AMENORRHEA IN EXERCISING WOMEN RESULTS IN ADAPTATIONS IN vBMD, BONE GEOMETRY, AND ESTIMATED BONE STRENGTH

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SPRING 2014

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Kinesiology
with honors in Kinesiology

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ABSTRACT

Exercise is known to be an osteogenic stimulus, improving bone mineral density (BMD) and reducing the long-term risk of fracture. However, through metabolic and hormonal mechanisms, exercising women with menstrual cycle disturbances could be at risk for decreased BMD and negative adaptations in bone geometry that compromise bone strength secondary to insufficient caloric intake to compensate for energy expenditure. The purpose of this study was to compare volumetric BMD (vBMD), bone geometry, and estimated bone strength (bone strength index (BSI) and strength strain index (SSI)) between eumenorrhoeic (EU, n=9) versus amenorrhoeic (AM, n=18) exercising women. Bone variables were assessed at the tibia and radius using peripheral quantitative computed tomography. EU and AM women were similar in age (p>0.05) (20.7±0.5yrs), weight (57.2±1.5kg), BMI (20.6±0.4kg/m²), and body composition. Volumetric BMD, bone geometry, and estimated bone strength at the distal and proximal tibia and at the distal radius were not different (p>0.05) between EU and AM women. At the proximal radius, total vBMD, cortical vBMD, cortical thickness, endosteal circumference, muscle area, and the ratio of bone area to muscle area were also statistically similar (p>0.05) between the groups. However, EU women demonstrated a larger total area (p=0.045), cortical area (p=0.064), periosteal circumference (p=0.045),
and SSI (p=0.057) at the proximal radius compared with AM women. These findings were no longer significant after controlling for lean body mass. The results suggest that the alteration in reproductive hormones that typically results from an energy deficiency may negatively impact bone geometry and, consequently, bone strength. Because these findings were observed only at the radius, it may be that the osteogenic effect of exercise on the weight-bearing limbs is protective against the skeletal consequences of an energy deficiency in young, exercising women. However, this study may be underpowered and require a larger sample size to adequately address the research question.
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ACKNOWLEDGEMENTS

The completion of this honors thesis project would not have been possible without a tremendous support system.

To my thesis advisor, Dr. Mary Jane De Souza, who has guided me since my freshman year: you have provided me with more opportunities than I could have ever hoped for. Every day in the lab was a learning experience. You helped me become a critical thinker and even granted me the opportunity to attend a major conference. You pushed me when I needed to be pushed and believed in me even when I didn’t believe in myself. Most of all, you have given me a future, and I look forward to the many years to come during graduate school in the lab.

To my role model and mentor, Dr. Rebecca Mallinson: thank you for your constant guidance and leadership throughout my time as an undergrad. You have taught me more than I could ever say. You are always willing to help me with anything I need and are continuously full of knowledge and great advice. Your kindness, patience and understanding have been what pulled me through even the most stressful times in the lab. You have been an inspiration to me as a student and I aspire to follow in your footsteps. Thank you again for everything you have done for me, I assure you that it has not gone unrecognized.

Finally, to my family, friends, and friends that became family. I value all of your love and support more than you’ll ever know. A professor once told me, “For good or for bad, we are defined by the company we keep.” I couldn’t ask for better company.
Chapter 1
Introduction

