ASSESSING THE RELATIONSHIP AMONG PHYSICAL ACTIVITY, MUSCLE FORCE

CAPACITY, AND CORTICAL DIAPHYSEAL BONE STATUS: THE MUSCLE-BONE UNIT IN

YOUNG ADULTS.

by

SIMON HIGGINS

(Under the Direction of Ellen M. Evans)

ABSTRACT

Muscular forces associated with physical activity (PA) are the largest applied to the skeleton.

However, their relationship with bone status is unclear as proxy measures such as muscle cross sectional

area (MCSA) are typically assessed rather than direct measures of force. These proxy measures poorly

characterize the effect of muscle force and require participants to undergo costly and radiative

methodologies. There is a need to define the relationship between muscle force and bone status, and to

identify non-invasive, field-based measures of muscle force to be used for osteoporosis assessment and

research. Emerging research has used estimated power from vertical jump as a predictor of bone status;

however, this was not done in the context of PA, and did not assess sex-differences. Thus, this study

aimed to: 1) examine whether muscle force mediates the relationship between PA and bone status at the

mid-tibia in young adults (n=144, 18-20 yo), and whether this relationship is moderated by sex, and 2)

determine the utility of several lab-based and field-based measures of muscle force as predictors of bone

status compared to a common muscle force proxy. Bone status and MCSA were assessed via peripheral

quantitative computed tomography at the mid-tibia. Muscle force was estimated using dynamometry, leg

extension power, and vertical jump. PA was measured over ≥7-days via a waist-worn accelerometer.

Moderated mediation analyses revealed that sex moderates the relationship between PA and bone status

(Cortical Thickness; Coeff.(SE)=-.0088±.0039, LLCI -.0166, ULCI -.0010), with a positive relationship

existing in females (Cortical Thickness; Coeff.(SE)=.0088±.0027, LLCI .0034, ULCI .0142) but not

males. However, ankle dorsiflexor force did not mediate the relationship between PA and bone status (all $p>.05$). In further analyses knee extension peak torque and peak anaerobic power estimated from vertical jump emerged as the strongest predictors of bone status, independent of MCSA, with standardized effects ranging from $\beta=-.38$ to $.57$ (all $p<.05$). Measures of muscle force vary greatly in their utility as predictors of bone status, as such, future research should assess other methodologies such as knee extension torque and the field-based peak power estimate from a vertical jump as predictors of skeletal health outcomes.

INDEX WORDS: Cortical bone, Muscle specific force, Biodex, Isokinetic, Vertec, Vertical jump, Nottingham leg extensor power rig

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CHAPTER 1

INTRODUCTION

1.1 Significance

The incidence of fracture resulting from osteoporosis is predicted to exceed 3 million/year in the next decade, with the economic burden of medical costs rising to $25.3 billion (2). Historically viewed as an affliction of advancing age, it is now well established that osteoporosis prevention begins during youth via the adoption of health behaviors such as physical activity (PA), which contributes to the attainment of 20-40% of adult peak bone mass (22). Indeed, peak bone mass is a salient factor in the development of osteoporosis, with a 10% increase potentially delaying disease onset by 13 years (11). Interventions that promote PA have shown consistent positive results, with 6 months of weight-bearing PA promoting up to 6% greater gains in bone mass than controls, depending on the site, and participant age (22). Moreover, substantial bone structural differences among individuals with the highest vs. lowest habitual PA levels have been reported in numerous longitudinal studies (4, 13, 14). PA imparts most of its beneficial effect on bone status through the mechanical forces applied by skeletal muscles pulling on bony levers (3, 9). These forces are among the largest applied to the skeleton (17), yet despite the importance of muscular force on the PA-bone status relationship, few studies include this factor within their analyses (20). When muscle force is considered, it is often in the form of proxy measures such as muscle cross sectional area or mass, (8, 10, 18, 21) which only partially characterizes the force producing capacity of muscle, as evidenced by reports of direct effects of muscle power on bone strength, independent of cross sectional area (15). In further support of this partial characterization of the force producing capacity of muscle by proxy measures are findings that girls with lower muscle quality (high intramuscular fat content) had impaired bone mass gains over two years, suggesting that it is not simply muscle mass that accounts for

variance in bone status (6). Furthermore, factors such as fiber type and pennation angle, motor unit activation, and tendon length also contribute to a muscle's force producing capacity.

A prime example of the poor characterization of muscle force by measures of muscle mass, with regard to the influence on bone status, are the findings of lower muscle specific force (force output per unit of cross sectional area) in females compared to males (23). In this cohort, females had 15.7% lower bone strength than males (16). Whether a part of this observed bone strength difference is a result of lower mechanical forces on the female vs. male skeleton is not well defined, as much emphasis has been placed on the sex-specific hormonal environments (Estrogen, Testosterone, Growth Hormone, IGF-1, etc.) and their interaction with weight-bearing exercise (5, 7). Studies examining the muscle-bone relationship between sexes have shown that women have a larger bone area for a given muscle size in habitually loaded sites (12, 19). Implicated in this difference are both hormonal and mechanical characteristics such as a higher relative adiposity in women, leading to greater demand on muscles in everyday activities in order to carry the relative excess load. In light of the complicated landscape of factors affecting bone strength, there is a need to characterize the specific contribution of muscle specific force to the beneficial effects of PA on bone status, and to examine whether this differs in men compared to women at habitually loaded sites. Identifying the independent role of muscle force on bone strength will aid public health efforts in the prevention of osteoporosis by highlighting the potential of exercise interventions that increase muscle force capacity along with its regular application to the skeleton, such as combined resistance training and increased PA.

In addition, there is a need to identify non-invasive, feasible measures of muscle force that might be used for in both clinical and research settings in the place of often costly or radiative methods of assessing muscle mass (15). Emerging research has used muscle power estimated from vertical jump height as a predictor of bone status (1, 15); however, studies have not examined the predictive ability of muscle power in the context of PA, and more research is needed examining different ages, races, and

sexes. As such, the overarching aim of this project is to examine whether muscle specific force differentially mediates the relationship between PA and bone status at the mid-tibia in young adult males compared to females. Due to the reported differences in muscle specific force between men and women, the potential for sex to moderate the relationship between PA, muscle force, and bone status will also be explored. In this context, the specific aims are as follows:

1.2   Specific Aims

Specific Aim 1: To determine whether the relationship between objectively measured moderate-to-vigorous physical activity and cortical bone status, as measured by peripheral quantitative computed tomography, is mediated by muscle specific force at a habitually loaded site (mid-tibia), and whether this relationship is moderated by sex. *H1: Muscle specific force will mediate the relationship between physical activity and bone status. H2: The relationship between muscle specific force and bone status will be stronger in males than females.*

Specific Aim 2a: To determine whether the clinical lab standard measure of leg power, the Nottingham Leg Extensor Power Rig accounts for an equal portion of variance in bone status as the corresponding muscle cross sectional area at a habitually loaded site (mid-tibia) of young men and women. *H1: Muscle force capacity derived from the Nottingham Leg Extensor Power Rig scores will predict at least as much variance in bone status as the corresponding site-specific muscle cross sectional area derived from pQCT.*

Specific Aim 2b: To determine whether a non-invasive, feasible field measure of muscle function, the vertical jump, accounts for an equal portion of variance in bone status as the corresponding clinical standard measure of leg power, the Nottingham Leg Extensor Power Rig, in young men and women. *H1: Muscle force capacity derived from vertical jump scores will predict at least as much variance in bone status as the corresponding clinical standard measure of leg power.*

1.3 Scientific and Public Health Related Significance

Considering the ongoing public health effort to combat osteoporosis, there is a need to clarify the circumstances under which specific health behaviors, such as PA, will infer the greatest benefit to bone status. Together with the investigation of the specific type of PA intervention that is most beneficial in promoting bone accrual (20, 22), characterizing the contribution of muscle force capacity to the beneficial effects of PA on bone status, will allow for the creation of exercise interventions that increase both muscle force capacity, along with its regular application to the skeleton. This might include combined resistance training and osteogenic PA promotion; however further research is needed in the area. The proposed study is novel and timely in that it not only seeks to define the relationship among PA, muscle force capacity, and bone status, but also examines how it might differ by sex. Many physiological differences moderate bone accrual and loss in men and women, and as such, osteoporosis prevention methodologies should not follow a 'one size fits all' approach. In addition, the proposed study in young adults heeds the call for more research into the determinants of bone acquisition in the understudied period of young adulthood by the National Osteoporosis Foundation's recent position stand (22). Finally, the identification of non-invasive, field-based measures of muscle force capacity in place of radiative muscle mass based muscle force surrogates could reduce the risk and cost of osteoporosis screening and research for both patients and researchers.

1.4   References

1.   Baptista F, Mil-Homens P, Carita AI, Janz K, Sardinha LB. Peak vertical jump power as a marker of bone health in children. *Int J Sports Med*. 2016;37(8):653-58.

2.   Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. *J Bone Miner Res*. 2007;22(3):465-75.

3.   Burr DB. Muscle strength, bone mass, and age-related bone loss. *J Bone Miner Res*. 1997;12(10):1547-51.

4.   Duckham RL, Baxter-Jones AD, Johnston JD, Vatanparast H, Cooper D, Kontulainen S. Does physical activity in adolescence have site-specific and sex-specific benefits on young adult bone size, content, and estimated strength? *J Bone Miner Res*. 2014;29(2):479-86.

5.   Farr JN, Khosla S. Skeletal changes through the lifespan - from growth to senescence. *Nat Rev Endocrinol*. 2015;11(9):513-21.

6.   Farr JN, Laddu DR, Blew RM, Lee VR, Going SB. Effects of physical activity and muscle quality on bone development in girls. *Med Sci Sports Exerc*. 2013;45(12):2332-40.

7.   Farr JN, Laddu DR, Going SB. Exercise, hormones and skeletal adaptations during childhood and adolescence. *Pediatr Exerc Sci*. 2014;26(4):384-91.

8.   Forwood MR, Baxter-Jones AD, Beck TJ, Mirwald RL, Howard A, Bailey DA. Physical activity and strength of the femoral neck during the adolescent growth spurt: a longitudinal analysis. *Bone*. 2006;38(4):576-83.

9.   Frost HM. Muscle, bone, and the Utah paradigm: A 1999 overview. *Med Sci Sports Exerc*. 2000;32(5):911-7.

10.   Greene D, Naughton G, Briody J et al. Bone strength index in adolescent girls: Does physical activity make a difference? *Br J Sports Med*. 2005;39(9):622-7.

11.   Hernandez CJ, Beaupré GS, Carter DR. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. *Osteoporosis Int*. 2003;14(10):843-7.

12.   Hogler W, Blimkie CJ, Cowell CT et al. Sex-specific developmental changes in muscle size and bone geometry at the femoral shaft. *Bone*. 2008;42(5):982-9.

13.   Jackowski SA, Kontulainen SA, Cooper DM, Lanovaz JL, Beck TJ, Baxter-Jones AD. Adolescent physical activity and bone strength at the proximal femur in adulthood. *Med Sci Sports Exerc*. 2014;46(4):736-44.

14.   Janz KF, Letuchy EM, Burns TL, Eichenberger Gilmore JM, Torner JC, Levy SM. Objectively measured physical activity trajectories predict adolescent bone strength: The Iowa Bone Development Study. *Br J Sports Med*. 2014;48(13):1032-6.

15.   Janz KF, Letuchy EM, Burns TL, Francis SL, Levy SM. Muscle power predicts adolescent bone strength: The Iowa Bone Development Study. *Med Sci Sports Exerc*. 2015;47(10):2201-6.

16.   Leonard MB, Elmi A, Mostoufi-Moab S et al. Effects of sex, race, and puberty on cortical bone and the functional muscle bone unit in children, adolescents, and young adults. *J Clin Endocrinol Metab*. 2010;95(4):1681-9.

17.   Robling AG. Is bone's response to mechanical signals dominated by muscle forces? *Med Sci Sports Exerc*. 2009;41(11):2044-9.

18.   Sayers A, Mattocks C, Deere K, Ness A, Riddoch C, Tobias JH. Habitual levels of vigorous, but not moderate or light, physical activity is positively related to cortical bone mass in adolescents. *J Clin Endocrinol Metab*. 2011;96(5):793-802.

19.   Schoenau E, Neu CM, Mokov E, Wassmer G, Manz F. Influence of puberty on muscle area and cortical bone area of the forearm in boys and girls. *J Clin Endocrinol Metab*. 2000;85(3):1095-8.

20.   Tan VP, Macdonald HM, Kim S et al. Influence of physical activity on bone strength in children and adolescents: A systematic review and narrative synthesis. *J Bone Miner Res*. 2014;29(10):2161-81.

21.   Torres-Costoso A, Gracia-Marco L, Sanchez-Lopez M, Notario-Pacheco B, Arias-Palencia N, Martinez-Vizcaino V. Physical activity and bone health in schoolchildren: The mediating role of fitness and body fat. *PLoS One*. 2015;10(4):e0123797.

22.   Weaver CM, Gordon CM, Janz KF et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: A systematic review and implementation recommendations. *Osteoporos Int*. 2016;27(4):1281-386.

23.   Wetzsteon RJ, Zemel BS, Shults J, Howard KM, Kibe LW, Leonard MB. Mechanical loads and cortical bone geometry in healthy children and young adults. *Bone*. 2011;48(5):1103-8.

CHAPTER 2

LITERATURE REVIEW

2.1 Prevalence and Incidence of Osteoporosis in the United States

Recent statistics using census data from femoral neck and spinal dual energy x-ray absorptiometry (DXA) scan sites estimate that 10.3% of older adults in America suffer from osteoporosis, with a further 43.9% suffering from low bone mass (103). That is, over half of adults 50 years and older are afflicted in some way by low bone mass. Looking deeper into the prevalence of osteoporosis, we see women have a remarkably higher risk than men of the same racial ethnic group, with almost 16% of white non-Hispanic or Latino women being afflicted by osteoporosis, a relative prevalence that is ~75% higher than men (103). These values vary based on the measured site (femoral neck, spine, total hip, distal radius, etc.) and definition of osteoporosis used and as such may underestimate the true prevalence of osteoporosis at any site, with previous reports suggesting rates as high as 45% in postmenopausal women and 36% in age-matched men (72). Though recent data show osteoporosis rates holding constant (103), projections made in the past decade which were based on fracture rates suggested potential increases in both the occurrence and economic burden of osteoporotic fractures by ~48% (19). The cumulative cost of these fractures was suggested to rise from the already high $209 billion annually, to $228 billion from 2006-2015 to 2016-2025 (19). If this cost growth continues as projected, the outlook for the economic burden associated with osteoporosis is bleak which emphasizes the importance of research regarding bone accrual and maintenance.

2.2   Bone Accrual and Modifiable Health Behaviors

The development of osteoporosis is strongly driven by genetics with 60-80% of bone mass being attributed to heritable factors (51, 89, 90). Despite this, strong evidence suggests that adopting positive health behaviors can optimize the accrual of the remaining 20-40% of adult peak bone mass (99). Indeed, achieving a greater peak bone mineral density (BMD) has been highlighted as the most important factor in delaying the development of osteoporosis, with a 10% increase in peak BMD potentially delaying disease onset by 13 years (53). In contrast, a similar magnitude reduction in the rate of non-menopausal bone mass loss throughout life is predicted to delay osteoporosis onset by only 2 years (53). Moreover, data show that skeletal deficits present during youth appear to track through adulthood with men and women who sustained a distal forearm fracture under the circumstances of mild trauma during youth having diminished bone strength compared to those who fractured following moderate trauma (40, 41). These deficits in bone strength which predispose youth to fracture are again attributed to sub-optimal attainment of peak bone mass (41).

Given the importance of achieving optimal peak bone mass, research has focused on manipulating modifiable behaviors that are either detrimental or beneficial to bone accrual (99). Behaviors such as smoking and excess alcohol intake have been suggested as potential barriers to peak bone mass (16, 30, 34, 64, 68, 86). However, evidence is inconsistent, owing to a lack of randomized controlled trials and generally low-exposures during youth. In contrast, the two main behaviors that consistently stand out as strong positive affecters of bone status are PA and dietary factors, which have been shown to benefit bone status both individually and synergistically (25).

2.2a   Dietary Factors

Though many nutrients have been examined as being potentially beneficial to bone growth, including: fruits and vegetables (80, 97), adequate protein intake (2, 11, 96), specific fatty acids (27, 70),

and other micronutrients (Vitamin K, C, Zinc, etc.) (66, 77), adequate calcium and vitamin D intake are the major dietary factors known to affect bone accrual in otherwise healthy youths (21, 29, 31, 50, 62, 65, 74, 79, 98). Calcium supplementation usually occurs in the form of pills or chews, or within dairy products, with randomized controlled trials in youths supplementing between 1000 – 1200 mg/day of calcium carbonate for up to 24 months. Studies in youths to do so showed maximal increases in total body size-adjusted bone mineral content (BMC) of 4.6% above a placebo group after 18 months of supplementation (21), increases in BMC at the hip and spine of 2.3% and 2.5% greater than placebo (79), and distal radius size-adjusted BMC improvements of 5.5% over placebo following 13 and 12 months of supplementation, respectively (29). Despite consistent findings supporting the beneficial effects of calcium supplementation, the question remains as to whether calcium driven gains in bone mass are maintained long term, beyond cessation of supplementation (21, 65).

Vitamin D has a weaker base of evidence in support of its effects on bone accrual, with studies showing both positive (31, 62) and null (4, 73) effects following supplementation. Worthy of note is the fact that the only studies showing beneficial effects of vitamin D supplementation also supplemented their participants with calcium. Doses of vitamin D used within these studies ranged from 200 - 800 IU/day to 300,000 IU given 4 times/year, with 12-24 months of supplementation producing only small effects (1.2% greater size-adjusted BMC than calcium supplementation alone). The consensus surrounding vitamin D supplementation is that beneficial effects are likely confined to individuals with inadequate serum 25(OH)D concentrations <50 nmol/L (99).

2.2b   Physical Activity

Strong evidence supports the efficacy of weight-bearing PA, including intentional high impact exercise, in the augmentation of bone accrual during growth, with interventions of only 6 months promoting up to 6% greater gains in bone mass than controls, depending on the site and participant age

(99). An example of this enhanced bone accrual is an intervention utilizing only 40 minutes of school-based PA per day over two years, which led to 3% greater increases in lumbar spine BMC per year than a control group (3). Evidence also suggests that interventions using more intense PA or osteogenic jumping protocols show greater gains over a shorter period of time than lower intensity habitual PA (100). Paralleling the gains in bone mass, substantial benefits to bone structure are seen among individuals with the highest vs. lowest habitual PA levels in longitudinal studies (32, 56, 57). In one prospective study, when maturation and body size were accounted for, a 9% and 17% (boys and girls, respectively) greater gain in total body BMC was noted in the most active group compared to their inactive peers (10). Similar results were shown in a group of adolescents who had 8-15% greater height adjusted BMC depending on the site when compared to their inactive peers (15). Unlike dietary supplementation, the bone accrued through PA appears to track from childhood into adolescence (45), and from youth into adulthood, even in the case of cessation of the specific PA or sport (14, 35, 36, 78). Whether these benefits are maintained into middle-aged and older adulthood inferring a protective effect on osteoporosis risk requires further research, as the few studies to examine this question seem to show no difference between former athletes and controls (61, 69, 94). However, these studies are limited by either a cross-sectional approach, short follow-up periods, or self-report of youth PA.

2.3   Frost's Mechanostat Theory - Physical Activity as an Osteogenic Behavior

Clear evidence supports PA and exercise as behaviors that are beneficial for bone accrual, so much so that guidelines regarding the most osteogenic type of activity are taking shape; exercises must be dynamic, odd or non-repetitive in load direction, applied quickly, of a moderate to high load, and short in load duration (91, 93, 99). However, one key area that researchers often fail to address in intervention analyses is the effect of muscle on the PA-bone status relationship, with a recent review suggesting that approximately one third of studies examining the effect of PA on bone status controlled for muscle in

their statistical approach (91). Indeed, muscle is thought to be a key mediator in the PA-bone status relationship (91), imparting most of its beneficial effect on bone status through the mechanical forces applied by skeletal muscles pulling on bony levers (20, 47). These forces are among the largest applied to the skeleton, playing an important role in modulating bone strength, especially during growth (47, 85). This coupling of muscle and bone strength is a key proposition of the Utah paradigm (47), and is in part borne from Howard Frost's Mechanostat theory which postulates that bones adapt to the mechanical stressors placed upon them, and when a stressor surpasses the 'modeling threshold range', increases in bone strength occur (46).

When muscle force is considered in analyses it is often in the form of proxy measures such as muscle cross sectional area (MCSA) or mass (44, 49, 87, 92). These surrogates only partially characterize the force producing capacity of muscle, and thus the mechanical strain, as evidenced by direct effects of muscle power and muscle specific force on bone strength, independent of cross sectional area (58, 101). Some have argued that muscle mass is an adequate surrogate for muscle force, with measures of force only adding nominally to variance in bone status when included within predictive models (101); however, when considering the methodologies associated with the measurement of muscle mass and force some key concerns arise. Primarily, measures of muscle mass are most often derived from x-ray based methodologies which can be both costly and radiative, exposing participants to potentially harmful ionizing radiation. Conversely, commonly used measures of muscle force do not pose this same radiation exposure risk and are generally much more cost-efficient for both the researcher and clinician, as well as the patient. Thus, the utility of defining the specific contribution of muscle force to the PA-bone status relationship using non-invasive measures of muscular force capacity is of interest as it reduces risk to vulnerable populations during research and clinical testing, and provides opportunity for widespread, affordable clinical and research-based osteoporosis assessment.

