 2001). The most common osteoporotic fractures occur at the distal forearm, thoracic and lumbar vertebrae, and the proximal femur. As the population ages, we can expect a continued increase in osteoporosis cases in the next decade. Approximately two million Canadians are affected by osteoporosis annually and one in three women and one in five men will suffer from at least one osteoporotic fracture during their lifetime (Osteoporosis Canada, 2021). The high prevalence of osteoporosis causes a significant burden on the Canadian healthcare system, with annual costs of 2.3 billion dollars since 2010 (Tarride et al. 2012). Specifically, each hip fracture alone costs the healthcare system over $20,000 in the 1st year after hospitalization, and over $40,000 if the patient is institutionalized (Wiktorowicz et al. 2001)(Osteoporosis Canada, 2021). While some patients may return home, many individuals who suffer from a severe fracture are placed in a long-term care facility, incurring additional care and medication costs (Tarride et al. 2012).

There are two main types of osteoporosis: primary and secondary. Primary osteoporosis is the most common form of osteoporosis and is further divided into postmenopausal osteoporosis (type 1), and age-associated or senile osteoporosis (type 2) (Dobbs et al. 1999). Type 1 osteoporosis is characterized by a reduction in gonadal hormone production (specifically,

estrogen and progesterone from the ovaries), which results in an increased bone resorption relative to bone formation, and a net loss of bone mass (Dobbs et al. 1999). Type 2 osteoporosis is characterized by a gradual age-related bone loss in the presence of systemic senescence as stem cell precursors initiate bone loss, particularly through the loss and thinning of cortical bone and the reduction in the amount of and spacing between trabecular bone (Riggs & Melton, 1963). Secondary osteoporosis is defined as low bone mass and altered bone microarchitecture in the presence of a specific, diagnosed clinical disorder or medication (Dobbs et al. 1999). Secondary osteoporosis can occur in pre- and postmenopausal women and men. Secondary causes for osteoporosis include but are not limited to rheumatoid arthritis and other rheumatological conditions, genetic diseases such as cystic fibrosis, primary hyperparathyroidism, chronic kidney or liver disease, diabetes, malabsorption syndromes, and hypogonadism (deficiency in gonadal hormone production) (Cosman et al. 2014). Common causes of hypogonadism include eating disorders (i.e., anorexia nervosa), functional hypothalamic amenorrhea (typically seen in elite female athletes and dancers), premature menopause (before age of 45 years), chemotherapy, pituitary disease, and other chronic diseases. This literature review will primarily focus on the secondary causes of osteoporosis and the factors that play a role in its relationship with bone health, fractures, and bone stress injuries (BSI) in physically active individuals and athletes, particularly those participating in endurance sport (i.e., long-distance running).

2.1.1 Risk Factors for Osteoporotic Fractures

There are several well-known risk factors associated with osteoporosis and fractures including age, low body mass, family history, past fragility fracture, low calcium intake, smoking, alcohol use, history of falls, vitamin D deficiency, and physical inactivity (Papaioannou et al. 2010; Dobbs et al.1999).

Peak bone mass is typically attained by age 30 years, therefore it is crucial to maintain a proper diet (i.e., adequate calcium and vitamin D intake), as well as participate in regular, weight-bearing physical activity to maintain bone health. Bone mineral accrual during childhood and adolescence is a strong determinant of bone health in later life. Particularly, the amount of bone accrued through weight-bearing physical activity during childhood plays a large role in optimizing bone mineral density (BMD) throughout the aging process (Forwood et al. 2006). Using seven years of data from the Saskatchewan Pediatric Bone and Mineral Accrual Study in 109 boys and 121 girls aged 8-15 years, Forwood et al. evaluated the relationship between daily physical activity and peak bone mineral accrual throughout adolescence. Their findings revealed that children with higher physical activity levels during the adolescent growth spurt had larger bone cross-sectional area (CSA) and bone bending strength (Forwood et al. 2006), suggesting that physical activity during adolescence increases peak bone mass and strength. Therefore, the positive effects of physical activity on bone health during these critical years can lead to preserving bone strength throughout adulthood and reducing the risk of fractures throughout menopause and aging (Forwood et al. 2006).

Older age, low BMD, and previous fragility fracture also represent important risk factors for future osteoporotic fracture (Dobbs et al. 1999). The likelihood of sustaining a fracture increases with age (Dobbs et al. 1999; Ensrud, 2013; Kanis & Pitt, 1992). Evidence from population-based, prospective studies have shown that low BMD increases the risk of fracture among older adults, suggesting that age-specific bone loss is a major contributor to overall fracture incidence (Lewis et al. 2006; Nguyen, 2001; Stone et al. 2003). Additionally, age-related declines in balance and mobility increase the incidence of falls, and subsequently, the incidence of fractures in older individuals. Women are twice as likely to sustain a fracture as men (Dobbs

et al. 1999). Even though both females and males attain their peak bone mass at an average age of 30 years, women experience bone loss at a rate of 0.5% per year whereas men lose bone mass at a rate of 0.3% per year (Riggs & Melton, 1963). Furthermore, women experience a more accelerated bone loss due to estrogen deficiency during and after menopause. Estrogen plays a crucial role in the bone remodelling cycle and will be discussed later in the review.

2.2 Principles of Loading

Bone remodeling and modeling processes are stimulated by mechanical forces that act on the skeleton through skeletal muscle contraction (muscle or joint reaction forces) and through impact loading (gravitational or ground reaction forces) (Kohrt et al. 2009). Bone is responsive to local strains by routine stresses through activities of daily living and physical activity/exercise (Kohrt et al. 2009). However, depending on the environment, bone will adapt differently based on the strain magnitude, strain rate, strain frequency, strain distribution, number of loading cycles, and rest-recovery periods (Kohrt et al. 2009).

Bone adapts to stress from an external source which produces a strain, eventually causing a structural deformation (Hart et al. 2017). Specifically, stresses of varying intensities cause different magnitudes of strains due to the amount of force applied to the bone (Turner'.' et al. 1993; Yang et al. 2011). Stress is measured as a load per unit of area in Newtons/metres^2 and strain is expressed as a microstrain or percentage change in bone dimension. The stress-strain relationship suggests that higher levels of strain above the yield point deform bone material beyond its point of resilience, causing damage in the form of microcracks (Burr, 2011). Resilience refers to the amount of elastically stored energy in bone that can withstand stress before microdamage occurs. Cortical bone is stiffer than trabecular bone and can withstand higher levels of stress at a lower strain before fracture, whereas trabecular bone typically

withstands lower levels of stress at the same strain magnitude due to its porous nature (Currey, 2003)

The level of strain magnitude that bone undergoes will influence its ability to remodel. To maintain bone mass, a minimum effective strain is required (Ehrlich & Lanyon, 2002). When strain magnitude is above the minimum effective strain (1500-2500 microstrain), bone formation will be stimulated and subsequently, bone mass and CSA will increase (Turner & Pavalko, 1998). However, if strain magnitude is below the minimum effective strain, bone resorption is stimulated and subsequently, bone mass and CSA decreases (Turner & Pavalko, 1998). Strain frequency is the number of applied loading cycles to a given structure and plays a critical role in osteogenesis (Amidzic et al. 2001). Increased strain frequency causes a lower minimum effective strain, and therefore bone remodeling can occur at lower strain magnitudes, stimulating higher levels of bone formation (Amidzic et al. 2001).

Mechanical stimuli initiated by physical activity and exercise can positively affect bone modeling/remodeling (Frost, 1987). However, not all types of loading are optimal for bone turnover. Classic experimental studies in animals and humans have shown that higher magnitude, low-repetition modalities of physical activity create peak impact loads, resulting in improved bone outcomes (Frost, 1987). Furthermore, bone modeling occurs when strains of 2000 Newtons (average) are applied to bone (Nikander et al. 2006). Therefore, it is important to participate in activities that create these optimal strains to obtain osteogenic effects and improve bone health across the lifespan.

2.3 Bone Stress Injuries (BSI)

A BSI refers to when bone is unable to withstand chronic repetitive mechanical loading, causing structural fatigue and localized pain and tenderness (Warden et al. 2014). BSI varies in severity from grade 1 (periosteal edema) to grades 2 and 3 (bone marrow edema) to grade 4 (radiographically evident non-linear/linear cortical abnormalities). The initial response can potentially create a stress reaction, similar to a bone bruise, due to overuse. A stress reaction that goes untreated can lead to a stress fracture, leading to small cracks from repetitive trauma, and in more severe cases, a complete bone fracture, normally occurring around 7300 microstrains. The excessive bone strain stimulates bone remodeling to repair the substantial amount of microdamage. The remodeling process takes 3-4 months in which osteoblasts are deposited to initiate bone formation (Bennell & Brukner, 2005). Stress fractures develop if microdamage cannot be optimally repaired by the remodeling process (Bennell et al. 1999). Accelerated bone remodeling processes due to excessive strain may also weaken bone due to the increased bone resorption, resulting in a localized reduction in the energy absorbing capacity of bone and further microdamage accumulation (Bennell et al. 1999).

BSIs are commonly observed in individuals participating in a high volume of athletic or occupational loading of the skeleton (e.g., long-distance runners, military recruits). BSIs in long-distance runners are typically the result of the imbalance between load-induced microdamage formation and its removal. Specifically, bone is exposed to repetitive mechanical loading (like running), which causes bone to deform. The extent to which bone is deformed depends on the load magnitude and the ability of bone to resist load. The most common area for a BSI to develop in long-distance runners is the lower extremity, including the tibia, femur, fibula, tarsals, and metatarsals (Warden et al. 2014). The lumbar spine and pelvis are also common sites for a

BSI, with BSIs at the upper-limb and thoracic spine being relatively uncommon (Warden et al. 2014). Microdamage is threshold dependent and does not necessarily lead to a bone fracture. Therefore, if an individual has a higher minimum effective strain threshold, they will be less likely to develop a BSI. A higher threshold can be attained through higher amounts of bone-specific impact loading, accomplished through greater strain from mechanical loading.

According to Warden et al. (2014), there are two main categories of risk factors for a BSI. The first category is defined by the extrinsic application of mechanical loading to bone (Warden et al. 2014). A BSI can develop through biomechanical factors (e.g., abnormal motions and forces applied to bone), training factors (e.g., increased frequency and intensity of training), and muscle factors (e.g., lower muscle size and strength). Specifically, muscle size and strength play an important role during impact loading since muscle can respond to mechanical forces and act as an active shock absorber inhibiting the formation of a BSI. The second category of BSI risk factors is the intrinsic ability for bone to resist load without causing microdamage (Warden et al. 2014). As mentioned earlier, physical activity history is an important determinant of risk for BSIs as most bone adaptation occurs during the growing years (Warden et al. 2014). Regular weight-bearing physical activity during childhood and adolescence increases peak bone mass acquisition, leading to an increased resistance to bone fatigue and a lower risk of BSIs. Nutritional factors, particularly adequate energy availability (EA) and calcium and vitamin D intake, also play a fundamental role in reducing the risk of low BMD and BSIs. Lastly, reproductive hormones, such as estrogen, progesterone, and testosterone, stimulate bone growth and formation during childhood and adolescent years and can maintain bone health in adulthood. This review will summarize the effects of these hormonal and skeletal changes on bone's ability to resist load and repair microdamage in the presence of high-volume endurance training

(Warden et al. 2014). Due to the interrelationship between reproductive hormones (i.e., estrogen, progesterone), EA, and BMD, females are typically at a greater risk of developing a BSI (Warden et al. 2014).