Bone growth and development, though dependent on a myriad of factors, are notably influenced by the mechanical stimuli the bone experiences which are encountered to a great extent during purposeful exercise (Goodship 1992). It is well-established that high-impact exercise has an osteogenic effect on bone and is beneficial for improving bone mineral density (BMD) and preventing degenerative bone diseases, such as osteoporosis (Heinonen, Sievanen et al. 1996; Vainionpaa, Korpelainen et al. 2005). However, BMD is only one of the many factors that need to be considered when evaluating the integrity of bone. Bone geometry (i.e. size and shape) is also an important determinant of bone health and bone strength (Ducher, Hill et al. 2009). Among exercising women, another variable that impacts bone health is the presence of an energy deficiency, which is often indicated by menstrual dysfunction. Women experiencing menstrual disturbances such as amenorrhea, or the absence of menses for at least 90 days, have consistently demonstrated alterations in circulating concentrations of hormones indicative of energy status, to include decreased concentrations of triiodothyronine (TT3) (De Souza, Lee et al. 2007; Scheid, Williams et al. 2009), leptin (Christo, Cord et al. 2008; Corr, De Souza et al. 2011), and insulin-like growth factor-1 (IGF-1) (Christo, Prabhakaran et al. 2008) and increased concentrations of growth hormone (GH) (Waters, Qualls et al. 2001), ghrelin (Christo, Cord et al. 2008; Scheid, Williams et al. 2009), and peptide YY (PYY) (Scheid, Williams et al. 2009). These perturbations occur in concert with reductions in resting energy expenditure (REE), a metabolic indicator of an energy deficit (De Souza, Lee et al. 2007; Scheid, Williams et al. 2009). By evaluating bone
geometry and estimated bone strength in amenorrheic exercising women compared to eumenorrheic exercising women, we sought to gain further insight into the effects of exercise on bone when accompanied by the reproductive and metabolic hormone alterations indicative of an energy deficiency. In turn, our findings may identify factors that influence fracture risk among exercising women.

There has been thorough documentation in the literature regarding areal BMD (aBMD) in exercising women, obtained via dual energy x-ray absorptiometry (DXA)(De Souza, West et al. 2008; West, Scheid et al. 2009; Scheid, Toombs et al. 2011; Callreus, McGuigan et al. 2012). However, DXA is a two-dimensional (2-D) imaging technique that is not able to produce measurements of volumetric bone mineral density (vBMD), known as true BMD, or three-dimensional (3-D) bone geometry. In addition, it is not capable of evaluating the individual trabecular and cortical components of bone. More advanced techniques, such as peripheral quantitative computed tomography (pQCT), are able to produce three-dimensional images of bone, thereby providing measurements of bone geometry and vBMD for the total bone of interest as well as the separate cortical and trabecular components of the bone (Adams 2013). Bone geometry refers to the size of the bone, as well as the shape, or spatial distribution of the mass, of the bone (Bouxsein and Karasik 2006). Bone size and shape are desirable parameters for the evaluation of bone strength since a larger bone tends to be a stronger bone (Kanis, Melton et al. 1994), and furthermore, a bone that has its mass distributed farther from the center, a measurement known as cross-sectional moment of inertia (CSMI), tends to be a stronger bone (Bouxsein 2007). In addition to CSMI, section modulus is another geometric property obtained via pQCT. Together, these geometric and densitometric
variables are used in various combinations to calculate bone strength index (BSI) and strength strain index (SSI), which are estimates of bone strength against compressive and torsional/bending forces, respectively (Ducher, Eser et al. 2009). In fact, BSI was shown to be highly correlated with actual fracture load in a study of 103 excised rat femurs (Ferretti, Capozza et al. 1996), while SSI was shown to have a high prediction rate for fracture load in the forearm in a study using cadaveric human radii (Wilhelm, Felsenber et al. 2000). Therefore, these densitometric and geometric variables provide a comprehensive illustration of the structure of bone, which play a large role in bone strength, and thus may provide valuable information about properties of bone that are indicative of an increased risk of fracture.