2.4   Specific Measures and Surrogates of Muscle Force Capacity

The most common surrogates of muscle force capacity are MCSA (42), lean body mass (49, 87, 95), and total body mass or body mass index (22, 39), which are derived from peripheral quantitative computed tomography (pQCT) scans, DXA scans, and standard anthropometric measurements, respectively. Each surrogate captures a different portion of variance in bone status due to the inherent inclusion of factors such as adiposity, which contributes independent mechanical and hormonal effects (83, 84), and the whole body endocrine / localized paracrine effects of lean mass (17). The x-ray based methodologies also expose participants to radiation, with the dosages varying depending on the technology, scanning method, age of participant, and number of scans used (1, 26). DXA scans utilizing a fan beam have been reported to transfer between 4 - 27μSv of radiation in children, however more modern scanners used for adult whole body scans infer much smaller doses (~0.04μSv) (1, 26). Due to the faster scan time of pQCT scanners, similar radiation doses to DXA scans over multiple scan sites can be achieved, with 3 scans inferring approximately 1.4μSv per scan (9). These numbers, although ultimately amounting to no more than a few days of background radiation, expose participants to radiation that brings them closer to the guideline of <50uSV per year. When aiming to characterize the mechanical effects of muscle on bone status, the lack of specificity combined with the radiation exposure that accompany mass based surrogates provides a convincing argument against their use when specific measures of force are available.

A number of specific measures of muscle force capacity have been used to examine the relationships between muscle force and bone status, including the Nottingham Leg Extensor Power Rig (8, 24), the Biodex dynamometer (67, 101), peak handgrip force (54, 67), peak ground reaction force (GRF) via force plates (52, 75, 81, 82), the Wingate Anaerobic Power Test (102), and vertical jump assessments (12, 58), each measuring a different aspect of muscular force capacity at a specific muscular site. In contrast to surrogates, the only risk to participants from measuring muscle force capacity using

specific methodologies is the risk of musculoskeletal injury typically associated with maximal voluntary or explosive exercise. This injury risk is minimal for youth and young adult populations but does increase with age due to changes in connective tissue. Furthermore, a limitation of these methodologies is that some clinical lab-based estimates of muscle force capacity take slightly longer to assess than current muscle force surrogates.

Commonly used clinical lab-based assessment methodologies such as isokinetic dynamometry are highly reliable, providing accurate measures of isokinetic and isometric torque across a wide range of populations with little difference seen between dynamometer brands (28, 38). However, many testing methodologies require expensive equipment that preclude their use in widespread non-research testing. Accordingly, there is a need for translational non-invasive measures of muscle force capacity and as such, findings suggesting that muscle power predicted from a translational field-based vertical jump assessment accounted for up to 77% of the variance in bone status, depending on the site and participant gender, highlights it as a promising methodology in this regard (58). Furthermore, acceptable sensitivity and specificity of vertical jump in predicting bone status has also been shown in children (12); however, as with any instrument, the vertical jump needs to be assessed in comparison to more internally valid lab-based methodologies to ensure the validity of measurements in relation to the mechanical forces applied to bones. This type of criterion validation has been attempted using force platforms as a criterion methodology, comparing predicted height from flight time to measured height via multiple jumping methodologies, with conflicting results (18, 23).

In the context of bone status, it is the peak forces experienced by a bone that will lead to structural improvements and thus, emerging methodologies such as multiple single-leg hopping on a force plate provide potential context-specific criterion methodologies against which to assess translational measures (5-7, 52). The ground reaction forces associated with multiple single-leg hopping have been estimated to exceed 9 times an individual's body weight (7), a value almost double that expected during a

maximal eccentric contraction. Furthermore, in a varied sample (age range 8-82 yo) maximum force from multiple single-leg hopping predicted up to 84% of the variability in bone mass at the distal tibia and was a stronger predictor of bone mass than MCSA in both males and females (5). Future research comparing the validity of predicted peak power from translational measures to measured peak power using force plates and methodologies such as multiple single-leg hopping would therefore aid in answering currently ambiguous questions regarding the validity of translational measures of vertical jump.

2.5   Sex-Differences in the Muscle-Bone Relationship and Muscle Force Capacity

Just as bone accrual during growth is moderated by sex, as are other bone status determinants such as PA (37) and muscle force capacity (101). Sex-differences are also apparent in the muscle-bone relationship with studies showing that women have a larger bone area than men for a given muscle size in habitually loaded but not unloaded sites (55, 88). Two main hypotheses exist for this sex-moderated relationship between habitually loaded bone and muscle. First, the most obvious perspective is that the sex-specific hormonal environments (estrogen, testosterone, growth hormone, insulin-like growth factor-1; IGF-1, etc.) and their interaction with weight-bearing exercise (40, 43) influence bone status. Throughout pubertal growth it is IGF-1 that is majorly responsible for increases in bone structural size in growing girls, whereas testosterone plays the same role in growing boys (43). These differing hormonal concentrations and sex-specific roles continue throughout life, interacting with PA to lead to unique adaptive responses. An alternative hypothesis centers on differences in the mechanical forces applied to bones between men and women, with one suggested cause being a relative greater adiposity in women, leading to greater demand on a similarly sized muscle in everyday activities in order to carry the relative excess load (55, 88). Though these hypotheses are unique, sex-differences in the muscle-bone unit are likely a combination of multiple mechanisms working in parallel to produce a stable mechanical environment.

The research examining the former hypothesis, sex-differences in hormonally mediated bone accrual with exercise, is in agreement (13, 32, 33, 63); however, research regarding sex-differences in muscle force capacity is conflicting, with studies suggesting a lower muscle specific force (force per cross sectional area) in women compared to men (48, 59, 60, 101), and others suggesting no difference (59, 71, 76). If mechanical force differences were to result in a larger bone area in women compared to men, then logically these forces would have to be of a relatively greater magnitude to cause positive changes in bone geometry. More research is needed to clarify the conflicting findings regarding sex-differences in muscle force capacity and how this important factor relates to sex-differences in bone status.

2.6   Summary

The predicted rise in fracture incidence resulting from osteoporosis and sex-related disparity of these skeletal deficiencies highlight the importance of defining potential mechanisms through which beneficial lifestyle behaviors like PA might augment bone status in a sex-specific manner. PA has emerged as one of the most efficacious of these lifestyle behaviors, with much of its effect thought to be transmitted through skeletal muscle and the forces it applies to bones. As much research utilizes proxy measures of these muscular forces, it is unknown whether interventions specifically focused on improving muscle quality, and concurrently force production, would benefit individuals bone status through greater force generation during PA. Furthermore, the measurement of these muscle force proxies exposes patients to unnecessary risk via radiation and often increased medical costs, without certainty of improving the predictive value of an individual's bone status. To address these concerns future research must: 1) clearly define the role of muscle force in the PA and bone status relationship to inform potential therapeutic and preventative interventions, and 2) examine measurement tools that provide a strong muscle force based predictor of bone status without the need for costly, timely, or radiative scanning methodologies as this will aid in the widespread clinical and research-based assessment of osteoporosis.

2.7 References

1. Adams JE, Engelke K, Zemel BS, Ward KA. Quantitative computer tomography in children and adolescents: The 2013 ISCD Pediatric Official Positions. *J Clin Densitom*. 2014;17(2):258-74.

2. Alexy U, Remer T, Manz F, Neu CM, Schoenau E. Long-term protein intake and dietary potential renal acid load are associated with bone modeling and remodeling at the proximal radius in healthy children. *Am J Clin Nutr*. 2005;82(5):1107-14.

3. Alwis G, Linden C, Ahlborg HG, Dencker M, Gardsell P, Karlsson MK. A 2-year school-based exercise programme in pre-pubertal boys induces skeletal benefits in lumbar spine. *Acta Paediatr*. 2008;97(11):1564-71.

4. Andersen R, Molgaard C, Skovgaard LT et al. Effect of vitamin D supplementation on bone and vitamin D status among Pakistani immigrants in Denmark: A randomised double-blinded placebo-controlled intervention study. *Br J Nutr*. 2008;100(1):197-207.

5. Anliker E, Rawer R, Boutellier U, Toigo M. Maximum ground reaction force in relation to tibial bone mass in children and adults. *Med Sci Sports Exerc*. 2011;43(11):2102-9.

6. Anliker E, Sonderegger A, Toigo M. Side-to-side differences in the lower leg muscle-bone unit in male soccer players. *Med Sci Sports Exerc*. 2013;45(8):1545-52.

7. Anliker E, Toigo M. Functional assessment of the muscle-bone unit in the lower leg. *J Musculoskelet Neuronal Interact*. 2012;12(2):46-55.

8. Arden NK, Spector TD. Genetic influences on muscle strength, lean body mass, and bone mineral density: A twin study. *J Bone Miner Res*. 1997;12(12):2076-81.

9. Ashby RL, Ward KA, Roberts SA, Edwards L, Mughal MZ, Adams JE. A reference database for the Stratec XCT-2000 peripheral quantitative computed tomography (pQCT) scanner in healthy children and young adults aged 6–19 years. *Osteoporos Int*. 2009;20(8):1337-46.

10. Bailey DA, McKay HA, Mirwald RL, Crocker PRE, Faulkner RA. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: The University of Saskatchewan Bone Mineral Accrual Study. *J Bone Miner Res*. 1999;14(10):1672-9.

11. Ballard TL, Specker BL, Binkley TL, Vukovich MD. Effect of protein supplementation during a 6-month strength and conditioning program on areal and volumetric bone parameters. *Bone*. 2006;38(6):898-904.

12. Baptista F, Mil-Homens P, Carita AI, Janz K, Sardinha LB. Peak vertical jump power as a marker of bone health in children. *Int J Sports Med*. 2016;37(8):653-58.

13. Bass SL. The structural adaptations of cortical bone to loading during different stages of maturation. *J Musculoskelet Neuronal Interact*. 2003;3(4):345-7.

14. Baxter-Jones AD, Eisenmann JC, Mirwald RL, Faulkner RA, Bailey DA. The influence of physical activity on lean mass accrual during adolescence: A longitudinal analysis. *J Appl Physiol*. 2008;105(2):734-41.

15. Baxter-Jones AD, Kontulainen SA, Faulkner RA, Bailey DA. A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. *Bone*. 2008;43(6):1101-7.

16. Blum M, Harris SS, Must A, Phillips MS, Rand MW, Dawson-Hughes B. Household tobacco smoke exposure is negatively associated with premenopausal bone mass. *Osteoporos Int*. 2002;13(8):663-8.

17. Brotto M, Bonewald L. Bone and muscle: Interactions beyond mechanical. *Bone*. 2015;80:109-14.

18. Buckthorpe M, Morris J, Folland JP. Validity of vertical jump measurement devices. *J Sports Sci*. 2012;30(1):63-9.

19.	Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. *J Bone Miner Res*. 2007;22(3):465-75.

20.	Burr DB. Muscle strength, bone mass, and age-related bone loss. *J Bone Miner Res*. 1997;12(10):1547-51.

21.	Cameron MA, Paton LM, Nowson CA, Margerison C, Frame M, Wark JD. The effect of calcium supplementation on bone density in premenarcheal females: A co-twin approach. *J Clin Endocrinol Metab*. 2004;89(10):4916-22.

22.	Chastin SF, Mandrichenko O, Helbostadt JL, Skelton DA. Associations between objectively-measured sedentary behaviour and physical activity with bone mineral density in adults and older adults, The NHANES Study. *Bone*. 2014;64:254-62.

23.	Cheah PY, Cheong JPG, Razman R, Zainal Abidin NE. Comparison of vertical jump height using the force platform and the Vertec. In: F Ibrahim, JPG Cheong, J Usman, MY Ahmad, R Razman, VS Selvanayagam editors. *3rd International Conference on Movement, Health and Exercise: Engineering Olympic Success: From Theory to Practice*. Singapore: Springer Singapore; 2017, pp. 155-8.

24.	Cousins JM, Petit MA, Paudel ML et al. Muscle power and physical activity are associated with bone strength in older men: The osteoporotic fractures in men study. *Bone*. 2010;47(2):205-11.

25.	Daly RM, Duckham RL, Gianoudis J. Evidence for an interaction between exercise and nutrition for improving bone and muscle health. *Curr Osteoporos Rep*. 2014;12(2):219-26.

26.	Damilakis J, Adams JE, Guglielmi G, Link TM. Radiation exposure in X-ray-based imaging techniques used in osteoporosis. *Eur Radiol*. 2010;20(11):2707-14.

27.   Damsgaard CT, Molgaard C, Matthiessen J, Gyldenlove SN, Lauritzen L. The effects of n-3 long-chain polyunsaturated fatty acids on bone formation and growth factors in adolescent boys. *Pediatr Res*. 2012;71(6):713-9.

28.   de Araujo Ribeiro Alvares JB, Rodrigues R, de Azevedo Franke R et al. Inter-machine reliability of the Biodex and Cybex isokinetic dynamometers for knee flexor/extensor isometric, concentric and eccentric tests. *Phys Ther Sport*. 2015;16(1):59-65.

29.   Dibba B, Prentice A, Ceesay M, Stirling DM, Cole TJ, Poskitt EM. Effect of calcium supplementation on bone mineral accretion in gambian children accustomed to a low-calcium diet. *Am J Clin Nutr*. 2000;71(2):544-9.

30.   Dorn LD, Pabst S, Sontag LM, Kalkwarf HJ, Hillman JB, Susman EJ. Bone mass, depressive, and anxiety symptoms in adolescent girls: Variation by smoking and alcohol use. *J Adolesc Health*. 2011;49(5):498-504.

31.   Du X, Zhu K, Trube A et al. School-milk intervention trial enhances growth and bone mineral accretion in Chinese girls aged 10-12 years in Beijing. *Br J Nutr*. 2004;92(1):159-68.

32.   Duckham RL, Baxter-Jones AD, Johnston JD, Vatanparast H, Cooper D, Kontulainen S. Does physical activity in adolescence have site-specific and sex-specific benefits on young adult bone size, content, and estimated strength? *J Bone Miner Res*. 2014;29(2):479-86.

33.   Eastell R. Role of oestrogen in the regulation of bone turnover at the menarche. *J Endocrinol*. 2005;185(2):223-34.

34.   Eleftheriou KI, Rawal JS, James LE et al. Bone structure and geometry in young men: The influence of smoking, alcohol intake and physical activity. *Bone*. 2013;52(1):17-26.

35.   Erlandson MC, Kontulainen SA, Chilibeck PD, Arnold CM, Faulkner RA, Baxter-Jones AD. Former premenarcheal gymnasts exhibit site-specific skeletal benefits in adulthood after long-term retirement. *J Bone Miner Res*. 2012;27(11):2298-305.

36.   Erlandson MC, Kontulainen SA, Chilibeck PD, Arnold CM, Faulkner RA, Baxter-Jones AD. Higher premenarcheal bone mass in elite gymnasts is maintained into young adulthood after long-term retirement from sport: A 14-year follow-up. *J Bone Miner Res*. 2012;27(1):104-10.

37.   Evenson KR, Wen F, Hales D, Herring AH. National youth sedentary behavior and physical activity daily patterns using latent class analysis applied to accelerometry. *Int J Behav Nutr Phys Act*. 2016;13:55.

38.   Fagher K, Fritzson A, Drake AM. Test-retest reliability of isokinetic knee strength measurements in children aged 8 to 10 years. *Sports Health*. 2016;8(3):255-9.

39.   Farr JN, Blew RM, Lee VR, Lohman TG, Going SB. Associations of physical activity duration, frequency, and load with volumetric BMD, geometry, and bone strength in young girls. *Osteoporos Int*. 2011;22(5):1419-30.

40.   Farr JN, Khosla S. Skeletal changes through the lifespan - from growth to senescence. *Nat Rev Endocrinol*. 2015;11(9):513-21.

41.   Farr JN, Khosla S, Achenbach SJ et al. Diminished bone strength is observed in adult women and men who sustained a mild trauma distal forearm fracture during childhood. *J Bone Miner Res*. 2014;29(10):2193-202.

42.   Farr JN, Laddu DR, Blew RM, Lee VR, Going SB. Effects of physical activity and muscle quality on bone development in girls. *Med Sci Sports Exerc*. 2013;45(12):2332-40.

43.   Farr JN, Laddu DR, Going SB. Exercise, hormones and skeletal adaptations during childhood and adolescence. *Pediatr Exerc Sci*. 2014;26(4):384-91.

44.   Forwood MR, Baxter-Jones AD, Beck TJ, Mirwald RL, Howard A, Bailey DA. Physical activity and strength of the femoral neck during the adolescent growth spurt: A longitudinal analysis. *Bone*. 2006;38(4):576-83.

45. Francis SL, Letuchy EM, Levy SM, Janz KF. Sustained effects of physical activity on bone health: The Iowa Bone Development Study. *Bone*. 2014;63:95-100.

46. Frost HM. The mechanostat: A proposed pathogenic mechanism of osteoporosis and the bone mass effects of mechanical and nonmechanical agents. *Bone Miner*. 1987;2(2):73-85.

47. Frost HM. Muscle, bone, and the Utah paradigm: A 1999 overview. *Med Sci Sports Exerc*. 2000;32(5):911-7.

48. Goodpaster BH, Carlson CL, Visser M et al. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. *J Appl Physiol (1985)*. 2001;90(6):2157-65.

49. Greene D, Naughton G, Briody J et al. Bone strength index in adolescent girls: Does physical activity make a difference? *Br J Sports Med*. 2005;39(9):622-7.

50. Greene DA, Naughton GA. Calcium and vitamin-D supplementation on bone structural properties in peripubertal female identical twins: A randomised controlled trial. *Osteoporos Int*. 2011;22(2):489-98.

51. Guéguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J, Siest G. Segregation analysis and variance components analysis of bone mineral density in healthy families. *J Bone Miner Res*. 1995;10(12):2017-22.

52. Hardcastle SA, Gregson CL, Rittweger J, Crabtree N, Ward K, Tobias JH. Jump power and force have distinct associations with cortical bone parameters: Findings from a population enriched by individuals with high bone mass. *J Clin Endocrinol Metab*. 2014;99(1):266-75.

53. Hernandez CJ, Beaupré GS, Carter DR. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. *Osteoporosis Int*. 2003;14(10):843-7.

54. Herrmann D, Buck C, Sioen I et al. Impact of physical activity, sedentary behaviour and muscle strength on bone stiffness in 2-10-year-old children-cross-sectional results from the IDEFICS study. *Int J Behav Nutr Phys Act*. 2015;12:112.

55. Hogler W, Blimkie CJ, Cowell CT et al. Sex-specific developmental changes in muscle size and bone geometry at the femoral shaft. *Bone*. 2008;42(5):982-9.

56. Jackowski SA, Kontulainen SA, Cooper DM, Lanovaz JL, Beck TJ, Baxter-Jones AD. Adolescent physical activity and bone strength at the proximal femur in adulthood. *Med Sci Sports Exerc*. 2014;46(4):736-44.

57. Janz KF, Letuchy EM, Burns TL, Eichenberger Gilmore JM, Torner JC, Levy SM. Objectively measured physical activity trajectories predict adolescent bone strength: The Iowa Bone Development Study. *Br J Sports Med*. 2014;48(13):1032-6.

58. Janz KF, Letuchy EM, Burns TL, Francis SL, Levy SM. Muscle power predicts adolescent bone strength: The Iowa Bone Development Study. *Med Sci Sports Exerc*. 2015;47(10):2201-6.

59. Kanehisa H, Ikegawa S, Fukunaga T. Comparison of muscle cross-sectional area and strength between untrained women and men. *Eur J Appl Physiol Occup Physiol*. 1994;68(2):148-54.

60. Kanehisa H, Ikegawa S, Tsunoda N, Fukunaga T. Strength and cross-sectional area of knee extensor muscles in children. *Eur J Appl Physiol Occup Physiol*. 1994;68(5):402-5.

61. Karlsson MK, Linden C, Karlsson C, Johnell O, Obrant K, Seeman E. Exercise during growth and bone mineral density and fractures in old age. *Lancet*. 2000;355(9202):469-70.

62. Khadilkar AV, Sayyad MG, Sanwalka NJ et al. Vitamin D supplementation and bone mass accrual in underprivileged adolescent Indian girls. *Asia Pac J Clin Nutr*. 2010;19(4):465-72.

63. Kontulainen SA, Macdonald HM, Khan KM, McKay HA. Examining bone surfaces across puberty: A 20-month pQCT trial. *J Bone Miner Res*. 2005;20(7):1202-7.

64.   Korkor AB, Eastwood D, Bretzmann C. Effects of gender, alcohol, smoking, and dairy consumption on bone mass in Wisconsin adolescents. *WMJ*. 2009;108(4):181-8.

65.   Lambert HL, Eastell R, Karnik K, Russell JM, Barker ME. Calcium supplementation and bone mineral accretion in adolescent girls: An 18-mo randomized controlled trial with 2-y follow-up. *Am J Clin Nutr*. 2008;87(2):455-62.

66.   Laudermilk MJ, Manore MM, Thomson CA, Houtkooper LB, Farr JN, Going SB. Vitamin C and zinc intakes are related to bone macroarchitectural structure and strength in prepubescent girls. *Calcif Tissue Int*. 2012;91(6):430-9.

67.   Lorbergs AL, Farthing JP, Baxter-Jones AD, Kontulainen SA. Forearm muscle size, strength, force, and power in relation to pQCT-derived bone strength at the radius in adults. *Appl Physiol Nutr Metab*. 2011;36(5):618-25.

68.   Lorentzon M, Mellstrom D, Haug E, Ohlsson C. Smoking is associated with lower bone mineral density and reduced cortical thickness in young men. *J Clin Endocrinol Metab*. 2007;92(2):497-503.

69.   Magnusson H, Linden C, Karlsson C, Obrant KJ, Karlsson MK. Exercise may induce reversible low bone mass in unloaded and high bone mass in weight-loaded skeletal regions. *Osteoporos Int*. 2001;12(11):950-5.

70.   Mangano KM, Sahni S, Kerstetter JE, Kenny AM, Hannan MT. Polyunsaturated fatty acids and their relation with bone and muscle health in adults. *Curr Osteoporos Rep*. 2013;11(3):203-12.

71.   Maughan RJ, Watson JS, Weir J. Strength and cross-sectional area of human skeletal muscle. *J Physiol*. 1983;338:37-49.

72.   Melton LJ, 3rd, Khosla S, Achenbach SJ, O'Connor MK, O'Fallon WM, Riggs BL. Effects of body size and skeletal site on the estimated prevalence of osteoporosis in women and men. *Osteoporos Int*. 2000;11(11):977-83.