2.4 Overview of Bone Strength Determinants

Bone strength is the bone's resistance to fracture and there are multiple bone parameters that contribute to ultimate bone strength (Fonseca et al. 2014). Bone structure and geometry are two critical components that determine bone strength. The size, shape, CSA, and trabecular orientation are also important factors that determine the biomechanical properties of bone (Osteoporosis Canada, 2021). For bone to withstand large amounts of stress, there must be a higher BMD and a larger bone CSA to maintain its structure and function. There are two types of bone tissue: cortical (compact) bone and trabecular (spongy) bone. Cortical bone is the hard, outer layer of bone. In the presence of high-volume physical training, a person may experience micro-cracks in the cortical layer caused by strains on the bone, however, once the bone can no longer tolerate the constant strain, a fracture will occur (Fonseca et al. 2014). Trabecular bone is the spongy, inner layer of bone characterized by a network of horizontal and vertical struts or trabeculae. Trabecular bone is largely dependent on the thickness and density of its trabeculae. The loss of trabecular density highly affects the strength of the bone, a strong determinant of its overall density.

Not only are bone material properties crucial to maintaining bone strength, but the structural organization of bone tissue is just as important. Trabecular bone is commonly affected by bone resorption and elevated bone turnover may cause the trabeculae to become thinner, resulting in decreased bone strength (Fonseca et al. 2014). In a study by Legrand et al., 108 men with low bone mass and fractures had greater trabecular separation than, those without fractures.

Cortical bone parameters (thickness, porosity) are also significant predictors of bone strength (Fonseca et al. 2014; Legrand et al. 2000), and the thinning of and holes within the cortical bone can compromise bone health.

Bones are comprised of organic and inorganic composite materials. The way these materials align and interact between their relative composition contributes to the mechanical properties of bone. Mineral porosity also plays a role in determining the quality of bone material and how it will respond to load (Zebaze et al. 2010), specifically its ability to resist stress, absorb stress/energy, and resist load prior to failure (ultimate strength) (Zebaze et al. 2010).

2.5 Bone Mineral Density (BMD), Structure, and Strength

Bone mineral density, or BMD, is the mass of bone mineral per volume of bone tissue and is commonly measured using dual-energy X-ray absorptiometry (DXA) to diagnosis osteoporosis. A DXA test involves lying on a table while the x-ray detector scans the regions of interest (i.e., spine, hip, and/or wrist). A DXA scan involves minimal radiation exposure; wherein two low dose X-rays pass through the body. The rays are absorbed differently by bone tissue versus soft tissues, and the density profiles of these X-rays are used to calculate aBMD, which is the amount of bone mineral divided by the bone scanned area. To screen for osteoporosis, a T-score is calculated as the measured aBMD compared to the young normal reference mean. A T-score is expressed in units of standard deviations (SD), which indicates how the score deviates from a "normal" score for a young adult (Osteoporosis Canada, 2021). The World Health Organization (WHO) classifies osteoporosis as a T-score of less than or equal to -2.5 SD, osteopenia (low aBMD) as a T-score in between -1 SD and -2.5 SD, and normal aBMD as a T-score greater than -1 SD (Sozen et al. 2017). All women and men over 65 years, and all post-menopausal women and men aged 50-64 years with risk factors for fractures should have a

DXA scan. Young adults can also be at risk for low aBMD compared to individuals their age due to secondary causes (i.e., hypogonadism, nutritional/endocrine disorders) as described earlier in this review. The WHO uses Z-scores to compare aBMD to an age-matched normative value. Although clinical guidelines do not recommend routine bone density testing in younger age groups, a Z-score above -2.0 is considered normal. Young women and men under 50 years of age with a disease or condition associated with low bone mass are often referred for a DXA test (Osteoporosis Canada, 2021). The American College of Sports Medicine and Female and Male Athlete Triad Coalition consensus statements suggest that athletes involved in regular weight-bearing sports with an aBMD Z-score between 1 and 2 SD be referred for a DXA as they are expected to have higher aBMD due to their regular training patterns (de Souza et al. 2014).

Peripheral quantitative computed tomography (pQCT) is used in research settings to measure bone's true density, geometry, and macroarchitecture in cortical and trabecular compartments, which are stronger indicators of bone quality and fracture risk than DXA. pQCT is a three-dimensional imaging technique that acquires a transaxial image at peripheral measurement sites (i.e., tibia, radius) with low radiation dose (<0.5 μSV per slice at radius and <1.5 μSV per slice at tibia), and high sensitivity to detect statistically significant changes within short periods of time (Lala et al. 2012). pQCT can also measure CSA and density of muscle and fat tissues surrounding the bone in the lower limbs and forearms. Using the manufacturer's macro-based automated technique, an analysis of images derives total, trabecular, and cortical vBMD and area, cortical thickness, bone compressive strength index, and stress-strain index, all critical components of bone strength (Lala et al. 2012).

2.6 Overview of Bone Metabolism

Bones are primarily made up of three different cells: osteoblasts, osteoclasts, and osteocytes. Osteocytes are the longest living, most abundant bone cell (90-95% of the cells in bone tissue) and are commonly found in mature bone. Osteocytes are primarily responsible for responding to mechanical strain and sending signals for bone remodelling. Bone remodeling occurs when bone is broken down by osteoclasts (takes ~3 weeks), and then osteoblasts stimulate bone formation and produce new bone called osteoid, which is made up of bone collagen and other proteins (takes ~3-4 months) (Fonseca et al. 2014). A stable balance between bone resorption and bone formation is crucial during adulthood and highly influences bone strength. If there is greater bone resorption relative to formation, bone loss occurs, increasing bone fragility and risk of fractures. Excessive bone formation is also deleterious as old and damaged bone will not be removed, resulting in decreased bone elasticity and higher amounts of energy expenditure (Fonseca et al. 2014). Type 1 collagen is also an important contributor to maintaining adequate bone strength. Collagen is laid down by osteoblasts and is responsible for bone stiffness (Fonseca et al. 2014). If there is a lack of osteoblast recruitment from osteocytes, the result is often a lower bone stiffness and subsequently, a lower ultimate bone strength. Previous studies have shown that greater mechanical stimulation through exercise can improve collagen structure and overall bone health (Shiiba et al. 2002).

After an injury, skeletal tissue heals by producing new bone with a resilient structure and integrity. Different metabolic pathways exist to properly repair bone. Specifically, the Wnt-Beta Catenin pathway plays a critical role in skeletal tissue regeneration and promotes bone growth. Mutations in the Wnt signalling pathway can result in excessive bone growth and resorption, leading to alterations in BMD and an increased risk of fracture (Houschyar et al. 2019).

Sclerostin, produced by osteocytes, is an important protein involved in the Wnt signalling pathway since an increase in sclerostin levels inhibits the formation of new bone during osteogenic differentiation (Houschyar et al. 2019). With altered levels of sclerostin, the risk of osteoporosis is high due to the inhibition of bone formation and lower osteoblast activity. However, mechanical loading through physical activity and exercise inhibits sclerostin activity and upregulates the Wnt-Beta Catenin pathway, suggesting a key role in stimulating bone turnover. Recent studies have explored the notion of anti-sclerostin therapy as a potential treatment for osteoporosis (Maeda et al. 2019) and other skeletal disorders.

2.7 Muscle-Bone Relationship

One of the most direct stimuli to bone is locally applied muscle contractile forces (DiGirolamo et al. 2013). Muscle and bone share similar mechanisms in which they adapt, also known as muscle-bone cross-talk. Specifically, genetic pathways can similarly alter muscle and bone and hypothetically contribute to the parallel development of osteoporosis and sarcopenia in the absence of mechanical stimuli (Cianferotti & Brandi, 2014). Muscle is a key factor in the process of osteogenesis and the frequency, rate, magnitude, and distribution of the muscle forces applied to bone can determine the extent to which bone remodelling occurs (Ireland et al. 2014).

Traditionally, muscle-bone associations were evaluated by studying the relationship between DXA-based measures of lean body mass and aBMD. In a cross-sectional study in 152 premenopausal women, Greenway et al. demonstrated that higher lean mass and lower fat mass were associated with higher aBMD at weight-bearing sites (Greenway et al. 2015). Similarly, Greene et al. found positive associations between lean body mass and aBMD in female adolescents aged 11-16 years participating in track and field and gymnastics (Greene et al. 2012), and an inverse association between fat mass and aBMD (Greene et al, 2012). Therefore, the

regulation of bone mass may rely on lean-related muscle mass and its ability to positively influence the bone remodelling process. Recent advancements in bone imaging technology led to the investigation of the associations between muscle size and quality and indices of bone strength measured by peripheral quantitative computed tomography (pQCT)/QCT. In a ten-year prospective study of 112 healthy, premenarchal girls, Petit et al. explored predictors of adult bone structural geometry and strength measured by hip structural analysis. Petit et al. found that muscle and mechanical loading factors (specifically, lean mass and sport/exercise participation) were associated with bone strength while gonadal hormone concentration (estrogen, testosterone) was associated with bone geometry (Petit et al. 2004).

The fundamental association between muscle forces and regulation of bone mass has been classically described as the functional muscle-bone unit (Schoenau, 2002). The functional muscle-bone unit theory states that muscle forces apply strains to stimulate bone formation, which leads to favourable changes in bone mass and structure. Specifically, Schoenau et al. found that higher muscle CSA was associated with higher bone mineral content (BMC) (Schoenau, 2022). The mechanical interaction between muscle and bone in response to loading enables a constant relationship between both skeletal tissues on a functional and structural level (Novotny et al. 2015).

Muscle can also influence bone tissue via biochemical pathways. Myokines, such as insulin-like growth factor-1 (IGF-1) and myostatin, are muscle-derived peptides that mediate muscle and bone through endocrine-paracrine metabolic processes (Hamrick, 2011, 2012). Myokines can influence the local activity through which the muscle fiber inserts directly into the bone, whereby it delivers blood and nutrients to the skeleton. IGF-1 is stimulated by muscle-contraction (Hamrick, 2012) and helps to promote bone and tissue growth, suggesting its role in

both bone and muscle metabolism (Hamrick, 2012). Myostatin has an inverse relationship with muscle, such that a myostatin deficiency increases muscle mass. Myostatin is highly expressed in an upper- or lower-extremity injury and can inhibit bone repair (Hamrick, 2012). Muscle loss has also been shown to precede bone loss as disuse of the lower extremity can significantly alter the bone remodeling process (Bettis et al. 2018). Therefore, the mechanical and metabolic processes in which muscle can interact with bone further supports the functional muscle-bone relationship and its relevance to maintaining bone strength.