Many factors contribute to these structural properties of bone, including age, gender, nutritional status, and participation in physical activity. Physical activity may have different osteogenic effects depending on the developmental period or life stage during which the activity occurs. In women, fluctuations in reproductive hormones during childhood, adolescence, premenopausal adulthood, and postmenopausal adulthood alter the ways in which the skeleton responds to loading. For instance, habitual loading exercises performed during childhood and adolescence have been demonstrated to enhance the attainment of peak bone mass (Burrows 2007). In addition, Baxter-Jones et al. found that physical activity during adolescence augments the accrual of bone mineral content, an effect shown to persist into young adulthood (Baxter-Jones, Kontulainen et al. 2008). Adults who continue to exercise regularly have been able to maintain bone mass later in life (Andreoli, Celi et al. 2012), which may, in turn, help to prevent osteoporosis (Birge and Dalsky 1989). It is with the hope of gaining further insight into the factors
affecting bone strength that investigators continue to evaluate the impact that exercise has on these and other densitometric and geometric properties of bone when loading occurs at different time points throughout the lifespan.

The hormonal component of bone growth is also worthy of consideration. Bone growth and development are affected by a variety of hormones within the body to include both metabolic and reproductive hormones. While the effects of metabolic hormones, such as insulin-like growth factor-1 (IGF-1), leptin, ghrelin, and peptide YY are beyond the scope of this analysis and readers are encouraged to refer to other sources (Soyka, Grinspoon et al. 1999; Russell and Misra 2010; Mantzoros, Magkos et al. 2011; Scheid, Toombs et al. 2011; Sienkiewicz, Magkos et al. 2011), the potent effects of estrogen on bone turnover and, subsequently, bone health may be examined through the lens of differing menstrual statuses. Hypoestrogenism, or suppressed estrogen concentrations as is observed with amenorrhea, has been shown to negatively affect bone mass (De Souza, West et al. 2008). Due to the presence of estrogen receptors in osteoblasts, osteoclasts, and osteocytes, estrogen plays a large role in mediating bone turnover, specifically inducing osteoclast apoptosis while inhibiting apoptosis of osteoblasts (Weitzmann and Pacifici 2006). In turn, by shortening the lifespan of osteoclasts, estrogen helps to prevent bone resorption (Weitzmann and Pacifici 2006). Conversely, by inhibiting osteoblast apoptosis, estrogen helps to extend the lifespan of osteoblasts and increases their functional capacity for bone formation (Syed and Khosla 2005). These are effective strategies in promoting the accumulation of bone mass during childhood, adolescence, and young adulthood as well as preventing bone loss with aging. However, the hypoestrogenism that is characteristic of amenorrhea creates the opposite effect,
decreasing the rate of osteoclast apoptosis, thereby extending osteoclast lifespan and increasing the activation frequency of basic multicellular units (BMUs) (Weitzmann and Pacifici 2006). BMUs are responsible for bone remodeling such that an increased activation frequency expands the bone remodeling space, increases cortical porosity, and enlarges the resorption area on the trabecular surfaces. Furthermore, hypoestrogenism augments osteoblast apoptosis, thereby shortening the osteoblast lifespan and diminishing the potential for bone formation (Weitzmann and Pacifici 2006). Together, these effects of estrogen deficiency on the cellular level negatively impact the density and microarchitecture of bone, consequences that are likely to be observed among amenorrheic exercising women.

When an energy deficiency results in suppression of the hypothalamic-pituitary-ovarian (H-P-O) axis, menstrual dysfunction becomes an overt symptom of the resultant hypoestrogenic environment. However, a less conspicuous consequence of an energy and estrogen deficiency is compromised bone health. Through the use of 3-D bone imaging made possible with pQCT, we aimed to uncover the effects of such physiological conditions, i.e., an energy and estrogen deficiency, on the densitometric and geometric properties of bone when compounded with the supposed osteogenic effects of habitual exercise. Additionally, we intended to discover variables within bone that provide for estimates of bone strength, perhaps a surrogate indicator of fracture risk.

**Purpose of this Study**

The purpose of this study was to compare volumetric BMD (vBMD), total, trabecular, and cortical area, cortical thickness, periosteal and endosteal circumference,
and estimates of bone strength (BSI and SSI) in the proximal and distal radius and tibia of amenorrheic exercising women and eumenorrheic exercising women.