73.   Molgaard C, Larnkjaer A, Cashman KD, Lamberg-Allardt C, Jakobsen J, Michaelsen KF. Does vitamin D supplementation of healthy Danish Caucasian girls affect bone turnover and bone mineralization? *Bone*. 2010;46(2):432-9.

74.   Moyer-Mileur LJ, Xie B, Ball SD, Pratt T. Bone mass and density response to a 12-month trial of calcium and vitamin D supplement in preadolescent girls. *J Musculoskelet Neuronal Interact*. 2003;3(1):63-70.

75.   Munukka M, Waller B, Multanen J et al. Relationship between lower limb neuromuscular performance and bone strength in postmenopausal women with mild knee osteoarthritis. *J Musculoskelet Neuronal Interact*. 2014;14(4):418-24.

76.   Neu CM, Rauch F, Rittweger J, Manz F, Schoenau E. Influence of puberty on muscle development at the forearm. *Am J Physiol Endocrinol Metab*. 2002;283(1):103-7.

77.   O'Connor E, Molgaard C, Michaelsen KF, Jakobsen J, Lamberg-Allardt CJ, Cashman KD. Serum percentage undercarboxylated osteocalcin, a sensitive measure of vitamin K status, and its relationship to bone health indices in Danish girls. *Br J Nutr*. 2007;97(4):661-6.

78.   Pollock NK, Laing EM, Modlesky CM, O'Connor PJ, Lewis RD. Former college artistic gymnasts maintain higher BMD: A nine-year follow-up. *Osteoporos Int*. 2006;17(11):1691-7.

79.   Prentice A, Ginty F, Stear SJ, Jones SC, Laskey MA, Cole TJ. Calcium supplementation increases stature and bone mineral mass of 16- to 18-year-old boys. *J Clin Endocrinol Metab*. 2005;90(6):3153-61.

80.   Prynne CJ, Mishra GD, O'Connell MA et al. Fruit and vegetable intakes and bone mineral status: A cross sectional study in 5 age and sex cohorts. *Am J Clin Nutr*. 2006;83(6):1420-8.

81.   Rantalainen T, Nikander R, Heinonen A et al. Neuromuscular performance and body mass as indices of bone loading in premenopausal and postmenopausal women. *Bone*. 2010;46(4):964-9.

82.   Rantalainen T, Sievanen H, Linnamo V et al. Bone rigidity to neuromuscular performance ratio in young and elderly men. *Bone*. 2009;45(5):956-63.

83.   Reid IR. Relationships among body mass, its components, and bone. *Bone*. 2002;31(5):547-55.

84.   Reid IR. Fat and bone. *Arch Biochem Biophys*. 2010;503(1):20-7.

85.   Robling AG. Is bone's response to mechanical signals dominated by muscle forces? *Med Sci Sports Exerc*. 2009;41(11):2044-9.

86.   Rudäng R, Darelid A, Nilsson M et al. Smoking is associated with impaired bone mass development in young adult men: A 5-year longitudinal study. *J Bone Miner Res*. 2012;27(10):2189-97.

87.   Sayers A, Mattocks C, Deere K, Ness A, Riddoch C, Tobias JH. Habitual levels of vigorous, but not moderate or light, physical activity is positively related to cortical bone mass in adolescents. *J Clin Endocrinol Metab*. 2011;96(5):793-802.

88.   Schoenau E, Neu CM, Mokov E, Wassmer G, Manz F. Influence of puberty on muscle area and cortical bone area of the forearm in boys and girls. *J Clin Endocrinol Metab*. 2000;85(3):1095-8.

89.   Seeman E, Hopper JL. Reduced bone mass in daughters of women with osteoporosis. *New England Journal of Medicine*. 1989;320(9):554.

90.   Soroko SB, Barrett-Connor E, Edelstein SL, Kritz-Silverstein D. Family history of osteoporosis and bone mineral density at the axial skeleton: The Rancho Bernardo Study. *J Bone Miner Res*. 1994;9(6):761-9.

91.   Tan VP, Macdonald HM, Kim S et al. Influence of physical activity on bone strength in children and adolescents: A systematic review and narrative synthesis. *J Bone Miner Res*. 2014;29(10):2161-81.

92.   Torres-Costoso A, Gracia-Marco L, Sanchez-Lopez M, Notario-Pacheco B, Arias-Palencia N, Martinez-Vizcaino V. Physical activity and bone health in schoolchildren: The mediating role of fitness and body fat. *PLoS One*. 2015;10(4):e0123797.

93.   Turner CH, Robling AG. Designing exercise regimens to increase bone strength. *Exerc Sport Sci Rev*. 2003;31(1):45-50.

94.   Valdimarsson O, Alborg HG, Duppe H, Nyquist F, Karlsson M. Reduced training is associated with increased loss of BMD. *J Bone Miner Res*. 2005;20(6):906-12.

95.   Valtuena J, Gracia-Marco L, Vicente-Rodriguez G et al. Vitamin D status and physical activity interact to improve bone mass in adolescents. The HELENA Study. *Osteoporos Int*. 2012;23(8):2227-37.

96.   Vatanparast H, Bailey DA, Baxter-Jones AD, Whiting SJ. The effects of dietary protein on bone mineral mass in young adults may be modulated by adolescent calcium intake. *J Nutr*. 2007;137(12):2674-9.

97.   Vatanparast H, Baxter-Jones A, Faulkner RA, Bailey DA, Whiting SJ. Positive effects of vegetable and fruit consumption and calcium intake on bone mineral accrual in boys during growth from childhood to adolescence: The University of Saskatchewan Pediatric Bone Mineral Accrual Study. *Am J Clin Nutr*. 2005;82(3):700-6.

98.   Ward KA, Das G, Roberts SA et al. A randomized, controlled trial of vitamin D supplementation upon musculoskeletal health in postmenarchal females. *J Clin Endocrinol Metab*. 2010;95(10):4643-51.

99.   Weaver CM, Gordon CM, Janz KF et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: A systematic review and implementation recommendations. *Osteoporos Int*. 2016;27(4):1281-386.

100.   Weeks BK, Young CM, Beck BR. Eight months of regular in-school jumping improves indices of bone strength in adolescent boys and Girls: The POWER PE study. *J Bone Miner Res*. 2008;23(7):1002-11.

101.   Wetzsteon RJ, Zemel BS, Shults J, Howard KM, Kibe LW, Leonard MB. Mechanical loads and cortical bone geometry in healthy children and young adults. *Bone*. 2011;48(5):1103-8.

102.   Witzke KA, Snow CM. Lean body mass and leg power best predict bone mineral density in adolescent girls. *Med Sci Sports Exerc*. 1999;31(11):1558-63.

103.   Wright NC, Looker AC, Saag KG et al. The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine. *J Bone Miner Res*. 2014;29(11):2520-6.

CHAPTER 3

MODERATE-TO-VIGOROUS PHYSICAL ACTIVITY, MUSCLE SPECIFIC FORCES, AND

CORTICAL BONE IN YOUNG ADULTS: A MODERATED MEDIATION ANALYSIS[1]

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3.1 Abstract

Physical activity (PA) is well known as a beneficial behavior for promoting bone mineral gains and structural adaptations in youth, and muscle has been identified as a key mediator of this relationship. However, it is not fully understood whether the mechanical forces applied to bones by muscle directly influence the beneficial effects of PA more so than measures of muscle mass, nor whether muscular differences apparent between sexes influence this relationship. Thus, the aim of this cross-sectional analysis was to characterize the specific contribution of muscle force, independent of muscle size, to the beneficial effects of PA on bone status, and to examine whether this differed in men compared to women at habitually loaded and unloaded sites using a moderated mediation analysis. Young adults (n=144, 19.7 $\pm$ 0.7 yo, 52.1% female) had their diaphyseal cortical bone and muscle cross sectional area (MCSA) assessed by peripheral quantitative computed tomography at the mid-tibia. Moderate-to-vigorous physical activity (MVPA) was assessed via accelerometer and dorsiflexion muscle force of the non-dominant leg was measured using an isokinetic dynamometer. Muscle specific force (MSF) was calculated as peak dorsiflexion torque divided by MCSA. The index of moderated mediation suggested that MSF did not mediate the effect of MVPA on bone status (Coeff.(SE)=.0001±.0008, LLCI -.0013, ULCI .0018), and that this indirect effect was not moderated by sex. Furthermore, sex-specific indirect effects of MVPA on cortical thickness, through MSF, were also null for both sexes (p<.05). Contrary to these findings, the relationship between MVPA and cortical thickness, independent of MSF, was moderated by sex (Coeff.(SE)=-.0088±.0039, LLCI -.0166, ULCI -.0010), with a direct effect existing in females (Coeff.(SE)=.0088±.0027, LLCI .0034, ULCI .0142) but not males (Coeff.(SE)=.0000±.0028, LLCI -.0056, ULCI .0056). In a sample of young adults, MSF does not appear to mediate the relationship between MVPA and cortical bone status at any site, nor is this effect moderated by sex. Promoting MVPA in females of this age group may be beneficial for cortical structure; however, this relationship is not present in their male counterparts.

3.2   Introduction

Though osteoporosis is predominantly heritable, 20-40% of bone mineral accrual is also attributable to lifestyle behaviors (8, 44). There is strong evidence that in youth physical activity (PA), specifically activities that generate high impact loads, including moderate-to-vigorous physical activity (MVPA), are highly osteogenic (40), which in turn is one of the most promising ways to prevent osteoporosis in later life (20). Understanding the pathways through which PA exerts a beneficial effect on bone status is therefore crucial to optimizing interventions and recommendations across the lifespan.

The current hypothesis, stemming from Frost's mechanostat theory (16), is that PA modulates bone remodeling primarily though the mechanical forces applied to the skeleton by muscles (8, 17), forces which are among the largest experienced by bones (33). Indeed, it has been reported that muscle force transfers more than 70% of the bending moments onto the femur (29). Research comparing the independent contribution of muscle size and muscle force to bone status have shown that both strength and power-based measures of muscle force independently predict variance in bone status, but only marginally more so than muscle size (10, 25, 46). Studying the relationship between these measures of mechanical and non-mechanical muscle action (i.e. muscle force relative to muscle size vs. endocrine and paracrine action related to muscle mass) in the context of PA is important to shed light on potential mechanisms for optimizing the beneficial effects associated with PA. Furthermore, disproportionate rates of osteoporosis in women highlight the importance of considering whether the mechanisms of the effects of PA on bone status, through mechanical or non-mechanical means, might differ by sex (47).

Sex-specific hormonal environments drive bone accrual at different rates in girls and boys during childhood and adolescence, leading to differences in both bone mass and structure in adulthood (12). For example, women tend to have a larger bone area than men for a given muscle size, specifically at habitually loaded sites (21, 38). In the case of the mechanical relationship between muscle and bone status, data also suggest that women may have a lower relative muscle force (force output per unit of

cross sectional area) than their male counterparts (46). Whether these differences translate into altered

adaptation of bone status to PA between sexes is unknown.

Thus, there is a need to characterize the specific contribution of muscle force to the beneficial

effects of PA on bone status, and to examine whether this differs in men compared to women at habitually

loaded and unloaded sites. The primary aim of this study was to determine whether the relationship

between objectively measured MVPA and cortical bone status is mediated by muscle specific force

(MSF) in a cross-sectional sample of young adults, and furthermore, whether this relationship is

moderated by sex. We hypothesized that a) MSF would mediate the relationship between PA and bone

status, and b) sex would moderate the indirect relationship through MSF, with a greater MSF in males

leading to a stronger indirect effect compared to females.

3.3   Methods

Recruitment and Participant Characteristics. Participants included within this cross-sectional

analysis were recruited via email, posted flyer, and word-of-mouth advertising from fall 2016 to spring

2017. All participants (n=146; n=76 female) were white, non-Hispanic or Latino due to the known effect

of race on modeled variables, and age was restricted to $18 - 21$ yo to ensure that the majority of

participants had completed sexual maturation (>90% bone mineral accrual). Inclusion criteria also

dictated that participants were a) a currently enrolled University of Georgia student, b) free of orthopedic

limitations that precluded participation in exercise and PA, c) not current smokers (past 6 months), d) not

pregnant or planning to become pregnant for the duration of their participation, and had not given birth in

the last 12 months, e) not taking medications known to affect bone metabolism (i.e. glucocorticoids),

habitual dietary intake, or PA, f) free of any medical conditions know to affect bone metabolism (i.e.

Crohn's Disease), g) not currently diagnosed with an eating disorder, and h) had not undergone recent

weight loss surgery (bariatric or gastric bypass). All participants provided written informed consent prior to participation. The Institutional Review Board of the university approved all aspects of the protocol.

Anthropometric Measures. Standing height was measured by a stadiometer (Novel Products Inc., Rockton, IL) to the nearest 0.1 cm. Body mass was measured with a digital scale (Seca Bella 840, Columbia, MD) to the nearest 0.1 kg. Tibia length was measured with a sliding anthropometer to the nearest 0.1cm as the distance from the distal edge of the medial malleolus to the tibial plateau.

Physical Activity. Objectively measured PA was assessed using a tri-axial ActiGraph GT3X+ accelerometer (Firmware v3.2.1.) utilizing 15-s epochs. The GT3X+ measures accelerations within a range of magnitudes ($\pm 6g$) and digitizes output data by a 12-bit analog-to-digital converter at a user specified rate ranging between 30 and 100Hz, filtering out non-human movement with 30Hz used in the current study. Each participant was asked to wear the accelerometer on the mid axillary line of his/her right hip during all waking hours for a $\geq$7-day period, via an elastic band around the waist, except during water-based activities (e.g. showering and swimming). Data were included only if the participant had accumulated a minimum of 10 hours/day of recording determined through the automatic application of the wear time macro developed by the National Cancer Institute (CDC/National Center for Health Statistics) from the Troiano algorithm (41), for at least three days including one weekend day. Periods with consecutive raw activity values of zero (with a 2-min spike tolerance of $\leq$100 counts) for 60 min or longer were interpreted as "non-wear" and excluded from this analysis (9). Wear time analysis and data scoring was performed using Actilife software (v6.10.1; Actigraph, LLC, Fort Walton Beach, FL), using 15-s epochs and the VM3 vector magnitude approach (36). The VM3 cut points to classify PA intensity were 0-2690 for light, 2691-6166 for moderate, $\geq$6167 for vigorous, and $\geq$2691 for MVPA. A weighted average PA time [(weekday average *5) + (weekend average *2) /7] was used to represent mean weekly activity variables.

Bone Assessments. Peripheral quantitative computed tomography (pQCT; Stratec XCT-3000; Stratec Medizintechnic GmbH, Pforzheim, Germany) was used to assess bone strength and scans were taken of the non-dominant tibia at the 38% and 66% sites relative to the total leg length from the distal metaphysis. Each scan was acquired with a 0.4-mm voxel at a slice thickness of 2.4-mm. Scout view was used automatically by the software to position the two cross-sectional measurements, using the participant's medial end plate as an anatomic marker. Image processing and calculation of the various bone indices was performed using the Stratec software (version 6.20). The following parameters were assessed at the tibia 38% site: cortical volumetric bone mineral density (vBMD$_{Cort}$; mg/mm^3), cortical area (mm^2), cortical thickness (mm), periosteal circumference (mm), endosteal circumference (mm), and polar strength-strain index (pSSI, mm^3). Cortical bone parameters were assessed using cortmode 2 and the default threshold of 710 mg/cm^3, except for SSI which utilized a threshold of 480 mg/cm^3. The 66% site measurement was taken to assess muscle cross sectional area (MCSA, mm^2), which was determined by automated analyses utilizing edge detection, threshold techniques, and image filters to separate tissues. The automated analysis performs two steps to calculate MCSA (6):

1) Images are first filtered using contour mode 3 (-100 mg/cm^3 threshold) and peel mode 2 (40 mg/cm^3 threshold) to separate adipose (less than 40 mg/cm^3) and muscle/bone (greater than 40 mg/cm^3), and then further filtered using muscle smooth 3, manufacturer image filter (F03F05F05) to aid in distinguishing between muscle and subcutaneous fat. Subsequently, cortmode 4 (149 mg/cm^3 threshold, 40 mg/cm^3 inner threshold) is applied to measure cortical bone area, remove bone marrow, and measure any positive movement artifact (which would increase neighboring soft tissue density due to stretching of cortical bone into the soft tissues) by comparing to the area found with a 710mg/cm^3 threshold.

2) Secondly, to find the muscle area independent of subcutaneous fat, images are filtered using contour mode 31 (40 mg/cm^3 threshold) and peel mode 2 (40 mg/cm^3 threshold) and are filtered

again with an image filter (F03F05F05). This re-analysis of the image allows for the acquisition

of bone marrow and negative movement (movement artifact which decreases the density of

neighboring tissues, reducing muscle area). Cortmode 4 is then reapplied to the image (710

mg/cm^3 threshold, 40 mg/cm^3 inner threshold) to separate total bone from other tissues and

remove marrow. To calculate MCSA, total bone including marrow is subtracted from muscle area

plus bone, and any areas of negative movement are added back to MCSA.

All pQCT measures were performed and analyzed by one trained operator who was trained for acquisition

and analysis following guidelines provided by Bone Diagnostic (Spring Branch, TX, USA). The

manufacturer supplied phantom was scanned daily to maintain quality assurance (Stratec Medizintechnik

GmbH, Pforzheim, Germany).

Muscle Specific Force. Muscular isokinetic strength was assessed using a Biodex System Pro 4

isokinetic dynamometer (Biodex Medical Systems, INC., New York) with the participant positioned per

manufacturer guidelines. Using the non-dominant ankle, five maximal effort voluntary plantarflexion and

dorsiflexion contractions were performed at 60 deg/sec to assess peak torque (N·m). Muscle specific force

(N·m/mm^2) was then calculated by dividing peak torque by MCSA of the 66% tibia. Ankle dorsiflexion

via isokinetic dynamometry have been found to be highly reliable in young men and women (22), and

was chosen as the major force outcome as it reflects a muscle-bone unit with direct force transfer onto the

tibia.

Statistical Analysis. Statistical analyses were performed using SPSS for Windows (SPSS 22.0,

Chicago, IL) with statistical significance set at an α level of $p<.05$. The normal distribution of residuals,

linearity, and homogeneity of variance were examined across all combinations of outcome variables.

Multi-collinearity between independent variables was also assessed via variance inflation factor (VIF),

with a VIF of <10 indicating the absence of collinearity (30); no variables met this criteria. Two

participants were excluded from analysis after being deemed influential multivariate outliers based on

Mahalonobis distance critical values for a chi squared distribution with k degrees of freedom (k = number of predictors in the model). Means and standard deviations were calculated for all participant characteristics and primary outcome variables, and independent t-tests then identified any differences between sexes. Bivariate and partial correlations between variables of interest were calculated which subsequently informed stepwise linear regression analysis to assess the effect of potential confounders prior to inclusion in the final model. Moderated mediation analysis (model 59; see Figure 3.0) were performed using the PROCESS macro for SPSS, provided by Hayes (19). Moderated mediation analysis is used to determine whether the direct effect of an antecedent variable (X) on a consequent variable (Y), or the indirect effect of X on Y through a mediator variable (M) are conditional on the value of a moderator variable (W). Using bootstrapping, random samples were taken with replacement, 10,000 times, to construct 95% bias-corrected bootstrap confidence intervals which are reported in place of p-values, with a confidence interval entirely above or below zero highlighting a significant effect. To aid in interpretation of the final model, MVPA was mean-centered for all moderated mediation analyses. All data are expressed as mean ± standard deviation (M±SD), unless otherwise indicated.

Adequate power to detect mediation (indirect effect) via bias-corrected bootstrapping was ensured through the use of a report analyzing required sample size to achieve .80 statistical power across different simple mediation models (15). In order to address our primary aim of mediation we used unpublished data from our lab to calculate the expected $\beta = .65$ (estimated effect of muscle force on bone status, adjusted for MVPA), and the expected $\alpha = .40$ (estimated effect of MVPA on muscle force), leading to a required sample size of n=53 to achieve .80 power at p<.05. To ensure that power was maintained when moderation by sex was added to the model, the overall sample was doubled, with at least $n=53$ of each sex included in analyses. Thus, based on prior effect size estimates our sample of n=69 males and n=79 females should have adequate power to detect any indirect effects, as well as moderation of these effects.

3.4   Results

Recruitment. Recruitment is outlined via flow chart in Figure 3.1. In brief, a total of (*n*=452)

individuals completed the online screening survey between September 2016 and February 2017, of which

(*n*=108) were deemed ineligible based on inclusion/exclusion crieria: over the age of 20 (*n*=45), did not

complete the screening survey in enough detail to contact (*n*=30), taking medications known to affect

bone status (*n*=8), current smoker (*n*=7), diagnosed with a disease known to affect bone status (*n*=5), not

willing to wear a PA monitor (*n*=4), were currently injured and unable to complete testing (*n*=4), not

willing to meet the time commitments of the testing (*n*=3), or had an eating disorder (*n*=2). Thus, (*n*=344)

potential participants were contacted via email, of which (*n*=184) were scheduled for testing. Upon

scheduling or following the first of two testing visits participants (*n*=24) dropped from the study with

additional participants (*n*=6) excluded due to not having worn their PA monitors for the required time,

leaving a total useable sample of (*n*=154). Due to the known moderating effect of race on bone outcomes,

only white non-hispanic or latino participants were included (*n*=146), with participants (*n*=2) excluded as

multivariate outliers, leaving the final analyzed sample at (*n*=144).

Preliminary Analysis - Descriptives. As the main analysis examined moderation by sex,

participant characteristics are presented seperately for males and females in Table 3.0. As expected,

measures of body size, including: height, body mass, tibia length, and MCSA, were significantly greater

in males than females (8%, 21%, 8%, and 18%, respectively; all $p<.001$). MVPA did not differ between

males and females ($p>.05$) with both exceeding reccomendations by a substantial amount (42). Muscle

torque was 48% greater in males than females ($p<.001$). When expressed as MSF, a relative measure of

force output for the tibial muscles, males had 13% greater force output per mm^2 than females ($p<.001$).