There is substantial research on the muscle-bone relationship in young, healthy populations (Greene et al. 2012; Greenway et al. 2015; Mallinson et al. 2013), however, there are limited studies in endurance athletes, a notably vulnerable group to bone loss and BSI. Previous work by Popp et al. (2009) explored differences in tibial bone strength in 39 competitive female distance runners with and without a history of stress fractures. They found that female runners with a history of stress fractures had significantly lower cortical area, lower bone strength, and smaller muscle CSA at the tibia compared to their non-fractured counterparts (Popp et al. 2009). These findings suggest that muscle may explain the variability in bone strength outcomes in long-distance runners and serve as a modifiable target to reduce BSI risk in athletes participating in high-volume endurance training.

2.8 The Influence of Physical Activity and Exercise on Bone Strength

The positive effects of exercise and physical activity on bone parameters are well-documented (Hinton et al. 2015; Nikander et al. 2006; Rantalainen et al. 2011). There are several key loading principles that explain the influence of exercise on bone parameters. The principle of progressive overload states that the body must increase demands to improve bone mass, such as high-magnitude loading, in which bone will adapt and increase its ability to sustain

maximum strain. Heionen et al. (2001) examined the influence of high-impact loading on bone mass, size, and structural properties in 8 Finnish triple jumpers' lower limbs, and found that vBMD at the tibia was significantly higher than matched controls (Heinonen et al. 2001). As well, DXA-derived femoral neck and lumbar spine aBMD were higher in jumpers than in controls (Heinonen et al. 2001). Specificity of exercise training is crucial for bone adaptations as high levels of stress must be placed on the skeletal system to stimulate bone formation. Kontulainen et al. (2003) performed a longitudinal study of the effect of long-term impact-loading on bone parameters in 64 young (mean age of 26.5 years) and old (mean age of 44.4) female squash and tennis player. Their study compared side-to-side differences in the upper-arm bones of players who had started training during the growing years versus those who started their training in adulthood and nonplaying controls (Kontulainen et al. 2003). Kontulainen et al. (2003) determined that both groups of racquet players had greater BMC and total bone CSA in their playing arm compared to their non-playing arm. Thus, targeting odd-impact loading exercises (loading bones in many different directions and motions) may have positive effects on bone strength in the exposed limbs. In a meta-analysis of seven randomized controlled trials >24 weeks in length, (Kelley et al. 2013) determined the effects, as well as the potential moderators and predictors, of ground and joint reaction force exercise on femoral neck and lumbar spine aBMD in premenopausal women. Their findings demonstrated a small, yet statistically significant, improvement in aBMD at the femoral neck and lumbar spine (Kelley et al. 2013). However, it is unknown whether this magnitude of effect would translate to a clinically relevant decrease in fracture risk in those women who improved aBMD at these sites.

Resistance training is recommended as an effective strategy to maintain bone mass, geometry, and structure via the direct pulling action of muscle on bone and/or the increase in

gravitational loading forces. However, resistance training alone has small-to-no effects on hip and spine aBMD, despite improvements in muscle mass and strength (Hinton et al. 2015; Howe et al. 2011; Martyn-St James & Carroll, 2006; Watson et al. 2018; Zhao et al. 2015). Weight-bearing exercise, involving moderate-to-high magnitude loads (≥2-4 times body weight) and multidirectional movement patterns, is considered most effective at inducing changes in bone material and structural properties (Watson et al. 2018). In a meta-analysis of nine randomized controlled trials, Martyn-St James & Carroll (2006) found that exercise programs that combine odd-or high impact activity with high magnitude resistance training have positive effects on BMD in premenopausal women at the hip and spine compared to the control groups. Nikander et al. (2006) found that athletes who perform high- and odd-impact loading (volleyball, tennis, hurdling, soccer) have higher BMC at loaded bone sites (such as the tibia) compared to their non-athletic counterparts, further demonstrating the site-specific relationship between load intensity and bone adaptations. In a cross-sectional study of 117 female adolescents, Greene et al. (2012) demonstrated that girls who participated in gymnastics had the most skeletal benefits, including bone strength index measured by pQCT, compared to girls who participated in track and field and water polo (Greene et al. 2012). This finding is due to the high intensity nature of gymnastics as well as its production of higher muscle and impact forces than running- or water-based sports. Further, girls who participated in water polo did not show any change in bone strength index compared to the non-active control, demonstrating that non-weight-bearing activity does not induce favourable bone adaptations (Greene et al. 2012).

The most beneficial effects on BMD and bone strength typically result from concurrent exercise training, which includes resistance and high-impact training (de Avila et al. 2019). Resistance training and high-impact training have positive influences on aBMD (de Avila et al.

2019). Specifically, high-impact training causes an immediate effect on aBMD, whereas resistance training has more of a delayed effect over weeks and even months. In a longitudinal intervention study, Lambert et. al compared the effects of a 10-month, twice-weekly, high-impact loading and high-intensity resistance training on pQCT and DXA measures of bone strength at the upper and lower limbs of 22 physically inactive women aged 18-30 years. Their results suggest that impact training, such as jumping, promotes superior bone responses at distal sites compared to resistance training (Lambert et al. 2020). However, resistance training had a greater effect on DXA-derived bone indices at the shaft (Lambert et al. 2020). Both resistance and high-impact training are important as they provide different site-specific effects to improve bone health.

aBMD has also been shown to be related to physical activity history (Greenway et al. 2015). In girls aged 10-19 years, a higher amount of weight-bearing activity (≥3 METs) was associated with a higher aBMD (Greenway et al. 2015). For women aged 20-29 years, moderate to vigorous physical activity (MVPA) and weight-bearing activity were positively related to aBMD at the tibial shaft (Greene et al. 2012). However, women aged 30-39 years had the strongest benefits when participating in very vigorous activity compared to MVPA in the younger groups (Greene et al. 2012). This may be because peak bone mass is achieved around 30 years of age, and thereafter limited improvements in aBMD are typically observed. Therefore, long-term participation in bone-targeted physical activity and exercise at the adolescent and adult stages of the lifespan is a key predictor of later-life bone health.

2.9 Bone Fragility in Endurance Athletes: An Overview of Mechanisms

While weight-bearing physical activity is typically considered protective of bone health across the lifespan, endurance athletes (i.e., long distance runners, cyclists) tend to be at a higher

risk of bone loss and BSI due to high-volume training and elevated energetic demand. Specifically, the mechanistic pathways in which bone health is compromised in long-distance runners include low EA, suppressed gonadal hormone concentrations, and repetitive mechanical loading during high-volume training (described in-depth earlier in this literature – see **Sections 2.2 and 2.3**).

2.9.1 Energy Availability and Other Nutritional Factors

Endurance athletes expend large amounts of energy due to prolonged, high-intensity exercise training and often practice dietary energy restriction (both intentionally and unintentionally), making them more susceptible to low EA. EA is defined as the amount of energy readily available from dietary intake minus energy expenditure from exercise (Loucks et al. 2011). EA is considered low when there is a lack of energy to support the physiological demands of the human body (Loucks et al. 2011). Low EA is a common consequence in sports that favour leanness for optimal performance, such as long-distance running and gymnastics. Specifically, 31% of female distance runners and 25% of male athletes have experienced low EA during peak periods of training (Heikura et al. 2018; Loucks et al. 2011). The link between low EA and bone health is characterized by a perturbation to several hormones involved in bone remodelling, including but not limited to estrogen, testosterone, leptin, and IGF-1 (Loucks & Thuma, 2003). Furthermore, low EA is an etiological factor in the development of menstrual disturbances in female athletes (Williams et al. 2001) and hypogonadism in male athletes (Hackney et al. 1988; Lane et al. 1998). Low EA and related menstrual disturbances have been shown to cause serious consequences to bone health, including lower BMD and bone CSA, and an uncoupling in bone turnover (Heikura et al. 2018; Ihle & Loucks, 2004; Papageorgiou et al. 2018). Hutson et al. performed a systematic review and investigated bone outcomes in over 100

female weight-bearing endurance athletes (WBEA) with FHA. They discovered that 56% of weight-bearing endurance athletes with amenorrhea had lower aBMD at all measured sites compared to their male counterparts (Hutson et al. 2020). Additionally, males who participated in rigorous endurance exercise demonstrated lower aBMD compared to male athletes in non-endurance sports (Hutson et al. 2020). Therefore, both female and male athletes who experience low EA have an increased risk of sustaining fractures and BSIs due to reduced BMD and bone strength.

Low EA can alter bone turnover due to high training volumes and restricted dietary intake relative to exercise energy expenditure. Elevated bone resorption and lower bone formation due to low EA is further demonstrated through the work of (Ihle & Loucks, 2004). They determined a dose-response relationship between EA and bone turnover markers by severely restricting EA in 29 exercising women (Ihle & Loucks, 2004). In a randomised, crossover study by Papageorgiou et al. (2017), 11 eumenorrheic women and 11 men completed two 5-day protocols of controlled and restricted EA and daily exercise. Papageorgiou et al. (2017) found a significant increase in bone resorption and a decrease in bone formation in response to low EA in women. However, there were no effects of low EA shown in men (Papageorgiou et al. 2017). Further research is needed to better understand the influence of EA on bone turnover in physically activity individuals and athletes, particularly the sex-specific differences. Overall, correcting EA through adequate nutrition is of paramount importance to optimize bone health and decrease BSI risk over the course of an athlete's career and overall lifespan.

Calcium and vitamin D are also critical nutrients for optimal bone health due to their ability to affect bone metabolism. Bones are the main storage site of calcium in the body (~99%

of the body's total calcium). As such, calcium is an essential element for skeletal mineralization, providing bone strength and structure and acting as a metabolic reservoir to maintain intra- and extracellular calcium balance. A decrease in serum calcium concentration stimulates the release of parathyroid hormone, which causes the release of calcium from bone and reabsorption of calcium in the kidneys (Tønnesen et al. 2016). An optimal calcium intake is necessary for bone healthy at all stages of life. Calcium is obtained through two main sources: through the diet or from supplements. The recommended dietary allowance of calcium ranges from 700-1200 mg/day. Vitamin D is a hormone essential for calcium absorption and bone mineralization in the human body, and is positively associated with BMD (Holick, 2007). If individuals do not consume adequate amounts of vitamin D, only 10-15% of dietary calcium and about 60% of phosphorus would be absorbed (Holick, 2007). Unlike calcium, vitamin D is often obtained through supplementation since most individuals do not get enough from food and sun exposure (its primary source). The recommended vitamin D intake is at least 600 IU/d and should be even higher in older adults and individuals with osteoporosis (800-2000 IU/d). Thus, in addition to achieving a healthy EA, athletes should prioritize achieving an adequate vitamin D and calcium intake to reduce bone loss and the risk of fractures and BSIs.

2.9.2 Overview of the Female and Male Athlete Triad

The female athlete triad is defined as the interrelationship between low EA, menstrual dysfunction, and low BMD (Nattiv et al. 2007). Specifically, female athletes who participate in leanness-focused sports, such as long-distance running, are at an increased risk of developing the Triad, with up to 60% of female athletes reporting at least one of the Triad components (Gibbs et al. 2013). Menstrual disturbances are reported in up to 50-65% of female athletes participating in leanness sports, with functional hypothalamic amenorrhea being the most severe type of

menstrual disturbance (absence of menses for >90 days) (Dušek, 2001; Melin et al. 2015). Amenorrheic athletes report more BSIs compared to their eumenorrheic counterparts, which can lead to significant health and performance consequences (Ackerman et al. 2015; Barrack et al. 2014). Like female athletes, male athletes can also experience impaired bone health, suppression of gonadal hormones, and compensatory metabolic alterations in the presence of energy deficiency/low EA (Nattiv et al. 2021).

While both energy and estrogen status are crucial in maintaining adequate bone health, limited research has compared their independent and combined influence on bone outcomes. In a cross-sectional study of 109 exercising women, Southmayd et al. (2017) described pQCT measures of vBMD, bone geometry, and estimated bone strength in participants grouped according to energy status (energy replete vs. energy deficient), and estrogen status (estrogen replete vs. estrogen deficient). Women who were both energy and estrogen deficient had the greatest suppression of bone formation and rate of bone turnover (Southmayd et al. 2017). Additionally, there was a significant interaction between energy status and estrogen status that was negatively associated with total and trabecular bone CSA at the distal tibia (Southmayd et al. 2017). However, low EA often precedes menstrual disturbances, and thus, when aiming to reverse the Triad, correcting EA is critical for the recovery of menstrual function and subsequent improvements in bone health.

The literature is saturated with studies that explore the implications of the Female Athlete Triad, however emerging research suggests male athletes can experience similar consequences of low EA and hypogonadism. In a study by Julian-Almarcegui, male adolescent cyclists consumed lower dietary energy intake, and therefore presented with lower EA, compared to their sedentary counterparts (Julián-Almárcegui et al. 2013). Alternatively, Koehler et al. (2006) demonstrated

that a short-term reduction of EA (15 kcal/kg fat-free mass/day) was associated with reductions in leptin and insulin concentrations, but not IGF-1, free triiodothyronine, and testosterone, in 6 physically active men (Koehler K et al. 2016). Therefore, the magnitude and duration of low EA required for alterations in metabolic hormone concentrations is likely more severe in males than females. Male endurance athletes have also been shown to demonstrate lower levels of gonadal hormones, such as testosterone, an important hormone regulating the gain and maintenance of BMD in men (Hackney & Constantini, 2020). Julián-Almárcegui et al. (2013) reported significantly lower serum levels of testosterone in endurance male athletes compared to sedentary men (Julián-Almárcegui et al. 2013). Wheeler and colleagues found an inverse relationship between running mileage and total testosterone levels in 49 male runners (Wheeler et al. 1986). Hypogonadal men typically have accelerated bone turnover and increased fracture/BSI risk (Adam S. Tenforde et al. 2016). Low BMD has been reported in male distance runners (Barrack et al. 2017; Fredericson et al. 2007; Tenforde et al. 2015) and in male athletes engaged in low-impacts sports (e.g., cycling, swimming) (Barry & Kohrt, 2007; Nichols & Rauh, 2011; Palmer et al. 2003; Taafe & Marcus, 1999). Like female athletes, risk factors for low BMD in male athletes include energy deficiency/low EA, low body weight, high training volume, and history of BSI (Barrack et al. 2017)). Further work exploring the mechanisms underlying the male athlete triad is necessary, particularly sex-specific comparisons in the determinants of EA, reproductive function, and bone health (Bischoff-Ferrari et al. 2006; Holick, 2007; Tønnesen et al. 2016).

2.10 Conclusion

Regular weight-bearing physical activity and adequate nutrition typically confer beneficial effects on bone health. However, endurance athletes consistently tend to have

significantly lower BMD and bone CSA than athletes participating in high-impact (e.g., gymnastics, volleyball) or odd-impact loading sports (e.g., soccer, racquet sports) (Nikander et al. 2004; Rantalainen et al. 2010, 2011). The associations between self-reported physical activity and BMD are well-established, however, few studies have objectively measured physical activity levels to explore its relationship with bone strength, with most evidence reported in children/adolescents and older adults (Ng et al. 2020; Scott et al. 2018). Therefore, the main objective of this research was to evaluate the relationships between muscle size and strength, physical activity, and bone strength in healthy endurance-trained males and females aged 18-35 years.

CHAPTER 3. METHODS AND PROCEDURES

3.1 Study Design

The purpose of this study was to examine the associations between muscle size and strength, physical activity (MVPA and bone-specific physical activity), and bone strength in endurance-trained individuals aged 18-35 years. This study used a cross-sectional design to examine the primary and secondary objectives (see **Table 1.1**). Data collection took place over a rolling basis, in which participants completed two study visits, one at the Center of Innovative Medicine (CIM) (study visit 1; approximately 2 hours), and the other at the Currie Gymnasium at McGill University (study visit 2; approximately 2 hours) (see **Table 3.1** for a list of study outcomes and assessments). Written informed consent was obtained prior to the first study visit and participants arrived fasted overnight, having abstained from caffeine, strenuous exercise, and alcohol for ≥12 hours to standardize the DXA and pQCT protocols for assessing body composition and bone health (Nana et al. 2016). During study visit #1 at the CIM, participants underwent pQCT and dual-energy X-ray absorptiometry (DXA) imaging scans. Following the imaging tests, participants filled out a series of questionnaires using the Research Electronic Data Capture (REDCap) web-based system with the assistance of a research assistant, including a demographic and health history questionnaire, International Physical Activity Questionnaire (IPAQ), Bone-specific Physical Activity Questionnaire (BPAQ), Low Energy Availability in Females Questionnaire, Drive for Muscularity Scale, Drive for Thinness, Body Dissatisfaction, and Bulimia subscales from the Eating Disorder Inventory-3 and Three Factor Eating Questionnaire-Revised 21-Item. Prior to the second visit, participants arrived fasted, having abstained from caffeine, strenuous exercise, and alcohol for >3 hours to standardize the muscle strength and VO2max protocols for assessing physical performance. During the study visit #2 at

Currie Gym, participants completed a series of performance-based tests of upper- and lower-limb muscle strength, and maximal aerobic capacity (in the same order with at least 5-10 minutes rest in between each test). After the study visits, participants were asked to: 1) wear a triaxial accelerometer on their waist for 7 consecutive days; 2) wear a polar heart monitor during every exercise training session in the 7-day period; and 3) complete a 24-hour dietary recall using the Automated Self-Administered 24-hour web-based dietary assessment tool over three days (two weekdays, one weekend day). For this thesis, only relevant outcome measures are presented. This research was conducted according to the Tri-Council Policy Statement, second edition, and approval from the McGill University Health Centre Research Ethics Board was obtained.

3.2 Participants

We recruited physically active men and women aged 18-35 years (n=25 each) that were non-obese (body mass index (BMI) $\leq$30 kg/ m^2), free of metabolic, neuromuscular, or intestinal disorders known to affect bone metabolism, and performed high-volume, weight-bearing endurance exercise (i.e., running) $\geq$180 mins/week in the past 6 months. We recruited only female participants with a naturally occurring menstrual cycle (i.e., between 21-35 days) or those using oral contraceptive pills at the time of study enrolment. To minimize confounding effects of reproductive hormones across the menstrual cycle, female participants were tested during the early follicular phase of the menstrual cycle (days 1-5) or during the non-active pill phase if on oral contraceptives. Additional exclusion criteria included medication known to affect bone metabolism (e.g., hormonal contraception use other than oral contraceptives within last 3 months prior to study participation, glucocorticoids, anti-hypertensive drugs, anti-epileptic drugs, osteoporosis therapy), orthopedic or musculoskeletal injury/disease that limits the capacity to exercise, pQCT scan impossible to perform, current diagnosis of an eating disorder, current

smokers, female participants with self-reported or diagnosed hypothalamic amenorrhea, polycystic ovarian syndrome, hyperprolactinemia, or primary ovarian insufficiency, and pregnant or breastfeeding.

3.3 Outcome Measures

3.3.1 Anthropometry

Height was measured to the nearest 0.1 cm using a calibrated wall-mounted scale. The participants' heels, buttocks and head were placed flat on the wall, and eyes looking forward. After an exhalation, the head plate was brought down to the top of the participants head to determine their height. This process was repeated twice for accuracy. Weight was measured to the nearest 0.1 kg using a clinical, calibrated electronic weighing scale (Scale-Tronix, Welch Allyn, Skaneateles, NY). The scale was zeroed, and the participant stepped onto the scale ensuring shoes, heavy jewelry, or any heavy clothing was removed. Participants stood straight, looked forward, and still. The results were recorded, and this process was repeated for accuracy. Body mass index (BMI) was calculated as body weight (kg) divided by height squared (kg/m^2).

3.3.2 DXA

aBMD was determined at the lumbar spine (L1-L4), femoral neck and total hip, and body composition was measured using DXA (GE Lunar iDXA scanner) according to the manufacturer's guidelines. Each DXA scan involved lying on an open scanner for 1-7 minutes, depending on the scan, while two X-ray beams passed through the body aimed at the participants bones, fat mass, and lean mass. Participants were asked to remove any metal or jewelry to avoid confounding the results of the scan. Whole-body and regional fat and lean mass (kg), and aBMD at each site were determined using the GE Lunar encore software. Daily machine calibration

using periodic phantom scans, daily and weekly quality assurance tests and longitudinal stability were monitored. All data were converted to standardized aBMD values.

3.3.3 pQCT Imaging

Peripheral quantitative computed tomography (pQCT) imaging is used in research and clinical settings to assess bone's true density (in mg/cm^3), macroarchitecture in both cortical and trabecular compartments, and estimated strength. A trained bone densitometry technologist performed pQCT scans at the tibia using the XCT 3000 scanner (Stratec Medizintechnik, Pforzheim, Germany). pQCT acquisition parameters are 2.5 mm slice thickness, 0.5 x 0.5 mm in-plane pixel size and a tube voltage 60 kV operated at 0.3 mA. Images were analysed using the Stratecsoftware (Orthometrix Inc., White Plains, NY) to derive the following variables at the 4%, 38%, and 66% sites of the tibia (measured from the distal end of the medial malleolus to the proximal end of the medial tibia plateau): trabecular and cortical vBMD and area, and stress-strain index (SSI) (Lala et al. 2012; Lala et al. 2014). Trabecular vBMD and area were analyzed from the 4% site using the CALCBD analysis - contour mode 1 with a threshold of 180 mg/cm^3 and a trabecular area of 45%. Cortical vBMD and area, and cortical thickness were analyzed at the 38% and 66% sites using the CORTBD analysis – contour mode 1 and a threshold of 710 mg/cm^3. SSI was analyzed at the 66% site using a threshold of 280 mg/cm^3. Calf muscle CSA was analysed at the 66% site using a threshold of 280 mg/cm^3 with contour mode 1 (Wong et al. 2015; Scott et al. 2018). Segmentation of muscle from subcutaneous fat used a threshold of 40 mg/cm^3 with contour mode 3. To determine muscle CSA, bone area was subtracted from total bone area + muscle area.

3.3.4 Medical History, Physical Activity, Lifestyle Behaviour, and Body Image Questionnaires

The International Physical Activity Questionnaire (IPAQ) and the Bone-Specific Physical Activity Questionnaire (BPAQ) were administered by the research team using REDcap at study visit 1.