Bone geometric properties including cortical area, cortical thickness, and periosteal circumference were

greater in males than females (29%, 15%, and 13%, respectively; all $p<.001$) which is indicative of a

wider, stronger bone. As a bone's diameter increases, its bending and compression strength increase to a

much greater magnitude than if it's density were to increase by the same relative amount (7). This was reflected in the 41% larger pSSI seen in males compared to females ($p<.001$). In contrast, females had smaller, more dense bones with a smaller medullary cavity, as reflected by a 3% greater vBMD$_{cort,}$ and 11% smaller endosteal circumferences, coupled with the already identified smaller periosteal circumference (all $p<.001$).

Preliminary Analysis – Bivariate and Partial Correlations. Although age was significantly related to vBMD$_{cort}$ ($r=.20$, $p=.019$) it was not related to any other bone status variables, which, combined with the relatively narrow age range, negated it from inclusion in further analysis. In contrast, tibia length was significantly related to all bone status outcomes (r-.501 to .693, all $p<.001$) and was retained as a covariate as previously suggested (46). Partial correlations controlling for tibia length, accelerometer wear time, and sex are shown in Table 3.1. MVPA was positively related to cortical thickness ($p=.02$), and the relationship with cortical area approached significance ($p=.08$). MVPA was also positively related to MCSA at the lower leg ($r=.24$, $p=.004$), however when MCSA and peak dorsiflexion torque were combined to create the MSF outcome variable, this relationship was attenuated ($r=.01$, $p=.937$). Though traditional mediation analysis such as Barron and Kenney's causal step approach (3) requires the proposed *a* path (*X*; MVPA → *M*; MSF, Figure 3.0) to be significant, Hayes (19) suggests that this reasoning should be abandoned for multiple reasons, not limited to the fact that the indirect effect is a product of both *a* and *b* paths and thus an inferential test for an indirect effect should be predicated on the product rather than on the testing of individual *a* and *b* paths. For this and other reasons cited, MSF was kept in the overall model.

Moderated Mediation Analysis. As is depicted both conceptually and statistically in Figure 3.0, the proposed model tests the hypothesis that: 1) the relationship between MVPA and bone status is mediated by MSF; that is, the effect of MVPA on bone status is carried through MSF, and 2) this indirect effect and its components (*a* and *b*), as well as the direct effect of MVPA on bone status independent of

MSF, are moderated by sex; that is, the effects differ based on the level of the moderator (males=1 vs. females=0).

Results of the moderated mediational analysis can be found in Tables 3.2 and 3.3, with Table 3.2 reporting the tests of individual paths and interactions, whilst controlling for tibia length and accelerometer wear time, and Table 3.3 reporting the tests of indirect and direct effects, as well as the index of moderated mediation. As the confidence interval for the index of moderated mediation contains zero, the overall hypothesis that the effect of MVPA on bone status through MSF differs between males and females is rejected. The specific bone status variable modeled in these tables is cortical thickness as it had the strongest relationship with MVPA. However analysis at other sites were performed producing similar results for cortical area, but not vBMDcort, periosteal circumference, endosteal circumference, or pSSI (see Appendix A, Tables 3.4 and 3.5).

Taking a step back to understand why there is a lack of moderated mediation, we see that indirect effects for both males and females are non-significant, suggesting that MSF did not mediate the relationship between MVPA and cortical thickness in either sex. This null result is mirrored throughout all tests of individual paths that comprise the indirect effect, with no relationship between MVPA and MSF (a_1= .0000, $p>.05$) in females and no indication of sex moderating this relationship (a_3=.0000, $p>.05$). Furthermore, MSF appeared not to affect cortical thickness (b_1=-96.37, $p>.05$), again with a lack of moderation by sex (b_2=10.23, $p>.05$).

In contrast, a signficant conditional direct effect of MVPA on cortical thickness was demonstrated in females (c_1'+ c_3'W=.0088, LLCI=.0034, ULCI=.0142) but not males (c_1'+ c_3'W =.0000, LLCI=-.0056, ULCI=.0056), while holding constant MSF. These results are supported by a signfiicant interaction between MVPA and sex, suggesting that, independent of MSF, greater participation in MVPA would lead to increased cortical thickness in females (c_3=-.0088, p=.026) but not in males. In an attempt to further probe the conditional direct effect, a graphical representation of the relationship between

MVPA and cortical thickness was plotted using data derived from a model 1 analysis (simple moderation of the relationship between *X* and *Y* by *W*), whilst removing the non-significant mediation pathways but still controlling for the same covariates. Figure 3.2 represents the slope of the regression line predicting cortical thickness from MVPA in females, supporting the notion that as MVPA increases cortical thickness also increases.

3.5   Discussion

The aim of this study was to determine whether the relationship between objectively measured MVPA and cortical bone status, measured by pQCT at the 38% tibia, was mediated by MSF, and furthermore, whether this relationship was moderated by sex. We hypothesized that: a) MSF would mediate the relationship between MVPA and bone status, and b) sex would moderate the indirect relationship through MSF, with a greater MSF in males leading to a stronger indirect effect. The major findings of the moderated mediation analysis were that both of our hypotheses were rejected, with MSF failing to mediate the relationship between MVPA and cortical thickness (or other bone status variables). Furthermore, as there was no mediation there could also be no moderation of this indirect pathway by sex. Contrary to the null findings of moderated mediation, a moderation of the direct effect of MVPA on bone status was found, with females who participated in more MVPA having thicker cortices. Regarding our mediation approach, to our knowledge no other studies have assessed the indirect effect of MVPA on bone structure (cortical thickness, cortical area, etc.) or mass (vBMD$_{cort}$) through a measure of relative force capacity such as MSF. However, the lack of an indirect effect is still unexpected and somewhat contrary to past research which examined the components of MSF: muscle size and absolute force.

Mediation - Physical Activity and Muscle. Currently no studies have examined the effect of PA on MSF, however, many studies agree that PA is related to both absolute muscle force and size across the lifespan (2, 5, 13, 14, 26-28, 32, 34). One of the most convincing studies to show the effect of prolonged

moderate-intensity PA on absolute muscle force followed a cohort of boys and girls over seven years following randomization to interventions of 200 minutes/week of school-based PA, or a control group of 60 minutes/week (14, 28). At follow-up, high-active participants were ~14 yo and had gained between 5.3 - 11.7% more isokinetic knee peak torque (both extension and flexion; $p<.05$) compared to their control group counterparts. Importantly, there was no reported difference in outside school PA which suggests that torque increases were wholly accountable to the intervention-imposed PA. Furthermore, both cross-sectional and longitudinal studies agree that there is a positive relationship between PA and lean mass, or lean mass development, throughout the childhood and adolescent years (2, 5, 26, 27). Baxter-Jones et al. (5) followed 222 participants from childhood into adolescence over 6-years assessing their PA and body composition twice each year and concluded that habitual PA, independent of maturity and stature, significantly influenced the accrual of lean body mass. Within the cohort, boys who increased their PA by 1 SD above the mean were estimated to accrue almost ~350g more lean mass per year, with similar results seen in girls, however to a ~37% smaller magnitude. Thus, the link between habitual PA and muscle mass and/or absolute force output is well established throughout childhood and adolescence, including the current population of study.

Mediation - Muscle and Bone Status. Previous research has focused on the use of both MSF as well as the component parts, absolute muscle force and size, in relation to bone status (10, 25, 45). In a similarly designed study to the present one, Wetzsteon et al. (46) assessed the relationship among multiple predictors of bone strength, including dorsiflexion torque controlling for MCSA, in a racially diverse sample (32% black, 47% male, n=321, 5 to 35 yo). In support of our findings, muscle force was greater in males compared to females when controlling for age, race, MCSA, and tibia length. Further, when muscle force was used to predict cortical bone strength and structure, a significant relationship was seen at three sites (polar section modulus, cortical area, and periosteal circumference; all $p<.05$), independent of age, sex, race, tibia length, and pubertal status assessed via Tanner stage. The presence of

significant effects of both muscle torque independent of MCSA, as well as MCSA alone, on cortical

geometry is in direct opposition to the results of the present study. Such findings are mirrored in other

well-designed studies, highlighting the independent predictive ability of muscle force derived from

vertical jump assessments on cortical bone status (25), as well as isokinetic torque in predicting DXA-

derived bone mass and structural outcomes such as femoral neck BMD or section modulus (10). Coupling

these results with the well-known genetically driven relationship between muscle and bone, accounting

for 55-85% of the co-variance between tissues (39), it seems that our inability to define a mediation,

which negated potential for moderated mediation, could be in-part due to methodological issues. For

example, the inability to isolate only active muscles directly contributing to torque output in pQCT-

derived MCSA estimates which may have limited sensitivity; however, as previous research has

successfully used this same combination of predictors to assess MSF, we were confident in our selection

of variables (46). Moreover, objectively measured MVPA during this transitional college period may not

being indicative of typical activity accrued during childhood and adolescence which predominantly drives

PA related bone accrual, as evidenced by average MVPA being substantially higher than expected across

sexes.

Moderation. The literature concerning the specific aspect of bone strength that MVPA imparts

beneficial effects upon is mixed, with both observational and longitudinal studies finding positive

relationships between MVPA and measures of overall bone strength (11, 18), structural measures such as

total area, cortical thickness, or periosteal circumference (11, 37, 43), as well as bone mass (1, 37, 43).

Thus, our findings of a direct relationship between MVPA and cortical thickness, independent of MSF,

but no relationship with bone strength (pSSI) or periosteal circumference is both in agreement with and

contrary to previous literature. Furthermore, the moderation of this direct relationship by sex, with

females appearing to benefit from greater time spent in MVPA (Figure 3.1) but males showing no

relationship, appears to conflict with the current view that both sexes tend to benefit from PA and that sex

differences are site-specific (mass vs. structure) due to differences in sex-hormones (12, 40). A majority

of studies have shown benefits that are independent of sex (18, 23, 24, 31, 35, 37). However, in some

instances effects of a relatively greater magnitude than that seen in girls matched for maturity have been

observed in both pre-pubertal and post-pubertal boys (24, 31), although none of these studies performed a

formal test of moderation. One such instance examined the relationship between MVPA split into impact

and non-impact loading on bone structural outcomes measured by high resolution pQCT in a sample of

adolescent and young adult males and females similar to the current study (31). Interestingly, males

tended to benefit from impact loading by increasing bone structure; with a 1-hour per week increase

leading to 0.6% greater total bone area, whereas females tended to benefit via increased whole body BMD

which, with a similar increase in weekly loading, was predicted to increase by 1%. One key factor to note

is that there is a scarcity of research in this more mature, young adult, population which could account for

these differences. During this post-pubertal period not only has longitudinal growth almost completely

ceased but also load induced bone modeling may occur in different surface-specific manners when

compared to pre- and mid-pubertal stages, with limited research suggesting that periosteal expansion

predominates in males, whereas endocortical contraction is primary in females (4).

Limitations & Future Directions. It is important to acknowledge that our results are not without

their limitations. Further to those listed within the previous sections, it is likely that such a highly active

sample of college students meant that we were unable to distinguish the effect of lower levels of MVPA

on bone status, or its mediating relationship through MSF, giving an incomplete picture of the effect of

PA in this age-group. Moreover, although the narrow age-range of this healthy white non-Hispanic or

Latino sample is a strength of this analysis by reducing variance and potential for confounding; however,

this also means that our data are likely not generalizable to other populations. With regards to our

methodologies, firstly, during the measurement of muscular torque a participant's motivation can heavily

influence their effort level when providing what is supposed to be a maximal voluntary contraction. To

standardize testing verbal and visual motivation were provided throughout the protocol, however we cannot guarantee that participants gave maximal effort. Secondly, waist worn accelerometers do not distinguish between activities of a high and low osteogenic value, nor do they measure all activities that might be beneficial to bone status (i.e. resistance training) which may have led to some PA being misclassified. Third and finally, though it is commonplace within the literature to use the MCSA of the lower leg as either a surrogate for muscle force or as a control variable for muscle size, not all the area included within the measure is active within a concentric dorsiflexion muscle action. This increased muscle area included within calculations of MSF would reduce absolute values and make the measure less specific.

Despite these limitations our study is one of the first to examine the relationships between MVPA and the muscle-bone unit, examining both muscle force as well as size, in a sample of young adults who are not yet fully skeletally mature. Future studies should build upon this foundation by using more specific measures of PA that assess impact-based activities over a longer period of time which are more indicative of the habitual loading likely to be driving bone accrual. Furthermore, researchers should examine multiple measures of muscle force and/or MSF to identify whether specific muscle actions (concentric, eccentric, or isometric), movement patterns, or loading characteristics contribute differently to the forces stimulating bone accrual.

3.6   Conclusion

In summary, it appears that a relative measure of dorsiflexion MSF did not mediate the effect of MVPA on bone status, though further research is needed examining the utility of alternative measures of muscular force capacity in more diverse samples of young adults. Moreover, MVPA appears to be beneficial to bone structure in young adult females, independent of the effect of MSF, a relationship that does not hold true in their male counterparts.

3.7   References

1. Alwis G, Linden C, Ahlborg HG, Dencker M, Gardsell P, Karlsson MK. A 2-year school-based exercise programme in pre-pubertal boys induces skeletal benefits in lumbar spine. *Acta Paediatr*. 2008;97(11):1564-71.

2. Baptista F, Barrigas C, Vieira F et al. The role of lean body mass and physical activity in bone health in children. *J Bone Miner Metab*. 2012;30(1):100-8.

3. Baron RM, Kenny DA. The moderator–mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *J Pers Soc Psychol*. 1986;51(6):1173-82.

4. Bass SL. The structural adaptations of cortical bone to loading during different stages of maturation. *J Musculoskelet Neuronal Interact*. 2003;3(4):345-7.

5. Baxter-Jones AD, Eisenmann JC, Mirwald RL, Faulkner RA, Bailey DA. The influence of physical activity on lean mass accrual during adolescence: A longitudinal analysis. *J Appl Physiol*. 2008;105(2):734-41.

6. Blew RM, Lee VR, Farr JN, Schiferl DJ, Going SB. Standardizing evaluation of pQCT image quality in the presence of subject movement: Qualitative versus quantitative assessment. *Calcif Tissue Int*. 2014;94(2):202-11.

7. Bouxsein ML, Karasik D. Bone geometry and skeletal fragility. *Curr Osteoporos Rep*. 2006;4(2):49-56.

8. Burr DB. Muscle strength, bone mass, and age-related bone loss. *J Bone Miner Res*. 1997;12(10):1547-51.

9. Choi L, Liu Z, Matthews CE, Buchowski MS. Validation of accelerometer wear and nonwear time classification algorithm. *Med Sci Sports Exerc*. 2011;43(2):357-64.

10.   Daly RM, Stenevi-Lundgren S, Linden C, Karlsson MK. Muscle determinants of bone mass, geometry and strength in prepubertal girls. *Med Sci Sports Exerc*. 2008;40(6):1135-41.

11.   Farr JN, Blew RM, Lee VR, Lohman TG, Going SB. Associations of physical activity duration, frequency, and load with volumetric BMD, geometry, and bone strength in young girls. *Osteoporos Int*. 2011;22(5):1419-30.

12.   Farr JN, Laddu DR, Going SB. Exercise, hormones and skeletal adaptations during childhood and adolescence. *Pediatr Exerc Sci*. 2014;26(4):384-91.

13.   Foong YC, Chherawala N, Aitken D, Scott D, Winzenberg T, Jones G. Accelerometer-determined physical activity, muscle mass, and leg strength in community-dwelling older adults. *J Cachexia Sarcopenia Muscle*. 2016;7(3):275-83.

14.   Fritz J, Rosengren BE, Dencker M, Karlsson C, Karlsson MK. A seven-year physical activity intervention for children increased gains in bone mass and muscle strength. *Acta Paediatrica*. 2016;105(10):1216-24.

15.   Fritz MS, MacKinnon DP. Required sample size to detect the mediated effect. *Psychol Sci*. 2007;18(3):233-9.

16.   Frost HM. The mechanostat: A proposed pathogenic mechanism of osteoporosis and the bone mass effects of mechanical and nonmechanical agents. *Bone Miner*. 1987;2(2):73-85.

17.   Frost HM. Muscle, bone, and the Utah paradigm: A 1999 overview. *Med Sci Sports Exerc*. 2000;32(5):911-7.

18.   Gabel L, Macdonald HM, Nettlefold L, McKay HA. Physical activity, sedentary time, and bone strength from childhood to early adulthood: A mixed longitudinal HR-pQCT study. *J Bone Miner Res*. 2017:[Epub ahead of print].

19.   Hayes A. *An introduction to mediation, moderation, and conditional process analysis: A regression-based approach.* New York: Guilford; 2013.

20. Hernandez CJ, Beaupré GS, Carter DR. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. *Osteoporosis Int*. 2003;14(10):843-7.

21. Hogler W, Blimkie CJ, Cowell CT et al. Sex-specific developmental changes in muscle size and bone geometry at the femoral shaft. *Bone*. 2008;42(5):982-9.

22. Holmback AM, Porter MM, Downham D, Lexell J. Reliability of isokinetic ankle dorsiflexor strength measurements in healthy young men and women. *Scand J Rehabil Med*. 1999;31(4):229-39.

23. Janz KF, Burns TL, Levy SM et al. Everyday activity predicts bone geometry in children: The Iowa Bone Development Study. *Med Sci Sports Exerc*. 2004;36(7):1124-31.

24. Janz KF, Gilmore JM, Levy SM, Letuchy EM, Burns TL, Beck TJ. Physical activity and femoral neck bone strength during childhood: The Iowa Bone Development Study. *Bone*. 2007;41(2):216-22.

25. Janz KF, Letuchy EM, Burns TL, Francis SL, Levy SM. Muscle power predicts adolescent bone strength: The Iowa Bone Development Study. *Med Sci Sports Exerc*. 2015;47(10):2201-6.

26. Janz KF, Levy SM, Burns TL, Torner JC, Willing MC, Warren JJ. Fatness, physical activity, and television viewing in children during the adiposity rebound period: The Iowa Bone Development Study. *Prev Med*. 2002;35(6):563-71.

27. Kennedy K, Shepherd S, Williams JE, Ahmed SF, Wells JC, Fewtrell M. Activity, body composition and bone health in children. *Arch Dis Child*. 2013;98(3):204-7.

28. Lofgren B, Daly RM, Nilsson JA, Dencker M, Karlsson MK. An increase in school-based physical education increases muscle strength in children. *Med Sci Sports Exerc*. 2013;45(5):997-1003.

29. Lu T-W, Taylor SJG, O'Connor JJ, Walker PS. Influence of muscle activity on the forces in the femur: An in vivo study. *J Biomech*. 1997;30:1101-6.

30. Marquardt DW. Generalized inverses, ridge regression, biased linear estimation, and nonlinear estimation. *Technometrics*. 1970;12(3):591-612.

31. McKay H, Liu D, Egeli D, Boyd S, Burrows M. Physical activity positively predicts bone architecture and bone strength in adolescent males and females. *Acta Paediatr*. 2011;100(1):97-101.

32. Paalanne NP, Korpelainen RI, Taimela SP et al. Muscular fitness in relation to physical activity and television viewing among young adults. *Med Sci Sports Exerc*. 2009;41(11):1997-2002.

33. Robling AG. Is bone's response to mechanical signals dominated by muscle forces? *Med Sci Sports Exerc*. 2009;41(11):2044-9.

34. Sandler RB, Burdett R, Zaleskiewicz M, Sprowls-Repcheck C, Harwell M. Muscle strength as an indicator of the habitual level of physical activity. *Med Sci Sports Exerc*. 1991;23(12):1375-81.

35. Sardinha LB, Baptista F, Ekelund U. Objectively measured physical activity and bone strength in 9-year-old boys and girls. *Pediatr*. 2008;122(3):728-36.

36. Sasaki JE, John D, Freedson PS. Validation and comparison of ActiGraph activity monitors. *J Sci Med Sport*. 2011;14(5):411-6.

37. Sayers A, Mattocks C, Deere K, Ness A, Riddoch C, Tobias JH. Habitual levels of vigorous, but not moderate or light, physical activity is positively related to cortical bone mass in adolescents. *J Clin Endocrinol Metab*. 2011;96(5):793-802.

38. Schoenau E, Neu CM, Mokov E, Wassmer G, Manz F. Influence of puberty on muscle area and cortical bone area of the forearm in boys and girls. *J Clin Endocrinol Metab*. 2000;85(3):1095-8.

39.   Seeman E, Hopper JL, Young NR, Formica C, Goss P, Tsalamandris C. Do genetic factors explain associations between muscle strength, lean mass, and bone density? A twin study. *Am J Physiol*. 1996;270(2):320-7.

40.   Tan VP, Macdonald HM, Kim S et al. Influence of physical activity on bone strength in children and adolescents: A systematic review and narrative synthesis. *J Bone Miner Res*. 2014;29(10):2161-81.

41.   Troiano RP. Large-scale applications of accelerometers: New frontiers and new questions. *Med Sci Sports Exerc*. 2007;39(9):1501.

42.   U.S. Department of Health and Human Services. *Physical Activity Guidelines for Americans*. Washington, DC: Office of Disease Prevention and Health Promotion; 2008. Available from: Office of Disease Prevention and Health Promotion.

43.   Wang QJ, Suominen H, Nicholson PH et al. Influence of physical activity and maturation status on bone mass and geometry in early pubertal girls. *Scand J Med Sci Sports*. 2005;15(2):100-6.

44.   Weaver CM, Gordon CM, Janz KF et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: A systematic review and implementation recommendations. *Osteoporos Int*. 2016;27(4):1281-386.

45.   Wetzsteon RJ, Petit MA, Macdonald HM, Hughes JM, Beck TJ, McKay HA. Bone structure and volumetric BMD in overweight children: A longitudinal study. *J Bone Miner Res*. 2008;23(12):1946-53.

46.   Wetzsteon RJ, Zemel BS, Shults J, Howard KM, Kibe LW, Leonard MB. Mechanical loads and cortical bone geometry in healthy children and young adults. *Bone*. 2011;48(5):1103-8.