Demographic and Health History Questionnaire: Participants filled out a demographic and health history questionnaire which assessed sociodemographic characteristics, current medication and supplement use, menstrual health (in females only), weight change patterns, and history of disease, illness, and musculoskeletal injury.

International Physical Activity Questionnaire: The International Physical Activity Questionnaire (IPAQ) short-form version evaluated the self-reported time (number of sessions in past 7 days, duration per session) spent in moderate and vigorous physical activity and sedentary behaviour (sitting, lying down awake). Additional questions assessed the self-reported time spent participating in strength/resistance training (on own or as a part of a fitness class) and balance/flexibility exercise (e.g., yoga, pilates, Tai Chi). Data from each activity were summed to provide a total amount of time spent in the respective physical activities over the 7 days. Reliability and validity of the IPAQ have been previously reported (CRAIG et al. 2003).

Bone-Specific Physical Activity Questionnaire: The Bone-Specific Physical Activity Questionnaire (BPAQ) was used to assess previous participation in bone-specific physical activity. The BPAQ measured self-reported lifetime physical activity (types of physical activity, age, and years of participation) as well as types and frequency of physical activity participation in the past 12 months. BPAQ responses were analysed using algorithms (current and past BPAQ algorithms) and effective load ratings (assigned to common sports and activities from ground

reaction force measures of fundamental actions observed in each sport/activity) (Weeks & Beck, 2008).

3.3.5 Muscle Strength

To assess muscle strength, validated isometric hand grip and knee extensor dynamometer tests were performed. Grip strength has been shown to be an indicator of whole-body strength and a significant determinant of aBMD in healthy adults (Greenway et al. 2015). We followed the Southampton protocol and used a Jamar hydraulic hand dynamometer (Model J00105), a valid and reliable assessment tool (Sammons Preston, Patterson Medical, IL, USA) (Mathiowetz et al. 1985). Participants were asked to sit with their shoulder adducted and neutrally rotated, elbow flexed at 90°, forearm in a neutral position, and wrist between 0° and 15° ulnar deviation. When ready, the participant squeezed the device as hard as possible without moving the arms (at the end of a full expiration). The test was performed three times per hand. The highest recorded and average value to the nearest 0.1 (in kilograms) was reported for each hand, and across all trials.

To assess lower limb strength, a validated isometric knee extensor test was performed. Knee extensor strength was measured with a Biodex System 4 Pro dynamometer (Biodex medical instruments, Shirley, NY, USA). The test-retest reliability is well-established (Feiring et al., 1990). Participants were seated and strapped into the Biodex and asked to perform a maximal force knee extension and hold the contraction for 5 seconds. Knee angle was set at 90-degrees. All participants started by performing a few knee extensions to warm-up (about 5 extensions). Each participant performed 4 contractions separated by 1 minute of rest. The highest recorded and average peak force (in Newtons metres) to the nearest 0.1 was reported for each leg, and across all contractions.

3.3.6 Cardiopulmonary Exercise Test

To test maximal aerobic capacity and endurance, participants performed a cardiopulmonary exercise test. Maximal aerobic capacity (VO_{2max} in $mL \cdot kg^{-1} \cdot min^{-1}$) and time to exhaustion (minutes) was measured during a progressive treadmill test to volitional exhaustion using the modified Astrand protocol (Astrand, P., & Rodahl, K. (1977). This protocol involved the participant maintaining a constant speed of 8 km/hr (5 mph), and the incline was increased by 2.5% each stage. Gas exchange was monitored continuously using a breath-by-breath indirect calorimetry system (SensorMedics Vmax metabolic cart, VIASYS Healthcare, CA, USA) (Herdy et al. 2016). We considered VO_{2max} to be achieved if three of the following four criteria were obtained: (1) attainment of age-predicted maximal heart rate; (2) respiratory exchange ratio ≥1.1; (3) plateau in oxygen consumption despite an increase in exercise workload; and (4) attainment of a rating of perceived exercise score ≥7.

3.3.7 Accelerometer

Accelerometers are wearable technology devices that can objectively measure physical activity levels (Loucks & Thuma, 2003). Participants wore a commercially available accelerometer (GT3X+ monitors, ActiGraph, FL, USA) over the hip for 7 consecutive days during waking hours. Tri-axial accelerometer data was used to compute the number of minutes spent in four intensity levels of activity (sedentary, light, moderate, and vigorous) based on standard counts/minute-based cut-points (Freedson et al. 1998; Santos-Lozano et al. 2013). Data was analyzed in 60 second epochs. Non-wear time was excluded if ≥60 minutes of continuous zeros (TROIANO et al. 2008). Data were analyzed in participants who wear the accelerometer for at least 4 days and 10 hours/day. Participants recorded the duration, intensity (rating of perceived exertion), mode, and distance (if applicable) of all exercise performed over the 7-day

period. Accelerometers have been shown to demonstrate positive associations between bone health and MVPA (Loucks & Thuma, 2003).

3.4 Statistical Analyses

Data analyses were conducted using the SPSS software package (version 24, Armonk, NY, USA). Participant characteristics and outcomes were summarized using descriptive measures: mean (standard deviation) or median (minimum-maximum or interquartile range) for continuous variables and number (percentage) for categorical variables. Data were screened for statistical outliers and normality and the assumptions for each statistical test were examined. Independent T-Test and Chi-Square analyses were used to compare continuous and categorical outcomes between sexes. Pearson correlation coefficients were determined to assess the correlations between independent and dependent variables of interest. Spearman correlation coefficients were reported in instances of non-normality. Partial correlations were conducted to determine the influence of sex on the primary and secondary associations. Exploratory subgroup analyses were also performed to describe and compare outcomes in participants classified by sex. Anticipating a moderate-to-strong association (effect size=0.33) between pQCT measures of calf muscle CSA and trabecular vBMD and area at the tibia (power=0.80 and alpha=0.05), we determined that 50 adults (25 men and 25 women) at minimum are needed to detect a significant result. Since the present study was analysed in a preliminary dataset (n=18 of the target 50 participants), we acknowledge that the primary and secondary objectives are of a hypothesis-generating, exploratory nature.

Table 3.1 Main study outcomes and assessments.

Bone Outcomes	
Bone strength	Trabecular and cortical vBMD and area, and SSI at the tibia by pQCT
aBMD	aBMD at the total body, lumbar spine, and proximal femur by DXA
Fracture/BSI history	Self-reported history of fracture and BSI
Muscle Outcomes	
Muscle size	Calf muscle cross-sectional area by pQCT.
Muscle strength	Knee extensor muscle strength (Biodex), grip strength (Jamar dynamometer)
Physical Activity Outcomes	
MVPA	MVPA levels by accelerometer and IPAQ short-version
Bone-specific physical activity	Total, past, and current score from the BPAQ

Note: aBMD=areal bone mineral density; DXA=Dual-Energy X-ray Absorptiometry; IPAQ= International Bone-Specific Physical Activity Questionnaire.

CHAPTER 4. RESULTS

4.1 Descriptive Statistics

Our study included 18 participants, 67% (n=12) were male and participants had a mean (SD) age of 25.8 (4.1) years (see **Table 4.1)**. Nearly the entire sample (n=17; 94%) participated in long-distance running (distances over 3000 metres) as their primary endurance sport. Notably, three out of those 17 participants (17.6%) regularly competed in triathlons as well. The participants' mean (SD) BMI was 22.0 kg/m^2 (2.1) and their mean (SD) body fat was 18.7% (6.2). Mean (SD) lean mass was 56.9 kg (7.7) and 40.7 kg (6.3) in males and females, respectively.

Fifty-five percent (n=10) of the sample were White/Caucasian, 27.8% (n=5) were Asian, 16.7% (n=3) were Black/African-American or Hispanic/Latino, and 1 participant classified themselves as "other". Forty-four percent (n=8) of the participants had a full-time job, 38.9 % (n=7) were full-time students, 11.8% (n=2) were students and worked part-time and the remaining participant (n=1) was a part-time student. In the past 6 months, only 16.7% of participants (n=3) reported weight gain, with 2 participants reporting weight loss, and most of the sample (72.2%, n=13) reporting a stable weight.

Although all participants were endurance-trained athletes, only 33.3% (n=6) reported a history of a fracture (diagnosed by a physician) and only 11.1% (n=2) reported having a stress fracture in their lifetime. None of the participants reported previous smoking and 72.2% (n=13) of participants consumed 1-7 alcoholic beverages per week.

4.2 Muscle and Bone Parameter Comparisons

18 participants completed pQCT and DXA scans **(Table 4.2).** Male participants had a higher trabecular vBMD (p=0.027), trabecular area (p=0.016), cortical areas at 38% and 66%

sites (p=0.006 and 0.002, respectively), SSI at 38% and 66% sites (p=0.004 and p=0.003, respectively) and calf muscle CSA compared to the females (p=0.044). Lastly, male participants had a higher lean mass (p<0.001), lower body fat percentage (p<0.001), and a higher total body (p=0.020), femoral neck (p=0.017), and total hip aBMD (p=0.019) compared to the females.

4.3 Physical Performance and Physical Activity Comparisons

Physical performance and physical activity data are found in **Table 4.3.** Male participants had a higher knee extensor strength than the female participants (p=0.009). Handgrip strength was also significantly higher in males compared to females (p<0.001). No statistically significant between-group difference was observed for VO_{2max}, however, male participants reported a greater RPE (p=0.022) compared to females.

For the IPAQ, there were no between-group differences (p>0.05) in MVPA, sedentary time, or walking time. There were also no between-group differences (p>0.05) for any of the BPAQ scores, nor accelerometer outcomes.

4.4 Associations between Muscle, Physical Performance, Physical Activity, and Bone

Moderate-to-strong positive correlations were found between muscle CSA and trabecular area (r=0.583, p=0.014), cortical area at 38% and 66% sites (r=0.737, p<0.001 and r=0.720, p=0.001, respectively), SSI at 38% and 66% sites (r=0.680, p=0.003 and r=0.667, p=0.003, respectively), femoral neck aBMD (r=0.505, p=0.038), and total hip aBMD (r=0.516, p=0.034) **(Table 4.4)**. No significant associations were found between any of the muscle outcomes and each of cortical and trabecular vBMD. Moderate-to-strong positive associations were also found between knee extensor strength and cortical area at 38% and 66% sites (r=0.708, p=0.001 and r=0.698, p=0.002, respectively), SSI at 38% and 66% sites (r=0.668, p=0.003 and r=0.664, p=0.004, respectively), femoral neck aBMD (r=0.615, p=0.009), and total hip aBMD (r=0.570,

p=0.017). Strong positive correlations were found between handgrip strength and cortical area at the 66% site (r=0.723, p=0.001) and SSI at the 66% site (r=0.710, p<0.001). Handgrip strength was also positively associated with trabecular area (r=0.627, p=0.007), cortical area at the 38% site (r=0.624, p=0.007), femoral neck aBMD (r=0.524, p=0.031) and total hip aBMD (r=0.536, p=0.030).

VO_{2max} was moderately correlated with SSI at the 66% site (r=0.551, p=0.022). No correlations (p>0.05) were found between total and current BPAQ scores and any bone outcomes. However, a modest correlation was found between past BPAQ score and cortical vBMD at the 66% site (r=0.600, p=0.011). There were no statistically significant associations between any of the accelerometer outcomes and bone variables (p>0.05).

When adjusted for sex, muscle CSA remained significantly correlated with cortical area at the 38% and 66% sites (r=0.63, p=0.009 and r=0.602, p=0.014, respectively), and SSI at the 66% site (r=0.519, p=0.039). Knee extensor strength also remained significantly correlated with cortical area at the 38% site (r=0.523, p=0.037) but not at the 66% site (p>0.05). When adjusted for sex, moderate correlations were found between total and past BPAQ scores and trabecular area (r=0.500, p=0.049 and r=0.555, p=0.026, respectively). Past BPAQ score was no longer associated with cortical vBMD, however strong inverse correlations were found between current BPAQ score and femoral neck aBMD (r= -0.724, p=0.002) and total hip aBMD (r= -0.747, r<0.001).

Table 4.1. Descriptive characteristics in study participants.

	All (N=18)	Male (n=12)	Female (n=6)	p-value
Age (years) – Mean (SD)	25.83±4.12	24.67±3.60	28.17±4.40	0.089
Height (cm) – Mean (SD)	172.14±9.27	177.18±9.27	162.88±7.75	**<0.001**
Weight (kg) – Mean (SD)	65.58±10.17	70.21±7.57	57.10±9.10	**0.006**
BMI (kg/m^2) – Mean (SD)	22.03±2.12	22.38±2.30	21.39±1.75	0.378
Lean mass (kg) – Mean (SD)	51.18±10.64	56.91±7.67	40.68±6.32	**<0.001**
Percent body fat (%) – Mean (SD)	18.69±6.15	14.93±3.82	25.60±1.87	**<0.001**
Fat mass (kg) - Mean (SD)	11.27±3.20	9.76±2.40	14.05±2.64	**0.004**
Race/ethnicity – N (%)				0.881
White/Caucasian	10 (55%)	7 (58.3%)	3 (50%)	
Black/African-American	1 (5.6%)	1 (8.3%)		
Asian	5 (27.8%)	3 (25%)	2 (33.3%)	
Hispanic/Latino	1 (5.6%)		1 (16.7%)	
Other	1 (5.6%)	1 (8.3%)		
Employment status				0.666
Full-time student	7 (38.9%)	5 (41.7%)	2 (33.3%)	
Part-time student	1 (5.6%)	1 (8.3%)		
Full-time job	8 (44.4%)	4 (33.3%)	4 (66.7%)	
Other	2 (11.1%)	2 (16.7%)		
Weight change in last 6 Months – N (%)				**0.025**
Gained	3 (16.7%)	3 (25%)		
Loss	2 (11.1%)	2 (16.7%)		
Stable	13 (72.2%)	7 (58.3%)	6 (100%)	
Fracture history – N (%)	6 (33.3%)	3 (25%)	3 (50%)	0.38
Stress fracture history – N (%)	2 (11.1%)	1 (8.3%)	1 (16.7%)	0.621

Sport/training type – N (%)				
Middle-distance running	2 (11.1%)	2 (16.7%)	0 (0%)	0.166
Long-distance running	17 (94.4%)	11 (91.7%)	6 (100%)	0.496
Triathlon	3 (16.7%)	1 (8.3%)	2 (33.3%)	0.309
Swimming	5 (27.8%)	3 (25%)	2 (33.3%)	0.729
Cycling	5 (27.8%)	3 (25%)	2 (33.3%)	0.745
History of smoking – N (%)	0 (0%)			
Alcohol use (servings per week) – N (%)				0.081
1-7 drinks/week	13 (72.2%)	7 (58.3%)	6 (100%)	
8-14 drinks/week	3 (16.7%)	3 (25%)		

Note: BMI=Body Mass Index; kg=kilogram

Table 4.2 Peripheral quantitative computed tomography (pQCT) measures of bone and muscle parameters in endurance-trained individuals.

	Males (n=12)	Females (n=6)	p-value
pQCT			
Trabecular vBMD (mg/cm^3) (4% site)	297.68±42.05	253.38±16.74	**0.027**
Trabecular area (mm^2) (4% site)	512.94±87.38	403.28±62.15	**0.016**
Cortical vBMD (mg/cm^3)			
38% site	1158.84±12.99	1165.62±32.01	0.637
66% site	1100.96±22.53	1117.22±36.74	0.272
Cortical area (mm^2)			
38% site	362.62±48.39	282.35±52.64	**0.006**
66% site	366.47±53.53	269.10±43.25	**0.002**
Stress strain index (mm^3)			
38% site	2200.66±390.29	1519.43±410.28	**0.004**
66% site	3509.10±603.66	2306.18±744.76	**0.003**
Total calf muscle CSA (mm^2) (66% site)	7654.96±1349.33	6282.75±951.17	**0.044**
DXA			
Total body aBMD (mg/cm^2)	1.35±0.13	1.19±1.11	**0.020**
Lumbar spine aBMD (mg/cm^2)	1.26±0.13	1.17±0.05	0.208
Femoral neck aBMD (mg/cm^2)	1.19±0.19	0.88±0.30	**0.017**
Total hip aBMD (mg/cm^2)	1.21±0.19	0.90±0.30	**0.019**

Note: pQCT= peripheral quantitative computed tomography; vBMD= volumetric bone mineral density; CSA= cross-sectional area; DXA= Dual X-ray Absorptiometry; aBMD=areal bone mineral density; mg=milligram; cm=centimeter; mm=millimeter

Table 4.3 Physical performance and physical activity outcomes in endurance-trained individuals.

	Males (n=12)	Females (n=6)	p-value
Muscle strength			
Peak knee extensor strength (N*m)	295.45±76.75	189.62±53.45	**0.009**
Maximal grip strength (Kg)	47.13±6.17	33.73±5.99	**<0.001**
Aerobic capacity			
VO_{2Max} ($ml*min^{-1*}Kg^{-1}$)	57.39±9.49	48.75±5.83	0.062
IPAQ			
MVPA (minutes/week)	426.25±295.61	245.83±135.44	0.095
Sedentary Time (hours/day)	7.09±3.08	7.92±2.42	0.580
Walking (minutes/week)	400.45±302.87	172.50±131.29	0.103
BPAQ			
Total BPAQ score	20.84±16.29	20.89±12.00	0.994
Current BPAQ score	4.44±2.73	16.08±30.71	0.396
Past BPAQ score	37.24±32.55	25.71±17.78	0.361
Accelerometer			
MVPA (minutes/week)	574.60±192.46	605.40±206.84	0.690
Light PA (minutes/week)	4158.8±1686.18	4512.60±1334.26	0.780
Sedentary Time (hours/day)	5.46±2.36	6.60±1.34	0.255

Note: MVPA=moderate to vigorous physical activity; IPAQ= International Physical Activity Questionnaire; BPAQ=Bone Specific Physical Activity; PA=physical activity; Kg=kilogram; N*m=Newton Meter; ml=millilitre; min=minute

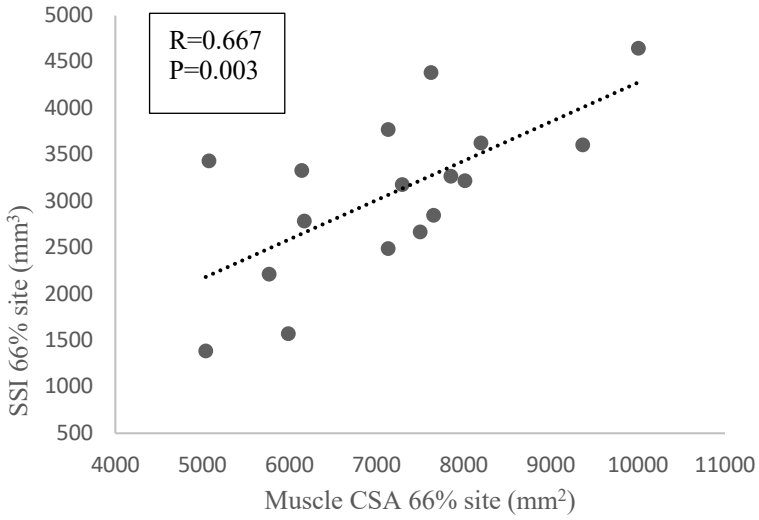
Table 4.4 Correlations between muscle function, physical performance, physical activity, and bone outcomes in endurance-trained individuals.

	Trabecular vBMD (mg/cm^3) (4% site)		Cortical vBMD (mg/cm^3) (66% site)		Stress strain index (mm^3) (66% site)		Femoral neck aBMD (mg/cm^2)		Total Hip aBMD (mg/cm^2)		Cortical area (cm^2) (38%)		Cortical area (cm^2) (66% site)		Trabecular area (cm^2) (4% site)	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Total calf muscle CSA (mm^2)	0.118	0.653	-0.206	0.428	**0.667***	0.003	**0.505**	0.038	**0.516**	0.034	**0.737***	<0.001	**0.720***	0.001	**0.583**	0.014
Knee Extensor Strength (Kg)	0.463	0.061	-0.094	0.72	**0.664**	0.004	**0.615**	0.009	**0.570**	0.017	**0.708***	0.001	**0.698**	0.002	0.445	0.073
Handgrip Strength (Kg)	0.389	0.123	-0.364	0.151	**0.71**	<0.001	**0.524**	0.031	**0.526**	0.030	**0.624**	0.007	**0.723**	0.001	**0.627**	0.007
VO_2 Max ($ml*kg^{-1}*min^{-1}$)	0.447	0.072	-0.462	0.062	**0.551**	0.022	0.210	0.420	0.217	0.403	**0.423**	0.091	0.467	0.058	0.2707	0.294
Accelerometer MVPA (minutes/week)	-0.173	0.537	0.403	0.136	0.099	0.726	0.168	0.551	0.114	0.685	0.204	0.465	0.059	0.834	0.255	0.359
Total BPAQ Score	0.12[a]	0.646	0.333[a]	0.191	0.167[a]	0.523	**-0.245[a]***	0.343	-0.186[a]	0.474	0.039[a]	0.881	0.032[a]	0.903	**0.252[a]***	0.328
Current BPAQ Score	0.229[a]	0.376	-0.229[a]	0.376	0.143[a]	0.583	**-0.17*[a]**	0.513	**-0.179[a]***	0.492	0.086[a]	0.743	0.021[a]	0.937	0.0.85[a]	0.402
Past BPAQ Score	0.02[a]	0.94	0.600[a]	0.011	0.066[a]	0.801	0.039[a]	0.881	0.098[a]	0.708	0.211[a]	0.417	0.127[a]	0.626	0.402*[a]	0110