47.   Wright NC, Looker AC, Saag KG et al. The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine. *J Bone Miner Res*. 2014;29(11):2520-6.

Table 3.0. Descriptive characteristics (*n*=144)

	Males (*n*=69)	Females (*n*=75)	*p*
Age (years)	19.7 ± 0.6	19.7 ± 0.8	.562
Body Mass (kg)	**76.1 ± 10.5**	**63.1 ± 11.9**	**<.001**
Height (cm)	**179.4 ± 6.2**	**166.2 ± 5.3**	**<.001**
Tibia Length (cm)	**39.7 ± 2.3**	**36.7 ± 1.9**	**<.001**
Muscle Cross Sectional Area (mm^2)	**8170.0 ± 1137.0**	**6934.9 ± 911.2**	**<.001**
Dorsiflexion Peak Torque (N·m)	**22.5 ± 5.1**	**15.2 ± 4.5**	**<.001**
Muscle Specific Force (N·m/mm^2)	**.0028 ± .0006**	**.0022 ± .0005**	**<.001**
MVPA (mins/day)	92.6 ± 27.6	86.0 ± 27.2	.153
pQCT - 38% Tibia Measures			
$vBMD_{cort}$ (mg/cm^3)	**1158.7 ± 19.9**	**1188.2 ± 19.6**	**<.001**
Ct.Ar (mm^2)	**346.8 ± 51.9**	**268.8 ± 31.3**	**<.001**
Ct.Th (mm)	**6.3 ± 0.7**	**5.5 ± 0.6**	**<.001**
Peri.Circ (mm)	**74.9 ± 4.9**	**66.3 ± 3.5**	**<.001**
Endo.Circ (mm)	**35.5 ± 4.1**	**31.9 ± 4.0**	**<.001**
pSSI (mm^3)	**2049.2 ± 384.0**	**1458.3 ± 211.5**	**<.001**

Note. Values are mean ± SD unless otherwise stated. **Bold** values = significant at *p*<.05. MVPA = moderate-to-vigorous physical activity. $vBMD_{cort}$ = cortical volumetric bone density. Ct.Ar = cortical area. Ct.Th = cortical thickness. Peri.Circ = periosteal circumference. Endo.circ = endosteal circumference. pSSI = polar stress strain index.

Table 3.1. Partial correlations between outcome variables (*n*=144)

		1	2	3	4	5	6	7	8	9	10
1	Muscle Cross Sectional Area (mm^2)	1									
2	Dorsiflexion Peak Torque (N·m)	**.36‡**	1								
3	Muscle Specific Force (N·m/mm^2)	**-.21***	**.83‡**	1							
4	MVPA (mins/day)	**.24†**	.13	.01	1						
5	vBMD$_{cort}$ (mg/cm^3)	-.13	**-.12**	-.05	-.11	1					
6	Ct.Ar (mm^2)	**.66‡**	**.26†**	-.12	.15	**-.17***	1				
7	Ct.Th (mm)	**.55‡**	**.23†**	-.08	**.19***	.03	**.88‡**	1			
8	Peri.Circ (mm)	**.59‡**	**.20***	-.13	.10	**-.35‡**	**.86‡**	**.52‡**	1		
9	Endo.Circ (mm)	-.02	-.06	-.05	-.11	**-.36‡**	-.12	**-.57‡**	**.40‡**	1	
10	pSSI (mm^3)	**.62‡**	**.21***	-.14	.06	**-.21***	**.91‡**	**.62‡**	**.96‡**	**.26†**	1

Note. $^‡p<.001$, $^† p<.01$, * $p<.05$. **Bold** values = significant at $p<.05$. Tibial length (cm), Accelerometer wear time (minutes/day), and Sex were used as covariates in all partial correlations. MVPA = moderate-to-vigorous physical activity. vBMD$_{cort}$ = cortical volumetric bone density. Ct.Ar = cortical area. Ct.Th = cortical thickness. Peri.Circ = periosteal circumference. Endo.circ = endosteal circumference. pSSI = polar stress strain index.

Table 3.2. Conditional process model coefficients – moderated mediation (Model 59; *n*=144)

		Outcome						
		M (Muscle Specific Force; MSF)				*Y* (Cortical Thickness)		
Predictor		Coefficient (SE)	LLCI	ULCI		Coefficient (SE)	LLCI	ULCI
X; MVPA	a_1	.0000 (.0000)	.0000	.0000	c'_1	.0088 (.0027)	**.0034**	**.0142**
M; MSF		-	-	-	b_1	-96.3682 (119.0027)	-331.7036	138.9672
W; Sex	a_2	.0004 (.0001)	**.0002**	**.0007**	c'_2	.5902 (.5027)	-.4039	1.5843
X × *W;* MVPA × Sex	a_3	.0000 (.0000)	.0000	.0000	c'_3	-.0088 (.0039)	**-.0166**	**-.0010**
M × *W;* MSF × Sex		-	-	-	b_2	10.2249 (191.8313)	-369.1336	389.5834
Constant.	i_1	.0012 (.0012)	-.0010	.0035	i_2	2.3555 (1.290)	-.1958	4.9068
		$R^2 = .46$				$R^2 = .59$		
		$F(5,138) = 7.35, p<.001$				$F(7,136) = 10.44, p<.001$		

Note. Values are unstandardized. **Bold** values = significant at *p*<.05. *SE* = Standard error. *LLCI* = Lower limit of 95% bias-corrected bootstrap confidence interval. ULCI = Upper limit of 95% bias-corrected bootstrap confidence interval. MVPA = moderate-to-vigorous physical activity. *X;* MVPA was mean centered to aid in interpretation. Tibial length (cm) and Accelerometer wear time (minutes/day) were included in the model as covariates.

Table 3.3. Conditional process model coefficients – indirect and direct effects, and moderated mediation index (*n*=144)

W; Sex	Indirect Effect			Direct Effect		
	Effect (SE) $(a_{1i} + a_{3i}W)(b_{1i}+b_{2i}W)$	LLCI	ULCI	Effect (SE) $c_1' + c_3'W$	LLCI	ULCI
0 = Female	-.0001 (.0005)	-.0015	.0006	.0088 (.0027)	**.0034**	**.0142**
1 = Male	.0000 (.0006)	-.0008	.0015	.0000 (.0028)	-.0056	.0056
Mediator	Index of Moderated Mediation					
	Index (SE)		LLCI		ULCI	
M; MSF	.0001 (.0008)		-.0013		.0018	

 Note. Values are unstandardized. **Bold** values = significant at *p*<.05. *SE* = Standard error. *LLCI* = Lower limit of 95% bias-corrected bootstrap confidence interval. ULCI = Upper limit of 95% bias-corrected bootstrap confidence interval. MVPA = moderate-to-vigorous physical activity. Tibial length (cm) and Accelerometer wear time (minutes/day) were included in the model as covariates.

A

M; Muscle Specific Force

X; MVPA

Y; Diaphyseal Bone

W; Sex

B

e_{M_i}

M_i; Muscle Specific Force

a_{1_i}

b_{1_i}

e_Y

a_{2_i}

X; MVPA

c'_1

Y; Diaphyseal Bone

a_{3_i}

c'_2

W; Sex

c'_3

b_{2_i}

XW; MVPA×Sex

MW; MSF×Sex

Conditional indirect effect of X on Y through $M_i = (a_{1_i} + a_{3_i}W)(b_{1_i} + b_{2_i}W)$

Conditional direct effect of X on $Y = c_1' + c_3'W$
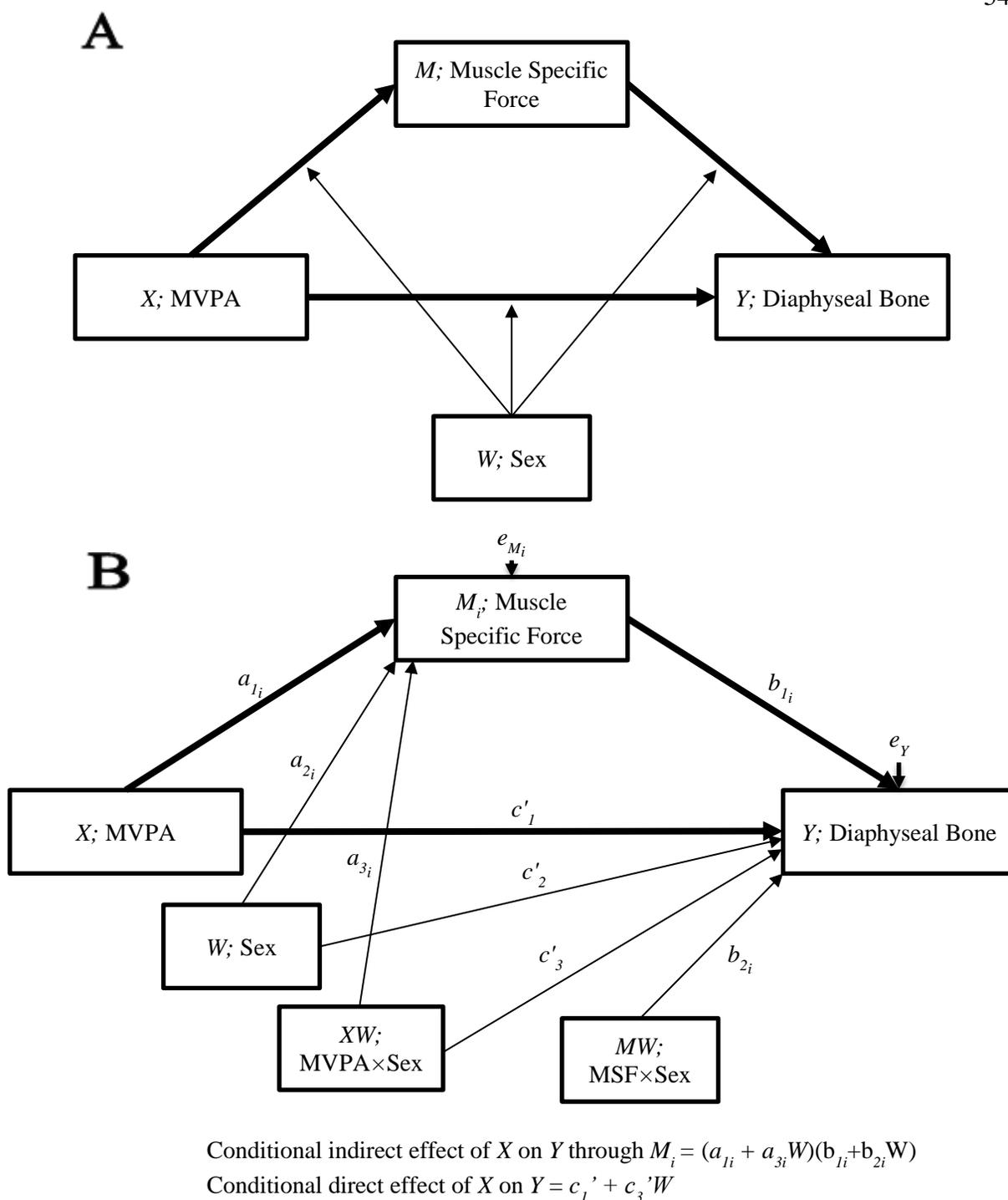
Figure 3.0. Conceptual (A) and Statistical (B) diagrams of a 'model 59' moderated mediation analysis. Note. All analysis included the covariates: tibia length and accelerometer wear time, not depicted in these diagrams. MVPA = moderate-to-vigorous physical activity. MSF = muscle specific force.
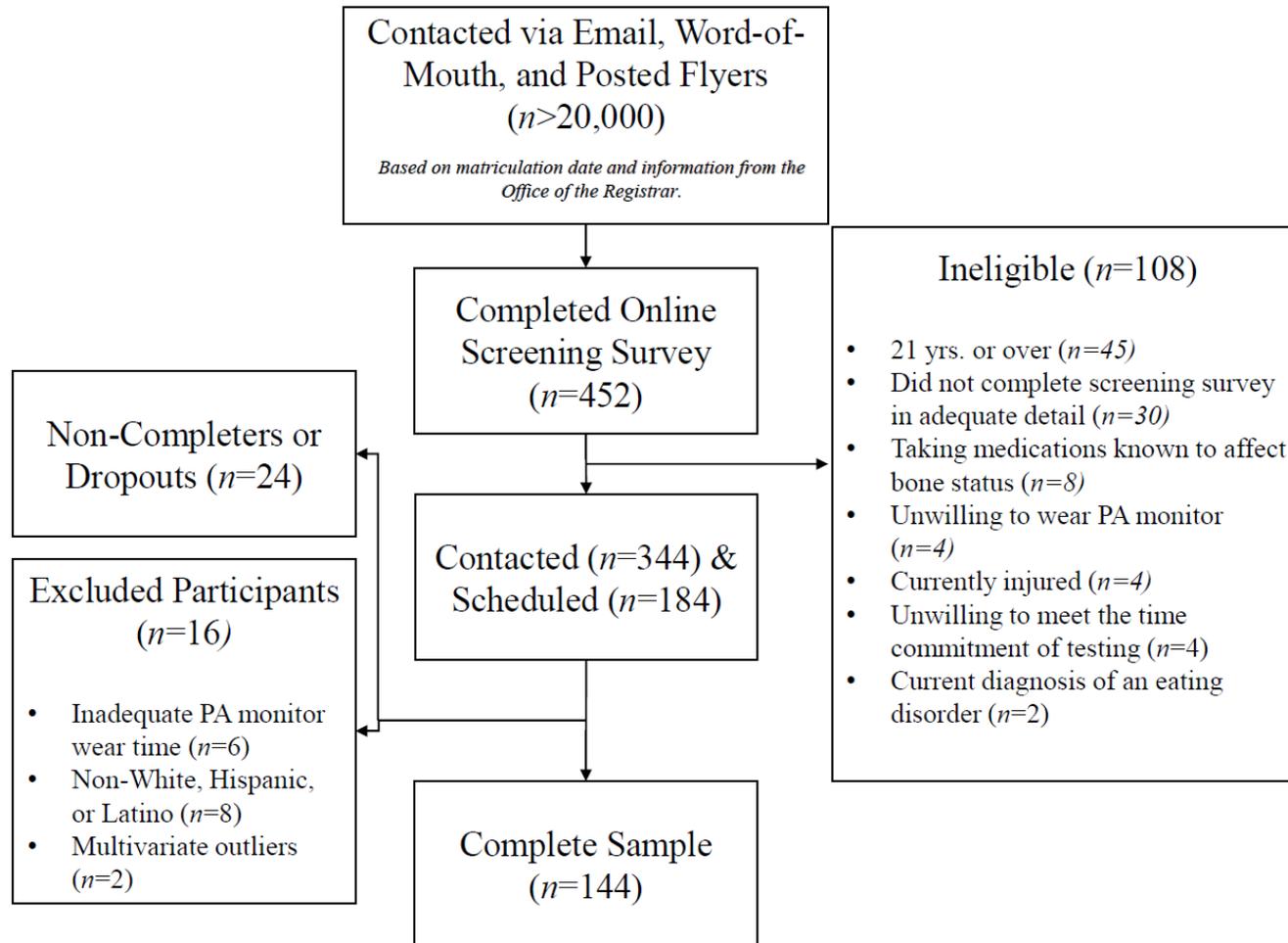
Figure 3.1. Participant recruitment flow chart.
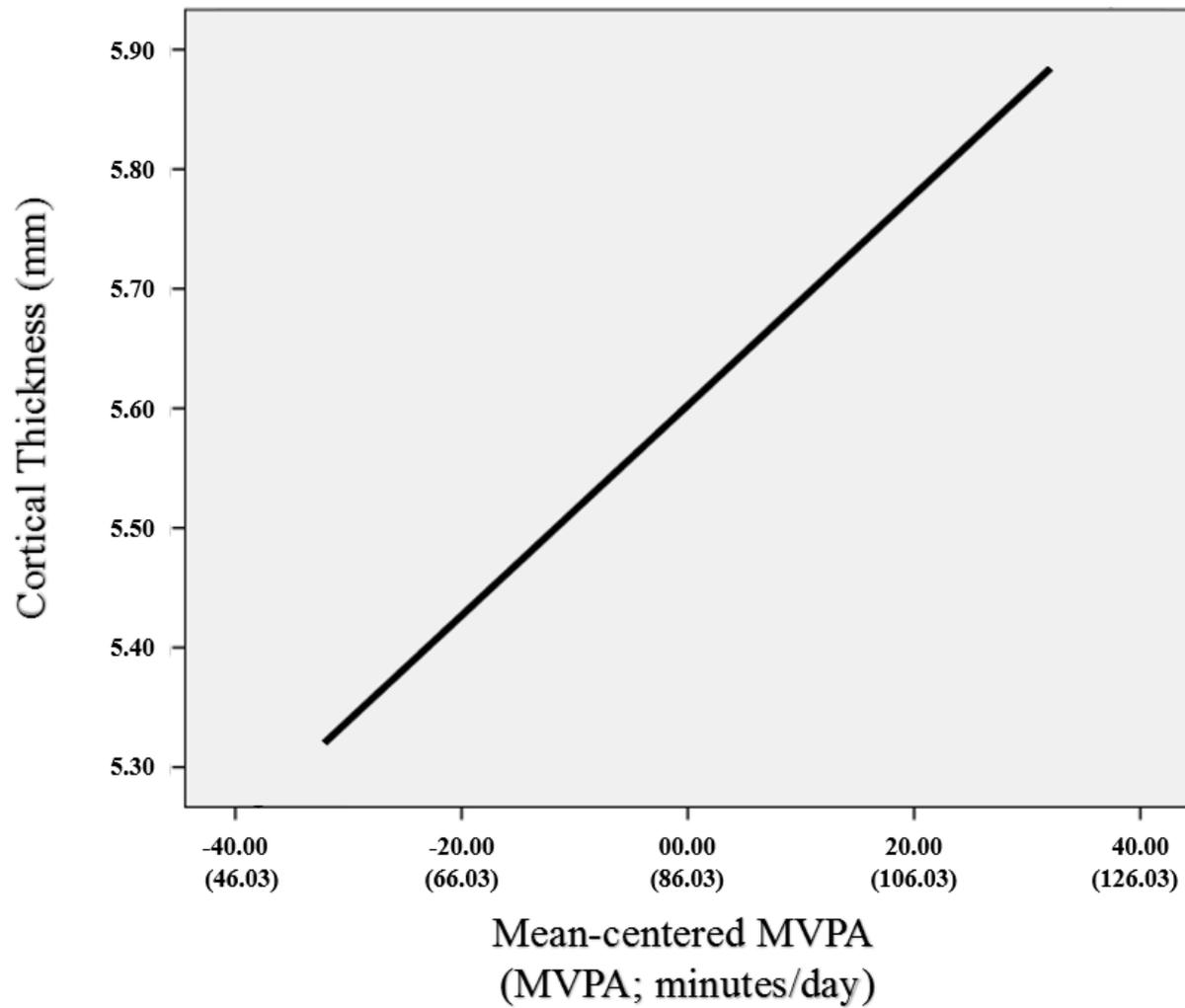
Figure 3.2. Depiction of the positive relationship between MVPA and cortical thickness in females. Note. MVPA = moderate-to-vigorous physical activity.

CHAPTER 4

COMPARISON OF MULTIPLE MEASURES AND SURROGATES OF MUSCLE FORCE

CAPACITY IN PREDICTING DIAPHYSEAL CORTICAL BONE STATUS[1]

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[1]Higgins, S., Sokolowski, C.M., Vishwanathan, M., Anderson, J.G., Schmidt, M.D., Lewis, R.D., and

Evans, E.M. To be submitted to *Medicine & Science in Sports & Exercise*.

4.1   Abstract

Muscle cross sectional area (MCSA) is often used as a surrogate for the forces applied to bones during physical activity. Though MCSA has consistently been shown to be a strong predictor of cortical bone status, it makes assumptions about the linear relationship between muscle size and force production that are not entirely accurate. Furthermore, to measure muscle size and mass, expensive clinical laboratory equipment is needed and patients are often exposed to radiative methodologies such as dual energy x-ray absorptiometry which is a cause for concern for populations such as youths. Therefore, examining the utility of lab-based measures and field-based estimates of muscle specifc force in this context is pertinent. The aims of this study were two-fold: 1) to determine whether clinical lab-based methodologies for measuring muscular force capacity account for equal variance in diaphyseal cortical bone status as a commonly used muscular force surrogate; MCSA, at the mid-tibia in young men and women, and 2) to determine whether a non-invasive, feasible field measure of muscle force capacity; the vertical jump, accounts for equal variance in cortical bone status as corresponding clinical lab-based methodologies of muscle force capacity, in young men and women. Healthy males and females (n=142, 19.7±0.7 yo) were assessed via peripheral quantitative computed tomography at the mid-tibia for diaphyseal cortical bone status and MCSA. Muscle force capacity was measured via lab-based (Biodex dynamometer & Nottingham Leg Extensor Power Rig) and field (Vertec vertical jump) measures. Stepwise linear regression examined the independent variance predicted by each muscle force measure in comparison to MCSA, accounting for relevant confounders. MCSA, knee extension peak torque, and peak anaerobic power from vertical jump were all significant independent predictors of cortical structure with final models accounting for up to 78.4% of the variance explained depending on the measure (all p<.05). However, $vBMD_{cort}$ was unrelated to any measure or surrogate of muscle force capacity. In summary, MCSA remained a strong independent predictor of cortical bone status, however; both clinical

measures and field-based estimates of muscle force capacity are promising alternatives, explaining similar, and sometimes greater, variance than MCSA.

4.2   Introduction

Muscle and bone are inextricably linked, so much so that debate has surrounded whether the clinical outcomes of osteoporosis and sarcopenia are in fact the same disease (34). This relationship between muscle and bone begins in development with similar cellular origins and continues through growth into senescence, with the term 'the muscle-bone unit' being used to describe both the mechanical and biochemical coupling of the tissues (10, 18, 35). Much research has focused on the mechanical aspect of the muscle-bone unit as muscle force is suggested to account for many of bones adaptive responses, owing to the fact that internal muscle forces are the highest likely to be experienced by the skeleton, trauma aside (35). These forces, in the context of physical activity (PA), have highlighted potential avenues for the augmentation of bone accrual and prevention of osteoporosis later in life (42).