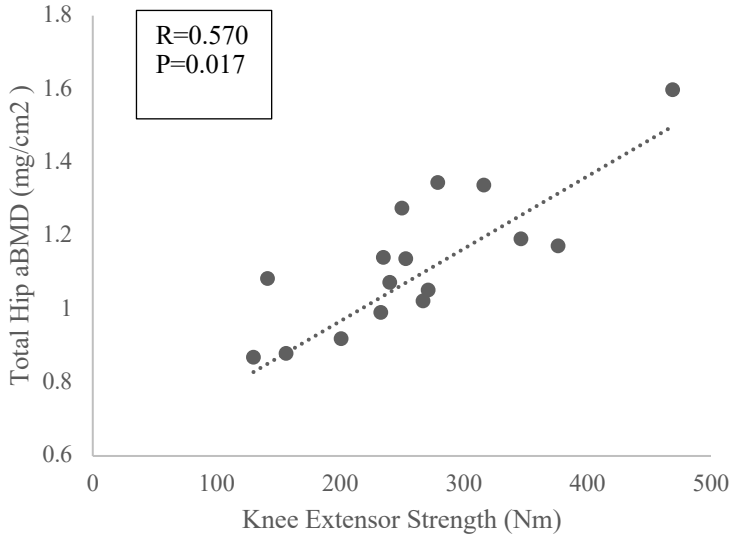
Note: CSA= cross-sectional area; MVPA=moderate to vigorous physical activity; BPAQ=Bone Specific Physical Activity Questionnaire; vBMD=volumetric bone mineral density; aBMD=areal bone mineral density; mm^2=millimeters squared; cm^2=centimeters squared; mg=milligram

r=Pearson/spearman correlation
a= Spearman
*= significant when adjusted for sex

A



B



C

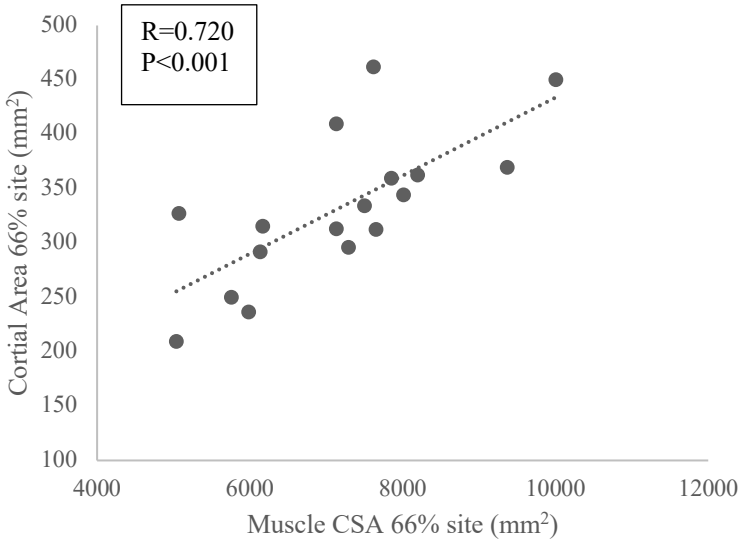


Figure 4.1. Scatterplots demonstrating the associations between A) Muscle CSA mm^2 and SSI 66% (mm^2) B) Knee extensor strength (Nm) and total hip aBMD (mg/cm^2) C) Muscle CSA 66% (mm^2) and cortical area 66% (mm^2) in endurance trained individuals

CHAPTER 5. DISCUSSION

5.1 Summary of Findings

The present cross-sectional study evaluated the relationships between muscle size and strength, physical activity levels (MVPA and bone-specific physical activity), and bone strength in healthy, endurance-trained males and females aged 18-35 years. Our results demonstrate that muscle CSA and isometric knee extensor strength were moderate-to-strong correlates of bone strength in both male and female endurance athletes, and thus muscle size and strength may represent modifiable targets for intervention for bone loss and BSIs in this at-risk population. We also found that lower-extremity muscle strength (a surrogate indicator of muscle force) may confer a positive influence on tibial bone strength (particularly SSI and cortical bone variables). Since bone adapts its strength to the peak voluntary muscle loads, the efficacy of high-magnitude, low-repetition resistance training targeting the muscles and bones of the lower-extremity in endurance athletes warrants investigation (Warden et al. 2014). Furthermore, MVPA and bone-specific physical activity (surrogate indicators of external reaction forces) may not be as strongly associated with bone outcomes as muscle factors (surrogate indicators of internal reaction forces) in endurance athletes. More sensitive measures of loading history (including frequency and intensity) are needed to better understand the influence of impact-based physical activity within the context of endurance training. Lastly, after adjusting for sex, several muscle-bone associations remained significant, suggesting that the contribution of sex to the muscle-bone relationship may not necessarily differ between males and females. These results mostly support our hypothesis that favorable patterns in muscle (not necessarily physical activity) variables are associated with better bone strength variables. However, we are unable to

make any conclusive inferences based on these preliminary findings until we perform our definitive analysis in the larger sample of participants.

5.2 Associations between Calf Muscle Size and Tibial vBMD and Bone Area

Our results suggest that muscle CSA may represent an important determinant of bone strength and morphology in endurance athletes, particularly SSI and cortical area in the tibial shaft. As expected, moderate-to-strong correlations were found between calf muscle CSA and pQCT bone outcomes, including trabecular and cortical area and SSI. Our findings are consistent with previous cross-sectional and prospective evidence of a muscle-bone relationship in young, healthy individuals and athletes (mostly, runners) (Beck et al. 2000; Bennell et al. 1996; Hoffman et al. 1999; Schoenau et al, 2007). However, these studies mostly focused their investigations on associations between muscle and surrogate measures of bone strength, specifically DXA-measured aBMD, rather that pQCT measures of vBMD and area and SSI which are more reflective of BSI/fracture risk. Although there were no significant correlations between muscle CSA and vBMD, strong associations were found with bone area outcomes, specifically trabecular area, femoral neck aBMD, total hip aBMD, and cortical area at the 38% and 66% sites. These results are consistent with other studies of the functional muscle-bone relationship in young adults (De Avilla et al. 2019; Greene et al. 2012), indicating that higher muscle size tends to associate with stronger bones, specifically cortical bone size (a strong indicator of the bone's resistance to BSI/fracture). Larger muscle CSA may also be associated with a greater ability to generate compressive forces, possibly reducing the excessive stresses and strains acting on the tibia during repetitive loading. Molecular crosstalk also mediates muscle and bone through secretory factors known as myokines (Hart et al. 2017), further suggesting a muscle-bone relationship independent of the mechanical link between these tissues.

In a previous study using pQCT to examine the relationship between muscle and bone in endurance athletes, Popp et al. (2009) compared bone geometry, vBMD, and muscle size in female competitive runners aged 18-35 years with and without a history of stress fractures. They found that the stress fracture group had significantly smaller cortical area and muscle CSA compared to the non-fractured group. Although our sample did not present with a history of BSI, muscle may play a protective role in the etiology of BSIs by dissipating energy or reducing bending moments during repetitive loading at weight-bearing sites like the tibia (Burr, 2000 ;2003). Similarly, Beck et al. (2000) explored how fracture susceptibility varies among military recruits aged 17-32 years stratified by sex. Consistent with our findings, Beck et al. (2000) suggested that those with a stress fracture (irrespective of sex) tend to have smaller thigh muscle CSA compared to those without a stress fracture. As mentioned earlier, larger cortical area is crucial to maintaining bone strength, and athletes that participate in high-volume endurance training often experience microcracks to the dense, outer layer of the bone. As cortical bone becomes thinner and more porous, the number of microcracks in bone will increase, contributing to an increase in fracture risk (Fonesca et al. 2014; McCalden et al. 1993). More research is needed to determine adjunctive training approaches to minimize the risk of these musculoskeletal declines in athletes participating in endurance sport.

5.3 Associations between Calf Muscle Size and Tibial Bone Strength

In our study, calf muscle CSA was also significantly correlated with SSI at both the 38% and 66% sites and remained significant after adjusting for sex. SSI is a measure of bone's ability to withstand deformation, specifically the act of bones bending and torsional strength, and a higher SSI value is indicative of higher bone strength. Notably, the risk for stress fracture at weight-bearing sites is higher in athletes with lower SSI (Popp et al. 2009) because of the bone's

lower fatigue resistance and failure load during repetitive loading. Adequate bone strength, density, and size is crucial for overall bone health, yet bone strength is more reflective of BSI/fracture risk than bone density and area. However, the density of the bone may be more sensitive to hormonal/nutritional deficits often demonstrated in endurance athletes (especially, females) as the trabecular bone has a larger surface area for mineral exchange and is more metabolically active than cortical bone. Further, fat infiltration within muscle and bone may be a better determinant of vBMD as less dense muscle is unable to generate as much contractile force to confer beneficial effects to bone and higher bone marrow adiposity has negative implications for bone formation in athletic populations (Rantalainen et al. 2003)Most research on the interaction between muscle, fat, and bone has focused on vulnerable populations such as older adults and those with disuse-related bone loss (i.e., spinal cord injury) and less is known in athletes. In a cross-sectional study of 179 young female athletes participating in impact and non-impact loading sports, tibial bone marrow density was associated with loading history and was an independent predictor of tibial bone strength (Rantalainen et al. 2013). These findings suggest that an exercise-induced increase in bone strength may be influenced by fat infiltration (or lower bone marrow density), and thus bone-specific loading is crucial to maintaining optimal bone strength at the weight-bearing tibia.

As predicted, moderate-to-strong positive associations were found between performance-based muscle measures (handgrip and knee extensor strength) and pQCT outcomes, particularly with cortical area and SSI, both at the 38% and 66% sites. Schnackenburg et al. (2011) investigated bone quality, including bone strength and muscle strength, in female athletes aged 18-45 years with a diagnosed stress fracture compared to those without a stress fracture. They found that female athletes with a stress fracture tend to have lower trabecular vBMD, cortical

area, and knee extensor strength compared to the non-stress fracture group. Similarly, Greenway et al. (2015) reported that both concentric and eccentric max strength were significantly correlated with aBMD at the total hip in 152 adult premenopausal women. Taken together, these findings indicate that muscle strength is a key contributor for overall bone fragility/bone strength in both healthy and clinical populations.

Due to the low magnitude, high-volume training that endurance athletes participate in, they are more susceptible to microdamage accumulation and subsequently, BSIs of varying severity. Previous studies have demonstrated that endurance athletes, specifically runners, are at increased risk for BSIs as they tend to have lower aBMD and vBMD and muscle CSA compared to athletes engaged in other sports (Bennell et al. 1999; Popp et al. 2009; Schnackenburg et al. 2011). Most participants in our sample engaged in more than 5 hours/week and more than 40 km/week of long-distance running, however 89% (n=16) of the participants also engaged in resistance training (muscle-generated loading), which has been shown to have protective effects on bone health . High-impact and odd-impact loading are the most beneficial exercise to bone (Nikander et al. 2006) and are predictors of total bone area, trabecular vBMD, and SSI (Nikander et al. 2006). While our study only focused on endurance athletes, we also found that a smaller calf muscle CSA was associated with lower SSI and cortical and trabecular area. These findings further implicate the relevance of muscle-generated loading and its influence on bone strength. Future prospective research in larger samples is needed to test the efficacy of resistance training approaches should be used to minimize injury and prevent bone loss in endurance athletes.