When assessing the effects of PA on bone strength, researchers often use absolute muscle mass or size as surrogates for the forces applied to bones by muscle due to the link between muscle cross sectional area (MCSA) and force production (39). However, the reasoning that muscle size and force output are interchangeable in their association with the forces applied to bone is flawed (3), with one of several reasons for this being that the potential to produce force in two muscles of the same size is dependent on factors such as their fiber type distribution, with fast-twitch isoforms producing 40-70% greater isometric forces than slow-twitch isoforms (24). Furthermore, to measure muscle mass accurately requires expensive clinical laboratory equipment and patients are often required to undergo methodologies that expose them to x-ray radiation, with risks in children being substantially higher than adults due to their greater susceptibility to the risk of radiation-induced biological effects coupled with a greater dose received in a majority of scans (15).

Thus, there is a need to compare the utility of different muscle force capacity measures against currently used surrogates to identify non-invasive, feasible measures that might be used within the clinical and research settings in place of often costly or radiative methods of assessing muscle mass (22). In this context, the primary aims of this study were two-fold: 1) to determine whether clinical lab-based methodologies for measuring muscular force capacity such as the Nottingham Leg Extensor Power Rig and the Biodex dynamometer account for an equal portion of variance in diaphyseal cortical bone status as a commonly used muscular force surrogate; MCSA, at the mid-tibia in young men and women, and 2) to determine whether a non-invasive, feasible field measure of muscle force capacity; the vertical jump, accounts for an equal portion of variance in cortical bone status as corresponding clinical lab-based methodologies in young men and women. We hypothesized that: a) muscle force capacity derived from clinical lab-based methodologies would predict at least as much variance in cortical bone status as the corresponding site-specific MCSA, and b) muscle force capacity derived from vertical jump scores would predict at least as much variance in cortical bone status as the clinical lab-based methodologies.

4.3   Methods

Recruitment and Participant Characteristics. This cross-sectional analysis included participants recruited between fall 2016 and spring 2017 at a major University in the southeast United States. Recruitment consisted of mass emails to university-provided accounts, posted flyers, and word-of-mouth advertising. All participants (*n*=146; *n*=77 female) were white, non-Hispanic or Latino due to the known effect of race on modeled variables, and age was restricted to 18 – 21 yo to ensure a similar skeletal maturation (>90% bone accrual). Participant recruitment and the final analyzed sample size is outlined via flow chart in Figure 4.0. Inclusion criteria required participants to a) be currently enrolled University of Georgia student, b) be free of orthopedic limitations that precluded participation in PA, c) not be current smokers (past 6 months), d) not be pregnant or planning to become pregnant for the duration of their

participation, and had not given birth in the last 12 months, e) not taking medications known to affect

bone metabolism (i.e. glucocorticoids), habitual dietary intake, or PA, f) be free of any medical conditions

know to affect bone metabolism (i.e. Crohn's Disease), g) not currently be diagnosed with an eating

disorder, and h) not have undergone recent weight loss surgery (bariatric or gastric bypass). All

participants provided written informed consent prior to participation. The Institutional Review Board of

the university approved all aspects of the protocol.

Anthropometric Measures. Body mass and standing height were measured via digital scale (Seca

Bella 840, Columbia, MD) and stadiometer (Novel Products Inc., Rockton, IL) to the nearest 0.1 kg and

0.1cm, respectively. Tibia length was measured with a sliding anthropometer to the nearest 0.1cm as the

distance from the distal edge of the medial malleolus to the tibial plateau.

Physical Activity. Objectively measured PA was assessed over a ≥7-day period using a tri-axial

ActiGraph GT3X+ accelerometer (Firmware v3.2.1.) utilizing 15-s epochs. Participants wore the

accelerometer on the mid axillary line of their right hip during all waking hours, except during water-

based activities (e.g. showering and swimming). Data were considered valid if the participant had accrued

≥10 hours/day of data on at least two weekdays and one weekend day, determined via the wear time

macro developed by the National Cancer Institute (CDC/National Center for Health Statistics) from the

Troiano algorithm (40). Periods with consecutive raw activity values of zero (with a 2-min spike tolerance

of ≤100 counts) for 60 min or longer were interpreted as "non-wear" and excluded from this analysis (11).

Data analyses were performed using Actigraph Software (v6.10.1; Actigraph, LLC, Fort Walton Beach,

FL), with 15-s epochs and the VM3 vector magnitude approach (37). The VM3 cut points were 0-2690

for light, 2691-6166 for moderate, ≥6167 for vigorous, and ≥2691 for MVPA. A weighted average PA

time [(weekday average *5) + (weekend average *2) /7] was used to represent mean weekly activity

variables.

Bone Assessments by pQCT. Peripheral quantitative computed tomography (pQCT; Stratec XCT-3000; Stratec Medizintechnic GmbH, Pforzheim, Germany) scans were taken at sites 38% and 66% of length of the non-dominant tibia using the distal metaphysis as the anatomical marker. Scans utilized a 0.4-mm voxel and slice thickness of 2.4-mm. A software-automated scout view was used to position the measurements, using the medial end plate as an anatomic reference. The following variables were derived from the 38% site scans: cortical volumetric bone mineral density (vBMD$_{Cort}$; mg/mm^3), cortical area (Ct.Ar; mm^2), cortical thickness (Ct.Th; mm), periosteal circumference (Peri.Circ; mm), endosteal circumference (Endo.Circ; mm), and polar strength-strain index (pSSI; mm^3). The 66% site scans were taken to acquire MCSA (mm^2), which was determined via automated analyses which utilizing edge detection, threshold techniques, and image filters to separate tissues, as described previously. Cortical bone parameters were assessed using cort mode 2 and the default threshold of 710 mg/cm^3, except for SSI which utilized a threshold of 480 mg/cm^3. All pQCT measures were performed and analyzed by one operator who was trained for acquisition and analysis following guidelines provided by Bone Diagnostic (Spring Branch, TX, USA), using Stratec software (version 6.20). The manufacturer supplied phantom was scanned daily to maintain quality assurance (Stratec Medizintechnik GmbH, Pforzheim, Germany).

Muscle Force Capacity. Muscular force output was assessed using several methods to characterize both strength and power, utilizing clinical and field-based methodologies. First, the Nottingham Leg Extensor Power Rig (Medical Engineering Unit, University of Nottingham Medical School, Nottingham, UK) was used as a clinical measure of lower body power (7). Participants pushed out on a pedal with the non-dominant leg as hard and fast as possible. Up to 10 trials were performed until a plateau in force output was achieved or all 10 trials were completed. Second, a Biodex System Pro 4 dynamometer (Biodex Medical Systems, INC., New York) was used as the clinical measure of muscular isokinetic strength at the knee and ankle joints. Participants were positioned per manufacturer guidelines and performed five maximal effort voluntary contractions at 60 deg/sec to assess peak torque (N·m) of

their non-dominant side. Ankle dorsiflexion and knee extension via dynamometry have been found to be highly reliable in young men and women (8), and were chosen as the major force outcomes as they reflect muscle-bone units with direct force transfer onto the tibia. Finally, the Vertec vertical jump system (Vertec, USA) was used as a field measure of peak anaerobic power. In short, participants were asked to jump as high as possible, tapping the rotating flags on the instrument to register jump height. The highest possible vertical jump was assessed over five attempts and peak power was obtained using the Sayer's equation (37), Peak Power (W) = 60.7 x (jump height cm]) + 45.3 x (body mass [kg]) - 2055. Jump height from Vertec testing is strongly correlated (r=.91) with criterion methodologies such as three-camera motion analysis (23). A single set of familiarization trials were performed of each movement within all methodologies prior to testing.

Dietary Calcium & Vitamin D. To assess micronutrients known to influence bone status, specifically dietary calcium (mg/day) and vitamin D_3 (IU/day) intakes, a 3-day diet record required participants to log all foods consumed over two weekdays and one weekend day. Trained interviewers utilizing food models to aid in estimation of portion sizing interviewed participants prior to receipt of the complete log. Dietary data were analyzed using the Nutrition Data Systems for Research software (University of Minnesota, Minneapolis, MN) and dietary supplements were included within all estimates based on responses to a simple questionnaire. All dietary data were checked by another trained interviewer for quality control.

Statistical Analysis. Statistical analyses were performed using SPSS for Windows (SPSS 22.0, Chicago, IL) with an α level of p<.05. The normal distribution of residuals, linearity, and homogeneity of variance were examined across all combinations of outcome variables. Multi-collinearity between independent variables was also assessed via variance inflation factor (VIF), with a VIF of <10 indicating the absence of collinearity (27); no variables met this criteria. Participants (n=4) were excluded from analysis after being deemed influential multivariate outliers based on Mahalonobis distance critical values

for a chi squared distribution with k degrees of freedom (k = number of predictors in the model), leaving the final analyzed sample at $(n=142)$.

Means and standard deviations were calculated for all participant characteristics and primary outcome variables. Independent t-tests then identified any differences between sexes for descriptive purposes and partial correlations assessed the relationship among proposed predictors and outcome variables, controlling for sex and age. Potential covariates were then entered into a regression model in a stepwise manner to assess their relationship with cortical bone status. Two potential covariates (calcium, mg/day and vitamin D_3 IU/day) were identified as having no relationship with any bone status variables in any model so were not included in final analysis. A base model predicting cortical bone status was assessed using multivariate regression, including the predictors: age (years), sex (female =0, male =1), tibia length (cm), body weight (kg), moderate-to-vigorous PA (MVPA; minutes/day). Muscular force variables and MCSA were then entered in a stepwise manner, assessing the individual predictive value of each variable and comparing the independent variance accounted for via squared semi-partial correlations. All results are presented as standardized beta coefficients (β) to aid in comparison between predictors and 95% confidence intervals are presented to highlight significant effects. All data are expressed as mean ± standard deviation (M±SD), unless otherwise indicated.

Adequate power to detect an effect was ensured through the use of G-power to run an a priori power analysis examining an R^2 increase in a fixed linear multiple regression model. Utilizing a 7-predictor model with a power of .8 and α=.05, to test the effect of adding of one of these predictors to the model with an expected effect size of $f^2=.15$ (moderate), a sample size of $n=55$ is required. As previous research suggests that partial correlations between muscle torque and cortical bone status outcomes, controlling for measures of body stature and maturity, range between $r=.56$ and .62 ($f^2=.45$ to .61), (22) we believed that this was a very conservative estimate. Thus, we were confident that we had adequate power for the analysis.

4.4   Results

Recruitment. In total (*n*=452) potential participants were screened via an online survey during Fall 2016 and Spring 2017. As depicted in Figure 4.0, (*n*=108) individuals were excluded based on inclusion/exclusion criteria, leaving (*n*=344) potential participants. Pre-scripted emails were sent to each pre-screened participant which led to (*n*=184) being scheduled for testing and (*n*=154) following through to study completion. The (*n*=30) participants lost between scheduling and completion either dropped out for reasons not disclosed (*n*=24) or did not accrue enough PA data to meet minimum usage standards (*n*=6). Due to the known moderating effect of race on cortical bone status, only white non-Hispanic or Latino participants were included (*n*=146), and with the (*n*=4) multivariate outliers also being removed, the final analyzed sample was determined (*n*=142).

Preliminary Analysis – Descriptive Statistics & Partial Correlations. Combined and sex-specific descriptive data are reported in Table 4.0 and Table 4.1. MVPA was similar between sexes with both groups exceeding public health PA recommendations (41). As expected, males were greater in stature and mass than females, with cortical bone status mirroring whole body differences. Bone outer diameter was 12.8% larger in males as reflected by Peri.Circ, and, although their medullary cavity was an average of 11.0% wider and $vBMD_{cort}$ 2.4% lower than their female counterparts, Ct.Th and pSSI suggested that their bones were 14.7% thicker and 40.2% stronger, respectively, compared to females (all *p*<.05). Direct measures of muscular force capacity and MCSA followed a similar pattern, with males having a 18.2% greater mid-tibial MCSA, and 50.6%, 58.0%, 47.9%, 64.1% greater lower body power, peak knee extension torque, peak ankle dorsiflexion torque, and peak anaerobic power, respectively, compared to females (all *p*<.05).

Partial correlations between predictors and cortical bone status are shown in Table 4.1, each of which control for age and sex. All body stature and muscle force predictors were negatively related to $vBMD_{cort}$, with relationships ranging from *r*=-.19 with lower body power, to *r*=-.28 with tibia length

($p<.05$), however, minutes per day of MVPA and MCSA were not significant predictors of vBMD$_{cort}$.

Contrary to the negative relationships observed with bone mass, bone structural variables were all

positively related to both body stature and muscle force variables, including MCSA. Tibia length and

body mass were positively related to all bone structural outcomes, with relationships ranging from $r=.17$

to $r=.61$ for tibia length and Ct.Th, and body mass and Peri.Circ, respectively (all $p<.05$). Furthermore,

muscle force estimates and MCSA were positively related to all cortical bone structural measures except

Endo.Circ, with the strongest of these relationships being between peak anaerobic power and Ct.Ar

($r=.63$, $p<.001$), and knee extension peak torque and Ct.Ar ($r=.59$, $p<.001$). Finally, MVPA was

positively related to Ct.Th ($r=.21$, $p=.013$) and a positive relationship with Ct.Ar approached significance

($r=.15$, $p=.074$), however, it was unrelated to any other bone structural variable.

Primary Analysis – Base Model & MCSA. The baseline model predicting cortical bone status is

presented in Table 4.2 and followed a similar pattern to partial correlations, with mechanical variables

(tibia length, body weight, and MVPA) negatively predicting vBMD$_{cort}$ and positively predicting cortical

bone structure, independent of other base model predictors. A one year increase in age predicted a .202

SD increase in vBMD$_{cort}$, but 95% confidence intervals suggested that age was unrelated to cortical bone

structure in this sample, likely due to the narrow age range recruited. Furthermore, as sex was coded as

female=0 and male=1, standardized beta (β) values represent the relationship in males, with male sex

being negatively related to vBMD$_{cort}$ (β=-.379, $p<.001$) but positively related to all bone structural

outcomes except Endo.Circ (β=.120, $p=.216$), as mirrored in descriptive data in Table 4.1. The baseline

model accounted for 43.9% of the variance in vBMD$_{cort}$ and between 29.0% (Endo.Circ) and 74.3%

(Peri.Circ) of the variance in cortical bone structural variables.

MCSA was added to the baseline model to assess the independent portion variance accounted for

by muscle size when used as a surrogate for muscle force. Model fit increased by 6.6%, 8.8%, 3.0%, and

4.3% for Ct.Ar, Ct.Th, Peri.Circ, and pSSI, respectively (all $p<.05$), however MCSA was not a significant independent predictor of $vBMD_{cort}$ or Endo.Circ (both $p>.05$).

  Primary Analysis – Nottingham Leg Extensor Power Rig. Models which build upon the baseline model by including muscle force capacity measures are reported in Table 4.3. Model 3 represents the individual portion of variance accounted for by each measure of muscle force when individually added to the base model, without accounting for MCSA. None of the measures of muscle force predicted $vBMD_{cort}$ either individually or when combined with MCSA, so the data for bone mass was not reported. When lower body power was used as the measure of muscle force no improvements in model fit were seen above baseline, as 95% confidence intervals spanned zero for all cortical bone status outcomes. Furthermore, when both lower body power and MCSA were included in the model together (model 4), lower body power remained a non-significant predictor of any cortical bone status outcome, independent of MCSA (all $p<.05$). Moreover, MCSA maintained a similar portion of variance explained at each structural site, independent of lower body power, as it predicted individually in model 2 with R^2 values also remaining within ~1%, suggesting that lower body power did little to improve the predictive value of the model.

  Primary Analysis – Biodex Dynamometer. Two measures of muscle force were derived from the Biodex dynamometer; peak ankle dorsiflexion torque and peak knee extension torque. When each was added to the baseline model individually, no relationship was present between either measure of force and Endo.Circ, however, knee extension torque emerged a significant predictor of all other diaphyseal cortical structural measures ($\beta=.247$ to $.501$, all $p<.05$). The addition of knee extension torque to the baseline model independently accounted for 3.73%, 5.91%, 1.44%, and 2.25% of the variance in Ct.Ar, Ct.Th, Peri.Circ, and pSSI, respectively. Furthermore, when MCSA was also added to the model these relationships were attenuated but remained significant, with some variance being shared between the two predictors. Together with the baseline model, the inclusion of knee extension torque and MCSA

accounted for 78.4% of the variance in pSSI, 78.3% of the variance in Peri.Circ, 76.1% of the variance in Ct.Ar, and 53.5% of the variance in Ct.Th, with the independent contributions of each muscular variable to each model being 1.6% and 3.7%, 1.0% and 2.6%, 2.8% and 5.6%, and 4.5% and 7.5%, for knee extension torque and MCSA respectively (all $p<.05$).

Primary Analysis – Vertec Vertical Jump System. The final measure of muscular force to be assessed was peak anaerobic power. Independent of baseline model variables, peak anaerobic power was a positive predictor of Ct.Ar, Ct.Th, and pSSI, and a negative predictor of Endo.Circ, accounting for 3.46%, 9.67%, 3.17%, and 2.13% of the variance in each, respectively. Interestingly, once MCSA was added to the model, not only did peak anaerobic power remain a significant predictor for each of the previously listed structural outcomes but also the magnitude of variance accounted for by peak anaerobic power was greater than when MCSA predicted Ct.Th (5.52% vs. 4.71%, respectively), and MCSA did not significantly predict Endo.Circ ($\beta=-.070$, $p=.549$). In contrast, MCSA accounted for a greater proportion of variance in Ct.Ar and pSSI than peak anaerobic power (4.00% and 2.99%, vs. 2.92% and 0.79%, respectively), and was a significant predictor of Peri.Circ ($\beta=.235$, p=.001) whereas peak power was not.

4.5 Discussion

The primary aims of this study were two-fold: 1) to determine whether clinical lab-based methodologies for measuring muscular force capacity such as the Nottingham Leg Extensor Power Rig and the Biodex dynamometer account for an equal portion of variance in diaphyseal cortical bone status as a commonly used muscular force surrogate; MCSA, at the mid-tibia in young men and women, and 2) to determine whether a non-invasive, feasible field measure of muscle force capacity; the vertical jump, accounts for an equal portion of variance in cortical bone status as corresponding clinical lab-based methodologies in young men and women. We hypothesized that: a) muscle force capacity derived from clinical lab-based methodologies would predict at least as much variance in cortical bone status as the

corresponding site-specific MCSA, and b) muscle force capacity derived from vertical jump scores would

predict at least as much variance in cortical bone status as the clinical lab-based methodologies. The

major findings of our analysis were that our primary hypothesis was rejected as none of the lab-based

measures of muscle force capacity predicted as much variance in cortical bone status as MCSA. However,

the measure of knee extension peak torque did predict variance in cortical bone status which was both

shared with- and independent of MCSA. Other clinical lab-based methodologies were unrelated to

cortical bone status when accounting for baseline covariates. Furthermore, a field estimate of muscle

force capacity derived from vertical jump height predicted cortical bone status to an equal and/or greater

extent than both Biodex knee extension torque and MCSA, depending on the bone status measure

assessed. This does not directly support our second hypothesis as the closest lab-based methodology to

the vertical jump was the Nottingham Leg Extensor Power Rig due to the similar lower body extensors

and mechanics involved in each movement. However, our data are novel as they provide basis for the

vertical jump to be used as a potential field-based measure of muscle force capacity for predicting cortical

bone status in young adults, without the need for expensive radiative methodologies for attaining muscle

mass. Moreover, the results add to previous research by comparing the predictive utility of multiple

commonly used and validated measures of muscle force capacity in a highly active population who are

not affected by maturational or major physical function differences.

Muscle Force Surrogate – MCSA. Since the discovery that bone mass and structure respond

directly to the forces placed upon it, (36) it has become commonplace for researchers to use mass or size

based surrogates of muscle force capacity, such as MCSA or total lean mass, to account for the

contribution of muscular forces in the prediction of bone status (13, 20, 26, 29, 39). This stems from data

showing strong relationships between measures of muscle size and corresponding bone status indicators

(20, 31), with correlations exceeding r=.81 in some reports (13, 20, 22). Our results agree with previous

research in that MCSA was one of the strongest predictors of cortical bone geometry, independent of

baseline mechanical and anthropometric predictors and, contrary to many dual energy x-ray absorptiometry (DXA)-based reports, MCSA was not a significant predictor of cortical density. One such example is a recent study by Wetzsteon et al. who employed similar methodologies for the assessment of MCSA at the mid-tibia to the present study in males and females (n = 31) with an average age of 16.5 yo (43). They found that independent of age, sex, race, tibia length, and maturity, MCSA independently predicted cortical bone geometrical properties with values of model fit up to R^2=.91, furthermore, they did not see a relationship between MCSA and cortical density in any model. Although reports such as these suggest that muscle size and mass measures are reasonable surrogates for muscle force in the prediction of cortical geometry, (14, 43) they over simplify the muscle-bone relationship with regards to loading. This oversimplification is eloquently highlighted in a study comparing the side-to-side differences between playing and non-playing arms in female tennis players (13). In this cohort, PA induced differences between forearm bone status as measured by magnetic resonance imaging were of the magnitude of 6-13%, and although these differences were related to muscle area (r=.36 to .40, p<.05), only 11.8-15.9% of the variance was independently accounted for by differences in muscle area. This example illustrates how, especially in an active cohort, many factors such as muscle fiber type distribution and pennation angle, motor unit activation, tendon length, and the site of insertion onto bony surfaces could also contribute to a muscle's force producing capacity as well as the transfer of this force onto bone surfaces (3). Thus, when aiming to characterize the mechanical effects of muscle on bone, the lack of specificity highlighted above, as well as the potential for radiation exposure that usually accompanies muscle force surrogates provides a convincing argument against their use when specific measures of force are available. These radiation doses have been reported to range between $1.4 - 27$ µSv (2.8 - 54.0% of the recommended yearly dose) depending on the methodology use, participant age, and number of scans required, (1, 5, 15). In this same way researchers have begun to examine the specific

utility of individual muscle force measures in the prediction of bone status by comparing their combined and independent predictive ability to that of muscle force surrogate measures (2, 6, 14, 22, 43).