5.4 Associations between Physical Activity and Tibial Bone Strength

Bone-specific physical activity is assessed by the BPAQ to measure previous participation over the span of one's lifetime and has been shown to have positive associations

with indices of bone strength (Weeks and Beck, 2008). BPAQ responses are analyzed using algorithms (current and past) and effective load ratings (Weeks and Beck, 2008). Long-distance runners tend to have lower vBMD and aBMD at lower limb sites than other athletic groups, particularly those with inadequate nutrition/chronic energy deficiency, and therefore are more susceptible to fracture (Popp et al. 2009). However, a substantial number of athletes in our study previously participated in high-impact and odd-impact loading sports during childhood and adolescence, which likely contributed positively to their achievement of peak bone mass. Specifically, exercises that impose high-magnitude, dynamic forces and high-frequency loading rates are the most osteogenic (Turner et al. 1995). Accordingly, we also found that past BPAQ score was associated with cortical vBMD in the tibial shaft. Consistently, several studies have demonstrated that BPAQ scores are predictive of indices of bone strength (Weeks and Beck, 2008). Sojung et al. (2016) and Jun Sung et al. (2019) found positive correlations between BPAQ scores and total hip and femoral neck aBMD in healthy women aged 18-26 years. These findings further support the importance of odd-impact (racquet sports) and high-impact loading (jumping, gymnastics) during early phases of life while maintaining BMD during adulthood and minimizing bone loss with aging, as peak bone mass is usually attained around 30 years of age.

However, when adjusted for sex, the association between past BPAQ score and cortical vBMD did not remain significant, but inverse correlations were found between current BPAQ score and femoral neck and total hip aBMD. This finding is unusual considering the significant amount of evidence showing an association between BPAQ and bone strength outcomes. This may be due to our small sample size (particularly the limited number of females), and lower statistical power to make conclusive inferences about the influence of physical activity on bone strength parameters.

Accelerometers are an objective way to assess physical activity, however our findings did not show any correlations between MVPA and bone outcomes. Ng et al. (2020) determined associations of current accelerometer-derived impact physical activity, and self-reported current, past, and total BPAQ scores with bone parameters. Ng et al. (2020) found that accelerometer-measured MVPA time was significantly and moderately positively associated with bone strength outcomes in 50 post-menopausal women with a mean age of 64. However, after adjusting for confounders, there were no relationships between sedentary, light, and MVPA time and HR-pQCT outcomes. Rowlands et al. (2020) used accelerometers to investigate the importance of volume and intensity of physical activity accumulated during late adolescence and young adulthood for bone health at age 23. Rowlands et al. (2020) found that both average acceleration and the intensity gradient were positively associated with all bone outcomes, except for spine aBMD. Interestingly, Ziebart et al. (2017) examined how system characteristics differ between accelerometers in 12 young male and female athletes. Their study concluded that measures of peak impact load during physical activities can differ across accelerometer systems. Further research should investigate the accuracy and reliability of the use of accelerometers as a validated assessment of physical activity and impact loading values. The IPAQ showed no statistically significant group differences between MVPA, walking time, and sedentary time. However due to its self-report nature, the IPAQ may not accurately capture the influence of physical activity on bone strength indices.

5.5 Sex Comparisons between Male and Female Endurance Athletes

Limited research has been conducted examining the sex-specific differences in muscle-bone associations in endurance athletes. Most research to date has been in female athletes, however we expected that the muscle-bone relationship would be influenced by the inherent

biological and hormonal differences between sexes. Female athletes that participate in weight-bearing endurance sports with low EA tend to have lower vBMD and aBMD, total and trabecular area, and estimated bone strength, each contributing to the risk of BSIs (Hutson et al. 2020). Although males are also affected by low EA, findings from Papargeorgiou et al. (2017) suggest that females are more sensitive to energy deficiency-related uncoupling in bone turnover, specifically lower bone formation markers (i.e., pro-peptide of type 1 collagen) relative to higher bone resorption markers (i.e., carboxy-terminal collagen crosslinks), than their male counterparts. As expected, in our preliminary analysis, male participants had higher pQCT muscle and bone outcomes, DXA, and muscle strength than females**.**

Our findings remained statistically significant even when adjusted for sex, including the associations between muscle CSA and SSI at 38% and 66% sites and cortical area at 38% and 66% sites, and knee extensor strength and SSI and cortical area at the 38% site, suggesting that the muscle-bone relationship may exist in endurance athletes regardless of sex. However, females are typically at a higher risk of stress fracture compared to males (Beck et al. 2000) due to the well-known interrelationships between estrogen deficiency/low EA, menstrual disturbances, and low aBMD, otherwise known as the Female Athlete Triad. In a cross-sectional study by Gibbs et al. (2013) in 437 young exercising women, late menarche and low BMI (surrogate indicators of a chronic energy deficiency) were associated with the highest percentage of low aBMD (Z-score score less than -1). Additionally, a cumulative number of Female Athlete Triad risk factors were associated with a higher prevalence of low aBMD, suggesting a dose-response between the number of Triad factors and BMD in exercising women, specifically those participating in leanness-focused sports (i.e., long-distance running, triathlon). These results were further supported from a study by Barrack et al. (2014), wherein the risk of BSIs was

higher when multiple Triad factors were present compared to a single Triad factor in 259 adolescent and adult female athletes. However, neither of these studies objectively evaluated the contribution of muscle-generated and impact loading to bone outcomes. Therefore, our results contribute new evidence on the muscle-bone relationship in endurance athletes.

5.6 Strengths and Limitations

There were many strengths to this cross-sectional study. Firstly, this exploratory study used advanced imaging technology to determine bone and muscle outcomes (DXA and pQCT), and both objective and self-report measures of physical activity to explore their associations with bone strength. Further, we collected data in the early follicular phase of the menstrual cycle for female participants to minimize the confounding effects of sex hormone levels. Most research on this topic focuses solely on female athletes, thus comparing sex differences is particularly novel as there is limited data on determinants of low BMD and BSIs in male athletes. Despite the study strengths, there are several limitations worth mentioning. The sample size for this cross-sectional study is quite small (18 participants) as it is a preliminary analysis for the present Master's thesis. As a result, we are unable to make any conclusive inferences based on these significant associations. While the capacity to compare sexes is a strength to the study, the specific timing of the study visits in the early follicular phase for female participants presented with several logistical challenges, including scheduling difficulties, and an exclusion of females with menstrual disturbances. Consequently, we have only recruited 6 female endurance athletes herein, limiting our ability to make robust statistical comparisons between males and females. Another limitation to the study is the reporting/recall bias inherent to the BPAQ and IPAQ, and the likelihood of an overestimation of physical activity. Lastly, five participants did not wear

their accelerometers for the entire 7-day period, requiring an adjustment to standardize the timeframe across participants.

5.7 Conclusions

In conclusion, our results demonstrate that muscle size and strength were strong correlates of bone strength in both male and female endurance athletes, suggesting muscle outcomes may represent modifiable targets for intervention for low BMD and BSIs in this athletic group. Also, MVPA and bone-specific physical activity were not as strongly associated with bone outcomes in this group as muscle variables, however a larger sample size is needed to make any conclusive inferences. Our findings revealed that after adjustment, most associations with bone parameters remained significant, including muscle CSA and SSI at the 66% site, and knee extensor strength and SSI at the 38% site. This research provides new knowledge regarding muscle- and activity-related determinants of low BMD and BSI risk in endurance-trained individuals, which will inform bone-targeted adjunctive training programs (i.e., resistance training, high-impact loading) to strengthen both the muscle and bone at the lower-extremity.

Notably, we will re-run these analyses in the target sample of the larger project (a minimum of 50 participants) and confirm the strong associations between muscle and bone outcomes mentioned herein. The larger study will more conclusively establish which muscle and physical activity related factors are associated with bone as well as the sex-specific differences in these associations in our sample of endurance athletes. Although the sample size is small for this thesis, the results appear to be promising and represent preliminary evidence underpinning our understanding of the muscle-bone relationship in endurance-trained individuals and will serve as pilot data for a larger interventional study to test the efficacy of high-intensity resistance training and high-impact loading on muscle and bone strength in this population.

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Formatting Checklist (See thesis format guidelines further explained under Preparation of a Thesis)		
☐	Font, spacing, and margins	The thesis is presented with uniform font size, line spacing, and margin sizes. A conventional font, size 12-point, 12 characters per inch is used. Line spacing is double or 1.5. Left- and right-hand margins are 1 inch.
☐	Footnotes, references, and appendices	Footnotes, references and appendices conform to the scholarly style appropriate to your discipline. Formatting is consistent throughout the thesis.
☐	Figures, illustrations, photographs, and images	If figures, illustrations, photographs, and digital images are used, they are positioned according to the publication conventions of your discipline. They are smaller than the standard page.

Section 2: Traditional thesis

All theses must include the components listed below. Please **check each box** (left hand side) to confirm your thesis contains the required sections.

Thesis Components (Each required section is further explained here)		**Important considerations**
☑	Title page	Consult https://www.mcgill.ca/gps/thesis/thesis-guidelines/preparation for the appropriate format
☑	Detailed table of contents	
☑	Brief abstract in English	If the language of the thesis is neither English nor French (only allowed for specific language Units) then a third abstract in the language of the thesis is required.
☑	Brief abstract in in French	
☑	Acknowledgements	Consult https://www.mcgill.ca/gps/thesis/thesis-guidelines/preparation for guidance
☐	**PhD theses only:** Contribution to original knowledge	A doctoral thesis must clearly state the elements of the thesis that are considered original scholarship and distinct contributions to knowledge.
☑	Contribution of Authors	• Contributions of the student to each chapter must be explicitly stated. • Contributions of any co-authors to each chapter must be explicitly stated.
☑	List of Figures and Tables	
☑	List of Abbreviations	
☑	Introduction	Clearly state the rationale and objectives of the research.
☑	Comprehensive review of the relevant literature	The literature review must be at least 20 pages and pertain to the entirety of the thesis
☑	Body of the thesis	The body of the thesis should encompass sections on methodology and research findings
☑	Comprehensive scholarly discussion of all the findings	The Discussion must be at least **10 pages** and pertain to the entirety of the thesis.
☑	Final conclusion and summary	Clearly state how the objectives of the research were met and discuss implications of findings.
☑	Reference list	Thorough master bibliography or reference list which includes all the references cited throughout the thesis
☑	Copyright	• Copyright approval has been obtained from the publisher for using figures, tables, and other diagrams from the literature • If you have adapted any figures from the literature, you must specify the figure (or table etc.) has been adapted from [source]

Formatting Checklist (See thesis format guidelines further explained under Preparation of a Thesis)		
☑	Font, spacing, and margins	The thesis is presented with uniform font size, line spacing, and margin sizes. A conventional font, size 12-point, 12 characters per inch is used. Line spacing is double or 1.5. Left- and right-hand margins are 1 inch.
☑	Footnotes, references, and appendices	Footnotes, references and appendices conform to the scholarly style appropriate to your discipline. Formatting is consistent throughout the thesis.
☑	Figures, illustrations, photographs, and images	If figures, illustrations, photographs, and digital images are used, they are positioned according to the publication conventions of your discipline. They are smaller than the standard page.