Muscular Force Capacity. A number of methodologies have been used to examine the predictive capacity of muscle force on bone status including the Nottingham Leg Extensor Power Rig (4, 12), the Biodex dynamometer (25, 43), peak handgrip force (22, 25), peak ground reaction force (GRF) via force plates (19, 28, 32, 33), the Wingate Anaerobic Power Test (44), and vertical jump assessments (6, 22), each measuring or estimating a different aspect of muscular force capacity at a specific muscular site across multiple populations. Few studies have directly compared these measures of force, with studies examining both handgrip and lower body power suggesting similar utility for predicting differences in bone status (4), or greater differences in bone status between the lowest and highest quartiles of handgrip force when compared to leg power (5% vs. 13%, for leg power and grip strength, respectively) (12). However, these measures examined different areas of the body (upper vs. lower) and are not directly comparable in the context of PA related mechanical forces which are predominantly applied to the lower body. Other studies have compared peak torque at the knee assessed via dynamometry to vertical jump (14, 28) or the Wingate Anaerobic Power Test (44) across multiple populations, again with conflicting findings. In pre-pubertal girls (*n*=103) isokinetic peak torque but not vertical jump height independently predicted whole leg and femoral neck bone mineral content (BMC), accounting for an additional 2-5% of the variance after controlling for lean mass (14). Contrary to this, in older women (*n*=139; aged 50-68 yo) with mild knee osteoarthritis after adjustment for relevant confounders, both peak countermovement jump power and knee extension force were among the strongest independent predictors of bone strength with comparative effect sizes of standardized β=.30 to .32 (*p*<.01) (28). These differing results are likely related to population differences in both physical function and bone status as well as methodological differences in muscle force and bone status assessment.

Clinical Methodologies – Nottingham Leg Extensor Power Rig. Contrary to previous research focused on middle-aged and older adults (4, 12), our data suggested that lower body power did not predict any facet of diaphyseal cortical bone status, despite positive partial correlations with cortical structural variables after controlling for age and sex. When MCSA and lower body power were both included within the model, model fit was like that of MCSA alone suggesting that leg power is not a meaningful predictor of cortical bone status in this population. The first of the two studies to examine lower body power and the relation to cortical bone status in older populations used a twin-study design which examined *n*=706 postmenopausal women aged 58.75±5.35 yo (4). Using DXA to assess BMC, leg power accounted for individual non-genetic variance as shown by a decrease in total additive variance once leg power was introduced into the model. The second and only other study to our knowledge to use lower body power to predict bone status did so using pQCT-derived cortical and trabecular bone indices in older men (*n*=1171; aged ≥65 yo). Participants were divided into quartiles based on leg power, with men in the highest (average lower body power; 254.5±34.1 W) compared to those in the lowest quartile (average lower body power; 108.5±20.4 W) having 5% greater total area and 5.3% greater bone strength index at the 4% site, and 3% greater total area along with 5% greater pSSI at 66% site, whilst controlling for clinic, age, race, weight, and limb length (12). Despite previous positive findings, the lack of research examining the relationship between lower body power from the Nottingham Leg Extensor Power rig and bone status in young healthy samples makes comparison to our current data difficult.

Clinical Methodologies – Biodex Dynamometer. Contrary to measures of lower body power, peak torque derived from dynamometry has been assessed as a predictor of both DXA-based and pQCT-derived bone status in a wide range of populations from youths (14, 16, 17, 29, 44) to young and older adults (28, 43). However, due to the wide range of populations, variability in joint assessed (knee, hip, and ankle) and muscle actions (concentric vs. isometric) used to acquire peak torque, as well as the small

number of studies employing pQCT based bone status measures, comparison of results remains challenging.

Our data suggesting that knee extension peak torque is a significant predictor of cortical bone geometric properties, accounting for variance independent of MCSA, agrees with one other study in which knee extension torque was used to predict pQCT-derived cortical bone status. In this study knee extension torque was the strongest predictor of distal tibia bone strength index (β= .30, p<.001) after adjustment for height, weight, and age in post-menopausal women (n = 139; 62±4 yo) with mild knee osteoarthrosis (28). Though these results mirror the effect size seen in our bone strength predictive models (pSSI; β= .31, p=.001), major limitations of comparing with our present study, population differences aside, are that: 1) their models did not account for any measure of lean mass or limb length; both of which are important predictors of cortical bone status, 2) only results at the distal tibia and not mid-tibia were reported, and 3) measures of knee extension torque were derived from isometric contractions which inhibits direct comparison with isokinetic contraction derived torque.

A similar difficulty arises when comparing the current ankle dorsiflexion results with the only paper to have assessed peak torque at this joint in the context of pQCT-based cortical bone mass, strength, and geometry prediction. Wetzsteon et al. utilized isometric peak torque during ankle dorsiflexion in a heterogenous sample (n=321; males and females aged 5 to 35 yo) and found, contrary to our results, that dorsiflexion peak torque significantly predicted both strength and structural cortical bone variables independent of MCSA, accounting for 92% of the variance in a combined model with MCSA, body weight, tibia length, and other baseline variables. However, similar to our findings ankle dorsiflexion was not related to vBMD$_{cort}$ (43). These results are intriguing and warrant further exploration as our data suggest that knee extension but not ankle dorsiflexion peak torque was associated with tibia diaphyseal cortical bone status. One explanation could be that in such a highly active sample as the present study, the greater involvement of knee extensors in eccentric contractions associated with jumping and sporting

movements, due to their crossing of the hip and knee, leads to forces arising from the quadriceps being applied to the proximal tibia at a greater magnitude and frequency than from ankle dorsiflexors. This theory is founded in data showing that plantarflexor peak forces during multiple single-leg hopping amount to ≥9 times bodyweight due to the ~50% greater force output during eccentric movements, coupled with an unfavorable lever arm relationship associated with the foot and ankle joints (3), both could logically be translated to movement controlled by the knee extensors that would not apply to ankle dorsiflexors in conventional human movement. Furthermore, the wide age range in the prior study of 5 to 35 yo may have introduced greater variance to outcome measures, potentially highlighting relationships not seen in our narrow age range.

A larger body of research has examined the relationship between isokinetic measures of torque and DXA-derived measures of bone mass, structure, and strength (14, 16, 17, 29, 44). In a study where the results closely mirror our own, Daly et al. reported in pre-pubertal girls (n=103, 7.8±0.6 yo) both leg lean mass and isokinetic knee extension torque were independently and equally predictive of bone geometry and strength at the leg and femoral neck, as well as BMC (14). Despite bone status being measured in a non-volumetric fashion by DXA, comparable additional variance of 2-5% was accounted for by knee extension peak torque once leg lean tissue mass was included in the model concurrently, which mirrors results seen in our knee extension models of 1.04 – 4.49%. This study is not alone in reporting that peak torque is related to bone geometric properties, with others agreeing that hip flexion isokinetic peak torque predicted femoral narrow neck strength in pre-menarcheal girls (n=76, 10.5±1.6 yo) after adjustment for maturity, and stature (16). This relationship did not account for lean mass and was attenuated when PA was added to the model, unlike the present study. However, most DXA-based studies only assess relationships between peak torque and measures of bone mass, with one finding that knee peak torque was independently predictive of bone mass at all sites independent of lean mass in adolescent boys (n=26, aged 15.9±0.3 yo) (29), but others suggesting that relationships were attenuated

when lean mass was added to the model alongside peak torque in post-pubertal girls (*n*=54, aged 14.6±0.5 yo) (44), or failing to include mass based surrogates in the model with peak torque in a sample of mixed-event elite emerging adult female athletes (*n*=75, aged 15-18 yo) (17). Despite the methodological differences in assessing muscle force and bone status, dynamometry-derived measures of peak torque have shown strong, consistent relationships with bone status across multiple populations, suggesting its utility as a predictor of bone status, independent of muscle surrogates.

Field Methodologies – Vertec Vertical Jump System. Though clinical measures of muscle peak torque show promise in replacing muscle force surrogates in the prediction of bone status, field measures are required for population-level application. The vertical jump was chosen for this study due to its ease of application, low cost, and the strong emerging research backing its validity as a predictor of bone status in multiple populations (6, 19, 22).

The most exciting finding of the present study was that peak anaerobic power estimated from vertical jump height independently predicted cortical structure and strength, explaining more variance in Ct.Th than MCSA when both were included in the model simultaneously. This important support for the use of vertical jump as a field-based predictor of bone status agrees with recent findings using a similar population and equation for estimating peak power (22). Janz et al. also predicted peak anaerobic power using the Sayer's equation in adolescent males and females (*n*=303, 17.5±0.4 yo), with mediation models suggesting that peak power was a direct predictor of bone strength and cortical area at the 66% tibia in both males and females, independent of MCSA (22). Moreover, standardized effect sizes were of a similar magnitude in both studies with a 1 SD increase in peak power predicting a .38 and .15 SD increase in Ct.Ar (in males vs. females, respectively) found by Janz, compared to a combined sex increase of .39 SD in Ct.Ar in the current study; effect sizes for pSSI mirrored these similarities.

A novel finding from our analysis is the positive relationship between peak power and Endo.Circ which is of interest as it highlights potential mechanisms whereby peak muscle force being applied to

bone might be beneficial to bone strength; in a highly active sample, peak forces applied regularly to cortical structures could be stimulating bone resorption on endosteal surfaces which supports the migration of bone mineral to bone's outer surfaces for bone formation, increasing overall diameter and strength (9). Though both studies, including the present, used the same equation to predict peak power (38), other researchers have employed different equations based on sample age and have found equally promising results. Baptista et al. predicted peak power from vertical jump height using sex-specific equations in a group of prepubertal children (n=114, 55% male, 8.6±0.4 yo), finding that peak power predicted all DXA-derived bone status variables with reasonable sensitivity and specificity after adjustment for skeletal age, accounting for 74.3-77.0% of the variance in simple linear regression models (all p<.001) (6). Furthermore, logistic regression suggested that the odds of having a low BMD (≤1 SD below the mean) decreased 1.2% per watt, with sex being a salient factor. Two major limitations in this context are that the researchers did not include important baseline variables such as body stature or muscle as covariates when examining effects.

Although the previously mentioned studies support the use of vertical jump as a predictor of bone status, not all have shown similar positive results. Two studies employing raw vertical jump height (cm) as the predictor of bone status in healthy prepubertal girls (n=103, 7.8±0.6 yo; (14)) and healthy pre- and early pubertal boys and girls (n=424, 10.2±0.6 yo; (26)), found that it was not a significant predictor of bone status at any site (measured by both DXA and pQCT), whereas MCSA was the strongest predictor at a majority of the sites after adjustment for appropriate stature and developmental covariates. Thus, it would follow that when utilizing vertical jump as a field-based predictor of bone status one should apply an appropriately validated prediction equation to convert raw scores into peak power estimates.

In agreement with findings from field measures of jump power, a multitude of emerging research has examined the relationship between the forces associated with double and single leg jumping using clinical methodologies such as force plates, and bone status, finding consistently significant positive

results (2, 19, 20, 28, 32, 33). In a series of studies, Rantalainen et al. employed samples of young (n=20, 24.0±2.0 yo) and older (n=25, 72.0±4.0 yo) men (33), as well as young (n=221, 23.0±5.0 yo) and post-menopausal women (n=82, 58.0±1.0 yo) (32), to examine the relationship between pQCT-derived bone status and GRF during bilateral hopping. In young men peak GRF (r=.47, p<.05) but not muscle volume (r=.41, p=.07) was positively related to bone strength at the distal tibia, and similar relationships were seen for total area and section modulus at the mid-tibia (r=.63 and .59, respectively, all p<.01). Moreover, in all women regardless of age, impulse; a measure of peak instantaneous power, was a significant independent predictor of bone strength at the distal and mid-tibia accounting for 8% and 9% of the variance independent of height, body mass, and age. Similar relationships between GRF during a vertical jump and bone strength have been reported in other studies (19, 28), strengthening the hypothesis that the peak force produced during jumping might be used as a predictor of bone strength. Though results from clinical methodologies confirm those reported with field estimates predicted from vertical jump height, for the widespread application of this methodology to skeletal health assessment and prediction further research is needed in samples other than healthy youths and young adults.

Limitations and Future Directions. Despite the comprehensive comparison among predictors of cortical bone status, our study is not without its limitations. The narrow age and racial/ethnic demographic of this study, though a strength in increasing statistical power, precludes generalization of results to other populations. Future testing across diverse samples is required to confirm the predictive ability and operational safety of vertical jump testing, especially in clinical or older populations. With regards to the testing of muscle force capacity, a participant's motivation can heavily influence their effort level when asked to produce a maximal effort. We are confident that our data were minimally affected as all testing protocols were standardized with verbal and visual motivation given to all participants. Moreover, sensitivity analyses (data not shown) suggested no effect of including participant motivation as a continuous variable (1 – 10 scale; derived from verbal questions immediately following each test) in the

regression model. Moreover, concentric isokinetic dynamometry testing cannot assess true maximal muscle force that a muscle could theoretically produce as this occurs during eccentric contractions; producing an average of ~50% greater absolute torque. However, eccentric testing using dynamometers is notoriously highly variable and would likely not add any benefit over more reliable concentric measures (30). Finally, bone status is a result of mass and structural accrual throughout youth, especially during the pubertal period. Thus, cross-sectional research makes a somewhat flawed assumption that current physiological and behavioral measures are indicative of similar habits and trajectories throughout growth. To avoid this assumption, future research must assess relationships between muscle force capacity and cortical bone status using prospective or intervention research designs.

4.6   Conclusion

In summary, our data suggests that surrogates of muscle force capacity such as MCSA are strong independent predictors of cortical bone status; however, both clinical measures and field estimates of muscle force capacity are promising alternatives explaining similar, and in some cases greater, variance than MCSA. Importantly, peak anaerobic power predicted from vertical jump height emerged as the strongest of these alternative predictors for measures of Ct.Ar and Ct.Th, whereas isokinetic knee extension peak torque prevailed in the prediction of Peri.Circ and pSSI at the mid-tibia. Neither MCSA nor any muscle force capacity measure predicted $vBMD_{cort}$ which is contrary to previous findings using DXA-derived bone mass estimates. Future research should aim to apply these findings to other populations, especially those where undergoing radiative measures of muscle size and mass might be contraindicated or in situations where expensive clinical laboratory equipment is not available.

4.7   References

1.   Adams JE, Engelke K, Zemel BS, Ward KA. Quantitative computer tomography in children and adolescents: The 2013 ISCD Pediatric Official Positions. *J Clin Densitom*. 2014;17(2):258-74.

2.   Anliker E, Rawer R, Boutellier U, Toigo M. Maximum ground reaction force in relation to tibial bone mass in children and adults. *Med Sci Sports Exerc*. 2011;43(11):2102-9.

3.   Anliker E, Toigo M. Functional assessment of the muscle-bone unit in the lower leg. *J Musculoskelet Neuronal Interact*. 2012;12(2):46-55.

4.   Arden NK, Spector TD. Genetic influences on muscle strength, lean body mass, and bone mineral density: A Twin Study. *J Bone Miner Res*. 1997;12(12):2076-81.

5.   Ashby RL, Ward KA, Roberts SA, Edwards L, Mughal MZ, Adams JE. A reference database for the Stratec XCT-2000 peripheral quantitative computed tomography (pQCT) scanner in healthy children and young adults aged 6–19 years. *Osteoporos Int*. 2009;20(8):1337-46.

6.   Baptista F, Mil-Homens P, Carita AI, Janz K, Sardinha LB. Peak vertical jump power as a marker of bone health in children. *Int J Sports Med*. 2016;37(8):653-58.

7.   Bassey EJ, Short AH. A new method for measuring power output in a single leg extension: Feasibility, reliability and validity. *Eur J Appl Physiol Occup Physiol*. 1990;60(5):385-90.

8.   Blew RM, Lee VR, Farr JN, Schiferl DJ, Going SB. Standardizing evaluation of pQCT image quality in the presence of subject movement: Qualitative versus quantitative assessment. *Calcif Tissue Int*. 2014;94(2):202-11.

9.   Bouxsein ML, Karasik D. Bone geometry and skeletal fragility. *Curr Osteoporos Rep*. 2006;4(2):49-56.

10.   Brotto M, Bonewald L. Bone and muscle: Interactions beyond mechanical. *Bone*. 2015;80:109-14.

11. Choi L, Liu Z, Matthews CE, Buchowski MS. Validation of accelerometer wear and nonwear time classification algorithm. *Med Sci Sports Exerc*. 2011;43(2):357-64.

12. Cousins JM, Petit MA, Paudel ML et al. Muscle power and physical activity are associated with bone strength in older men: The osteoporotic fractures in men study. *Bone*. 2010;47(2):205-11.

13. Daly RM, Saxon L, Turner CH, Robling AG, Bass SL. The relationship between muscle size and bone geometry during growth and in response to exercise. *Bone*. 2004;34(2):281-7.

14. Daly RM, Stenevi-Lundgren S, Linden C, Karlsson MK. Muscle determinants of bone mass, geometry and strength in prepubertal girls. *Med Sci Sports Exerc*. 2008;40(6):1135-41.

15. Damilakis J, Adams JE, Guglielmi G, Link TM. Radiation exposure in X-ray-based imaging techniques used in osteoporosis. *Eur Radiol*. 2010;20(11):2707-14.

16. Dowthwaite JN, Rosenbaum PF, Sames CA, Scerpella TA. Muscle function, dynamic loading, and femoral neck structure in pediatric females. *Med Sci Sports Exerc*. 2014;46(5):911-9.

17. Duncan CS, Blimkie CJ, Cowell CT, Burke ST, Briody JN, Howman-Giles R. Bone mineral density in adolescent female athletes: Relationship to exercise type and muscle strength. *Med Sci Sports Exerc*. 2002;34(2):286-94.

18. Frost HM, Schonau E. The "muscle-bone unit" in children and adolescents: A 2000 overview. *J Pediatr Endocrinol Metab*. 2000;13(6):571-90.

19. Hardcastle SA, Gregson CL, Rittweger J, Crabtree N, Ward K, Tobias JH. Jump power and force have distinct associations with cortical bone parameters: Findings from a population enriched by individuals with high bone mass. *J Clin Endocrinol Metab*. 2014;99(1):266-75.

20. Heinonen A, McKay HA, Whittall KP, Forster BB, Khan KM. Muscle cross-sectional area is associated with specific site of bone in prepubertal girls: A quantitative magnetic resonance imaging study. *Bone*. 2001;29(4):388-92.

21.   Herrmann D, Buck C, Sioen I et al. Impact of physical activity, sedentary behaviour and muscle strength on bone stiffness in 2-10-year-old children-cross-sectional results from the IDEFICS study. *Int J Behav Nutr Phys Act*. 2015;12:112.

22.   Janz KF, Letuchy EM, Burns TL, Francis SL, Levy SM. Muscle power predicts adolescent bone strength: The Iowa Bone Development Study. *Med Sci Sports Exerc*. 2015;47(10):2201-6.

23.   Leard JS, Cirillo MA, Katsnelson E et al. Validity of two alternative systems for measuring vertical jump height. *J Strength Cond Res*. 2007;21(4):1296-9.

24.   Linari M, Bottinelli R, Pellegrino MA, Reconditi M, Reggiani C, Lombardi V. The mechanism of the force response to stretch in human skinned muscle fibres with different myosin isoforms. *J Physiol*. 2004;554(Pt 2):335-52.

25.   Lorbergs AL, Farthing JP, Baxter-Jones AD, Kontulainen SA. Forearm muscle size, strength, force, and power in relation to pQCT-derived bone strength at the radius in adults. *Appl Physiol Nutr Metab*. 2011;36(5):618-25.

26.   Macdonald H, Kontulainen S, Petit M, Janssen P, McKay H. Bone strength and its determinants in pre- and early pubertal boys and girls. *Bone*. 2006;39(3):598-608.

27.   Marquardt DW. Generalized inverses, ridge regression, biased linear estimation, and nonlinear estimation. *Technometrics*. 1970;12(3):591-612.

28.   Munukka M, Waller B, Multanen J et al. Relationship between lower limb neuromuscular performance and bone strength in postmenopausal women with mild knee osteoarthritis. *J Musculoskelet Neuronal Interact*. 2014;14(4):418-24.

29.   Nordstrom P, Thorsen K, Nordstrom G, Bergstrom E, Lorentzon R. Bone mass, muscle strength, and different body constitutional parameters in adolescent boys with a low or moderate exercise level. *Bone*. 1995;17(4):351-6.

30. Olmo J, Jato S, Benito J, Martin I, Dvir Z. Identification of feigned ankle plantar and dorsiflexors weakness in normal subjects. *J Electromyogr Kinesiol*. 2009;19(5):774-81.

31. Puthucheary Z, Kordi M, Rawal J, Eleftheriou KI, Payne J, Montgomery HE. The relationship between lower limb bone and muscle in military recruits, response to physical training, and influence of smoking status. *Sci Rep*. 2015;5:9323.

32. Rantalainen T, Nikander R, Heinonen A et al. Neuromuscular performance and body mass as indices of bone loading in premenopausal and postmenopausal women. *Bone*. 2010;46(4):964-9.

33. Rantalainen T, Sievanen H, Linnamo V et al. Bone rigidity to neuromuscular performance ratio in young and elderly men. *Bone*. 2009;45(5):956-63.

34. Reginster J-Y, Beaudart C, Buckinx F, Bruyère O. Osteoporosis and sarcopenia: Two diseases or one? *Curr Opin Clin Nutr Metab Care*. 2016;19(1):31-6.

35. Robling AG. Is bone's response to mechanical signals dominated by muscle forces? *Med Sci Sports Exerc*. 2009;41(11):2044-9.

36. Rubin CT, Lanyon LE. Kappa Delta Award paper. Osteoregulatory nature of mechanical stimuli: Function as a determinant for adaptive remodeling in bone. *J Orthop Res*. 1987;5(2):300-10.

37. Sasaki JE, John D, Freedson PS. Validation and comparison of ActiGraph activity monitors. *J Sci Med Sport*. 2011;14(5):411-6.

38. Sayers SP, Harackiewicz DV, Harman EA, Frykman PN, Rosenstein MT. Cross-validation of three jump power equations. *Med Sci Sports Exerc*. 1999;31(4):572-7.

39. Tan VP, Macdonald HM, Kim S et al. Influence of physical activity on bone strength in children and adolescents: A systematic review and narrative synthesis. *J Bone Miner Res*. 2014;29(10):2161-81.

40. Troiano RP. Large-scale applications of accelerometers: New frontiers and new questions. *Med Sci Sports Exerc*. 2007;39(9):1501.

41.   U.S. Department of Health and Human Services. *Physical Activity Guidelines for Americans*. Washington, DC: Office of Disease Prevention and Health Promotion; 2008. Available from: Office of Disease Prevention and Health Promotion.

42.   Weaver CM, Gordon CM, Janz KF et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: A systematic review and implementation recommendations. *Osteoporos Int*. 2016;27(4):1281-386.

43.   Wetzsteon RJ, Zemel BS, Shults J, Howard KM, Kibe LW, Leonard MB. Mechanical loads and cortical bone geometry in healthy children and young adults. *Bone*. 2011;48(5):1103-8.

44.   Witzke KA, Snow CM. Lean body mass and leg power best predict bone mineral density in adolescent girls. *Med Sci Sports Exerc*. 1999;31(11):1558-63.

Table 4.0. Descriptive characteristics (*n*=142)

	Total Sample	Males (*n*=67)	Females (*n*=75)	*p*
Age (years)	19.7 ± .7	19.6 ± .7	19.7 ± .8	.449
Body Mass (kg)	68.1 ± 11.1	**75.4 ± 9.3**	**61.7 ± 8.1**	**<.001**
Height (cm)	172.3 ± 8.8	**179.3 ± 6.3**	**166.0 ± 5.3**	**<.001**
Tibia Length (cm)	38.1 ± 2.6	**39.6 ± 2.3**	**36.7 ± 1.9**	**<.001**
Calcium (mg/day)*	1134.1 ± 531.1	**1347.1 ± 568.7**	**950.6 ± 420.5**	**<.001**
Vitamin D_3 (IU/day)*	230.6 ± 229.2	**317.7 ± 254.3**	**155.7 ± 174.6**	**<.001**
Muscle Cross Sectional Area (mm^2)	7454.7 ± 1159.0	**8113.4 ± 1103.9**	**6866.2 ± 853.7**	**<.001**
Lower Body Power (W)	215.7 ± 68.5	**262.3 ± 66.1**	**174.1 ± 36.6**	**<.001**
Knee Extension Peak Torque (N·m)	129.8 ± 38.6	**161.1 ± 30.2**	**101.9 ± 18.9**	**<.001**
Dorsiflexion Peak Torque (N·m)	18.6 ± 6.1	**22.5 ± 5.2**	**15.2 ± 4.8**	**<.001**
Peak Anaerobic Power (W)	3594.0 ± 1082.6	**4528.2 ± 712.0**	**2759.4 ± 533.7**	**<.001**
MVPA (mins/day)	89.8 ± 27.7	93.0 ± 27.8	85.9 ± 27.4	.130

Note. Values are mean ± SD. **Bold values** = significantly different between sexes at *p*<.05. MVPA = moderate-to-vigorous physical activity. **n*=134 for dietary outcomes due to missing data.

Table 4.1 Partial correlations between predictors and outcome variables (n=142)

	$vBMD_{cort}$ (mg/cm^3)	Ct.Ar (mm^2)	Ct.Th (mm)	Peri.Circ (mm)	Endo.Circ (mm)	pSSI (mm^3)
Tibia Length (cm)	**-.28[†]**	**.43[‡]**	**.17***	**.54[‡]**	**.37[‡]**	**.53[‡]**
Body Mass (kg)	**-.20***	**.56[‡]**	**.36[‡]**	**.61[‡]**	**.25[†]**	**.60[‡]**
MVPA (mins/day)	-.09	.15	**.21***	.09	-.12	.06
Lower Body Power (W)	**-.19***	**.29[‡]**	**.23[†]**	**.30[‡]**	.06	**.26[†]**
Knee Extension Peak Torque (N·m)	**-.20***	**.59[‡]**	**.45[‡]**	**.57[‡]**	.12	**.58[‡]**
Ankle Dorsiflexion Peak Torque (N·m)	**-.20***	**.32[‡]**	**.25[†]**	**.30[‡]**	-.05	**.29[†]**
Vertec Total Body Power (W)	**-.27[†]**	**.63[‡]**	**.53[‡]**	**.56[‡]**	.03	**.57[‡]**
MCSA (mm^2)	-.14	**.61[‡]**	**.52[‡]**	**.54[‡]**	.01	**.56[‡]**
			Mean (SD)			
Total Sample	1174.3 (24.3)	303.4 (56.1)	5.8 (.8)	70.1 (5.6)	33.5 (4.4)	1718.2 (409.6)
Males (n=67)	**1159.3 (19.4)**	**344.1 (50.2)**	**6.3 (.7)**	**74.6 (4.6)**	**35.3 (4.1)**	**2024.7 (361.6)**
Females (n=75)	**1187.7 (20.2)**	**267.1 (30.2)**	**5.5 (.6)**	**66.1 (3.5)**	**31.8 (4.1)**	**1444.4 (204.7)**

Note. [‡]p<.001, [†]p<.01, * p<.05. **Bold values** = significant at p<.05 or lower for correlations and significantly different between sexes at p<.05 for mean data. Age and sex were used as covariates for all partial correlations. $vBMD_{cort}$ = cortical volumetric bone density. Ct.Ar = cortical area. Ct.Th = cortical thickness. Peri.Circ = periosteal circumference. Endo.circ = endosteal circumference. pSSI = polar stress strain index. MCSA = muscle cross sectional area. MVPA = moderate-to-vigorous physical activity.

Table 4.2. Multiple regressions comparing the predictive ability of baseline variables and a muscle force surrogate (n=142)

Model	Variable	vBMDcort (mg/cm³) β (95% CI)	R²	Ct.Ar (mm²) β (95% CI)	R²	Ct.Th (mm) β (95% CI)	R²	Peri.Circ (mm) β (95% CI)	R²	Endo.Circ (mm) β (95% CI)	R²	pSSI (mm³) β (95% CI)	R²
M₁; Base	Age	**.202 (.074, .331)**		.058 (-.041, .157)		.109 (-.024, .242)		.012 (-.075, .099)		-.102 (-.247, .043)		.045 (-.046, .135)	
	Sex	**-.379 (-.549, -.210)**		**.315 (.184, .445)**		**.274 (.099, .449)**		**.314 (.199, .428)**		.120 (-.071, .311)		**.305 (.187, .424)**	
	Tibia Length	**-.249 (-.426, -.071)**	.439	**.193 (.056, .329)**	.668	.025 (-.159, .208)	.402	**.276 (.155, .396)**	.743	**.340 (.140, .540)**	.290	**.274 (.149, .398)**	.725
	Body Weight	-.065 (-.251, .121)		**.412 (.269, .555)**		**.360 (.168, .553)**		**.398 (.272, .524)**		.139 (-.071, .348)		**.398 (.268, .529)**	
	MVPA	-.078 (-.208, .052)		.090 (-.010, .190)		**.155 (.021, .289)**		.046 (-.042, .134)		-.106 (-.252, .040)		.024 (-.067, .116)	
M₂; M₁ + MCSA	MCSA	-.040 (-.239, .158)	.440	**.400 (.263, .537)**	.734	**.464 (.274, .653)**	.490	**.269 (.142, .396)**	.773	-.144 (-.367, .078)	.299	**.325 (.197, .452)**	.768
	(Var%)	.07		**6.61**		**8.82**		**2.99**		.87		**4.33**	

Note. β = Standardized beta. **Bolded values** = statistically significant $p<.05$. *Var%* = Independent variance accounted for by each predictor variable based on squared semi partial correlation coefficients. vBMD$_{cort}$ = cortical volumetric bone density. Ct.Ar = cortical area. Ct.Th = cortical thickness. Peri.Circ = periosteal circumference. Endo.circ = endosteal circumference. pSSI = polar stress strain index. MCSA = muscle cross sectional area. MVPA = moderate-to-vigorous physical activity. M$_{1-2}$ = models 1 and 2.

Table 4.3. Multiple regressions comparing the predictive ability of muscle force direct measures and surrogates (*n*=142)

Model	Variable	Ct.Ar (mm²) β (95% CI)	R²	Ct.Th (mm) β (95% CI)	R²	Peri.Circ (mm) β (95% CI)	R²	Endo.Circ (mm) β (95% CI)	R²	pSSI (mm³) β (95% CI)	R²
		Leg Extensor Power Rig									
M₃; M₁ + Muscle Force	Lower Body Power	.077 (-.063, .217)	.671	.129 (-.059, .316)	.410	.048 (-.075, .172)	.744	-.075 (-.280, .130)	.293	.009 (-.119, .137)	.725
	(*Var%*)	.28		.81		.12		.27		.00	
M₄; M₂ + Muscle Force	Lower Body Power	.073 (-.053, .199)	.737	.125 (-.049, .298)	.498	.046 (-.070, .163)	.774	-.074 (-.279, .131)	.301	.006 (-.112, .124)	.768
	(*Var%*)	.26		.76		.10		.27		.00	
	MCSA	**.400 (.263, .536)**		**.465 (.274, .651)**		**.269 (.142, .395)**		-.144 (-.366, .079)		**.325 (.196, .453)**	
	(*Var%*)	**6.55**		**8.82**		**2.96**		.85		**4.33**	
		Biodex Isokinetic Dynamometer - Knee									
M₃; M₁ + Muscle Force	Knee Extension	**.397 (.207, .588)**	.705	**.501 (.243, .758)**	.461	**.247 (.074, .420)**	.757	-.214 (-.507, .079)	.301	**.309 (.133, .485)**	.747
	(*Var%*)	**3.73**		**5.91**		**1.44**		1.08		**2.25**	
M₄; M₂ + Muscle Force	Knee Extension	**.344 (.171, .517)**	.761	**.439 (.198, .681)**	.535	**.211 (.046, .376)**	.783	-.196 (-.490, .099)	.308	**.265 (.101, .430)**	.784
	(*Var%*)	**2.76**		**4.49**		**1.04**		.88		**1.64**	
	MCSA	**.372 (.241, .503)**		**.428 (.245, .610)**		**.252 (.127, .376)**		-.128 (-.351, .095)		**.303 (.178, .427)**	
	(*Var%*)	**5.62**		**7.45**		**2.56**		.67		**3.73**	
		Biodex Isokinetic Dynamometer - Ankle									
M₃; M₁ + Muscle Force	Ankle Dorsiflexion	.081 (-.052, .215)	.672	.117 (-.061, .296)	.409	.027 (-.090, .145)	.743	-.090 (-.285, .105)	.294	.026 (-.096, .148)	.725
	(*Var%*)	.36		.74		.04		.44		.04	
M₄; M₂ + Muscle Force	Ankle Dorsiflexion	.027 (-.095, .149)	.734	.054 (-.114, .223)	.492	-.010 (-.122, .103)	.773	-.072 (-.269, .125)	.301	-.019 (-.133, .095)	.768
	(*Var%*)	.04		.15		.01		.27		.02	
	MCSA	**.395 (.256, .534)**		**.454 (.262, .646)**		**.271 (.142, .399)**		-.131 (-.357, .094)		**.328 (.198, .458)**	
	(*Var%*)	**6.25**		**8.29**		**2.92**		.69		**4.33**	
		Vertec Vertical Jump System									
M₃; M₁ + Muscle Force	Peak Anaerobic Power	**.525 (.316, .734)**	.719	**.720 (441, .999)**	.499	.276 (.081, .470)	.757	**-.413 (-.738, -.088)**	.322	**.338 (.139, .536)**	.746
	(*Var%*)	**3.46**		**9.67**		1.42		**3.17**		**2.13**	
M₄; M₂ + Muscle Force	Peak Anaerobic Power	**.385 (.182, .588)**	.759	**.568 (.289, .847)**	.545	.174 (-.020, .369)	.778	**-.383 (-.723, -.043)**	.324	**.216 (.020, .412)**	.776
	(*Var%*)	**2.92**		**5.52**		.52		**2.50**		**.79**	
	MCSA	**.325 (.189, .462)**		**.353 (.165, .540)**		**.235 (.104, .366)**		-.070 (-.299, .159)		**.282 (.151, .414)**	
	(*Var%*)	**4.00**		**4.71**		**2.07**		.19		**2.99**	

Note. β = Standardized beta. **Bolded values** = statistically significant p<.05. *Var%* = Independent variance accounted for by each predictor variable based on squared semi partial correlation coefficients. vBMDcort = cortical volumetric bone density. Ct.Ar = cortical area. Ct.Th = cortical thickness. Peri.Circ = periosteal circumference. Endo.circ = endosteal circumference. pSSI = polar stress strain index. MCSA = muscle cross sectional area. MVPA = moderate-to-vigorous physical activity. M₁₋₄ = models 1-4.
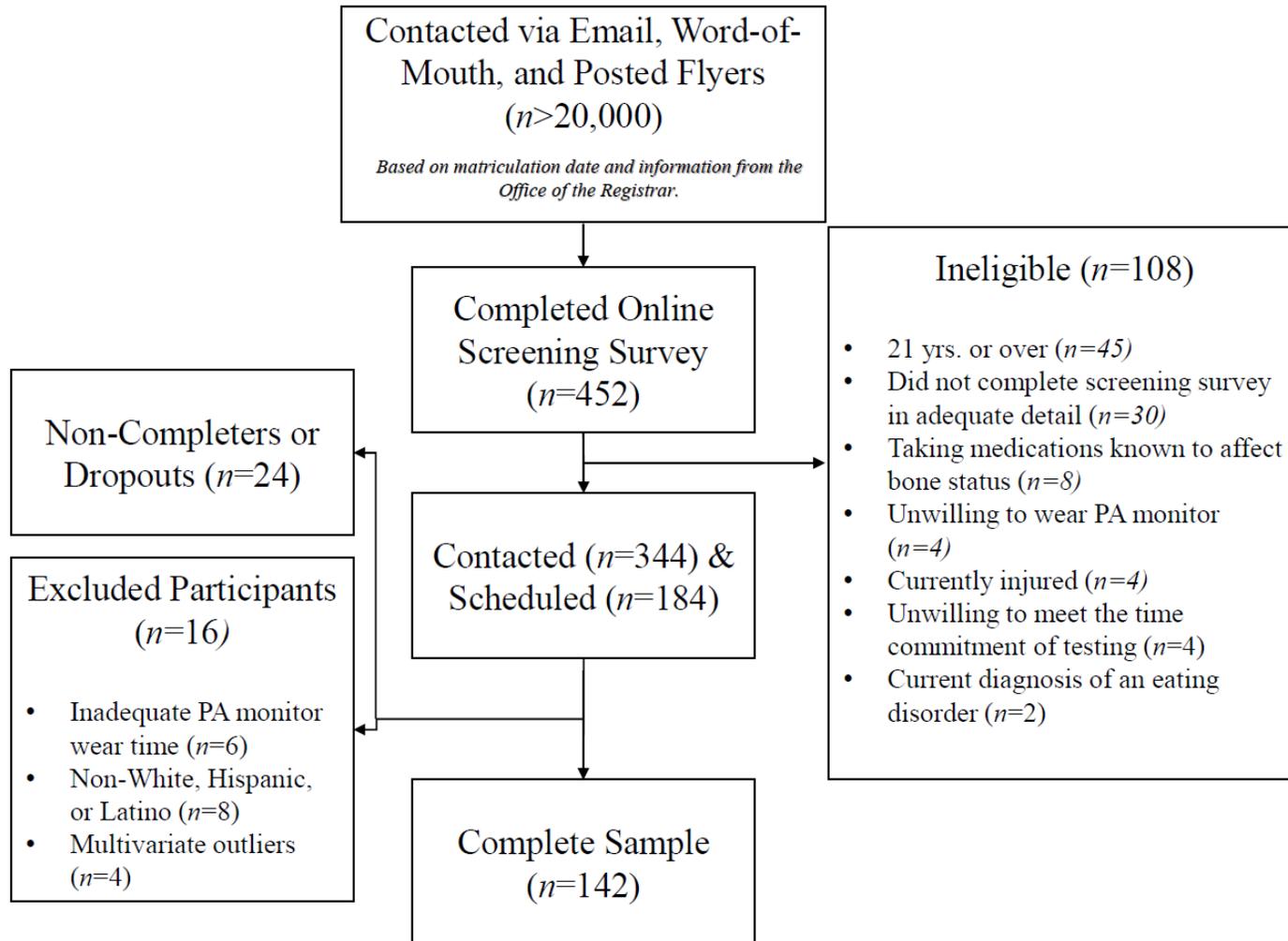
Figure 4.0. Participant recruitment flow chart.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The results from the present study add to our current understanding of the muscle-bone relationship by reporting that, in young adults, muscle specific force (MSF) measured in ankle dorsiflexion is not a means through which the beneficial effect of moderate-to-vigorous physical activity (MVPA) on cortical bone status is transmitted. Furthermore, MVPA appears beneficial to skeletal structural health in females of this age group but not males, a finding which is contrary to our view of physical activity (PA) being a beneficial behavior throughout the lifespan. These two findings may be linked, in that, the use of accelerometer-derived PA may not fully represent activities responsible for driving beneficial changes in bone status and therefore other, more-specific, measures may be needed to fully examine the relationship among PA, MSF, and bone status. In order to inform therapeutic and preventative interventions, future research should focus on confirming these findings in other populations, using measures of PA that specifically assess high-impact or high-load activities which are more likely to be driving bone accrual, and using multiple measures of muscle force to identify whether specific muscle actions (concentric, eccentric, or isometric), movement patterns, or loading characteristics contribute differently to the forces stimulating bone apposition.

Contributing to a growing literature examining the utility of direct measures of muscle force in the prediction of bone status, the findings that dynamometer and field-based measures of muscle force predicted structural aspects of cortical bone status independent of MVPA and muscle cross sectional area are important and exciting. The potential for field-based measures such as the vertical jump to be used as screening tools and/or as a predictor of the effect of muscles on bone status in place of radiative methodologies could help to reduce patient burden as well as medical costs associated with biomedical

imaging if developed further. Future work should aim to examine the apparent disconnect between structural and mass-based cortical bone outcomes in relation to muscle force, and to confirm the utility of these methodologies in a wider population.

Despite findings to the contrary, it is likely that muscle in fact does mediate the effect of PA on bone status as has been suggested in previous reviews of the literature, as the positive relationship between measures of muscle force and cortical structure support key mechanisms proposed for the beneficial effects of PA; periosteal apposition. Whether this mediation occurs more robustly throughout growth and development, explaining its apparent presence in younger age groups but not in young adults remains unanswered. Nonetheless, the utility of measuring muscle force rather than muscle mass in the prediction of bone status has promising use in reducing the radiative risk and cost associated with clinical and research-based osteoporosis assessment.

APPENDIX A

CONDITIONAL PROCESS ANALYSIS PREDICTING CORTICAL AREA

Table 3.4. Conditional process model coefficients – moderated mediation (Model 59; n=144)

Predictor		M (Muscle Specific Force; MSF) Coefficient (SE)	LLCI	ULCI		Y (Cortical Area) Coefficient (SE)	LLCI	ULCI
X; MVPA	a_1	.0000 (.0000)	.0000	.0000	c'_1	.4218 (.1656)	**.0944**	**.7493**
M; MSF		-	-	-	b_1	-8326.9353 (7210.6800)	-22586.508	5932.6376
W; Sex	a_2	.0004 (.0001)	**.0002**	**.0007**	c'_2	53.9826 (30.4592)	-6.2524	114.2177
X × W; MVPA × Sex	a_3	.0000 (.0000)	.0000	.0000	c'_3	-.4318 (.2377)	-.9018	.0382
M × W; MSF × Sex		-	-	-	b_2	309.4514 (11623.5503)	-22676.850	23295.7528
Constant.	i_1	.0012 (.0012)	-.0010	.0035	i_2	-89.8809 (78.1724)	-244.4718	64.7100
		R^2 = .46				R^2 = .57		
		$F(5,138) = 7.35, p<.001$				$F(7,136) = 25.95, p<.001$		

Note. Values are unstandardized. *SE* = Standard error. **Bold** values = significant at $p<.05$. *LLCI* = Lower limit of 95% bias-corrected bootstrap confidence interval. ULCI = Upper limit of 95% bias-corrected bootstrap confidence interval. *X*; MVPA was mean centered to aid in interpretation. Tibial length (cm) and Accelerometer wear time (minutes/day) were included in the model as covariates.

Table 3.5. Conditional process model coefficients – indirect and direct effects, and moderated mediation index (n=144)

W; Sex	Indirect Effect			Direct Effect		
	Effect (SE) $(a_{1i} + a_{3i}W)(b_{1i}+b_{2i}W)$	LLCI	ULCI	Effect (SE) $c_1' + c_3'W$	LLCI	ULCI
0 = Female	-.0048 (.0323)	-.0907	.0485	.4218 (.1656)	**.0944**	**.7493**
1 = Male	.0027 (.0423)	-.0563	.1236	-.0099 (.1719)	-.3498	.3299

Mediator	Index of Moderated Mediation		
	Index (SE)	LLCI	ULCI
M; MSF	.0075 (.0525)	-.0857	.1263

Note. Values are unstandardized. *SE* = Standard error. **Bold** values = significant at $p<.05$. *LLCI* = Lower limit of 95% bias-corrected bootstrap confidence interval. ULCI = Upper limit of 95% bias-corrected bootstrap confidence interval. Tibial length (cm) and Accelerometer wear time (minutes/day) were included in the model as covariates.