**Hypothesis 1: vBMD**

Amenorrheic exercising women will have significantly lower total and trabecular vBMD than eumenorrheic exercising women at the radius and tibia. Cortical vBMD will be similar between amenorrheic and eumenorrheic exercising women at the tibia and radius.

**Hypothesis 2: Bone structure**

*Tibia:* Total bone area at the tibia will not be different between amenorrheic and eumenorrheic exercising women; however, amenorrheic women will have a decreased cortical area and an increased trabecular area compared to their eumenorrheic counterparts. Accordingly, amenorrheic exercising women will have a decreased cortical thickness and thereby an increased endosteal circumference versus eumenorrheic exercising women; however, periosteal circumference will be similar between the groups.

*Radius:* At the radius, total area will be similar between the exercising women, regardless of menstrual status. However, it is hypothesized that bone geometry will be sport specific, i.e. sports that load the arms (gymnastics, racquet sports) will cause increases in radial bone area while lower body sports (like soccer and running) will not.
Hypothesis 3: Estimated bone strength

At the tibia and radius, amenorrheic exercising women will have significantly lower BSI than eumenorrheic exercising women. At the radius and tibia, amenorrheic and eumenorrheic exercising women will have similar SSI. However, radial differences in SSI may be observed among athletes of different sports (i.e. gymnastics or racquet sports vs. running or soccer)

Rationale for this Study

The energy deficiency that is the primary cause of FHA among exercising women has numerous physiological consequences that ultimately play a role in compromising bone health (Vescovi, VanHeest et al. 2008). Female runners with chronic amenorrhea demonstrate suppression of bone formation and elevation of bone resorption, resulting in a negative bone remodeling balance in amenorrheic runners, as compared to the positive bone remodeling balance observed in their eumenorrheic counterparts (Zanker and Swaine 1998). Additionally, correlations between decreased estradiol concentrations and decreased concentrations of osteocalcin and bone-specific alkaline phosphatase (BSAP) in amenorrheic distance runners has been observed, indicating that amenorrhea and its characteristic estrogen deficiency is linked to disturbances in bone turnover (Zanker and Swaine 1998). De Souza et al. added to this finding, stating that, when present in combination, hypoestrogenism and an energy deficiency create an uncoupling in bone turnover, characterized by an increase in bone resorption and decrease in bone formation.
Yet, regardless of estrogen status, an energy deficiency alone is enough to suppress bone formation (De Souza, West et al. 2008).

The uncoupling of bone turnover that occurs in an energy- and estrogen-deficient environment may contribute to decreased BMD (De Souza, West et al. 2008). Compromised vBMD has been observed in populations of amenorrheic adolescent athletes as well as retired gymnasts who were previously amenorrheic during their adolescent training period (Ackerman, Nazem et al. 2011) (Ducher, Eser et al. 2009).

Adolescent amenorrheic athletes have significantly lower cortical vBMD at the distal tibia as well as decreased trabecular and total vBMD at the ultradistal radius compared to nonathletic controls (Ackerman, Nazem et al. 2011). Similarly, Ducher et al. observed that at the distal radius, trabecular vBMD was significantly decreased among retired gymnasts with a history of amenorrhea compared to retired gymnasts without a history of amenorrhea. Furthermore, at the distal tibia, trabecular vBMD was lower (p<0.09) in retired gymnasts with a history of amenorrhea than in gymnasts without said history (Ducher, Eser et al. 2009). Based on these observations, we expect that amenorrheic exercising women will display similar deficits in total and trabecular vBMD when compared to eumenorrheic exercising women.

Bone area was also measured in amenorrheic and eumenorrheic adolescent athletes and non-athletic controls by Ackerman et al. (2011). Although both groups of athletes had significantly greater total bone area at the distal tibia than the nonathletic control subjects, amenorrheic adolescent athletes demonstrated similar total bone area compared to their eumenorrheic counterparts, indicating the osteogenic benefits of exercise were still incurred irrespective of an estrogen deficiency that presumably
occurred in conjunction with an energy deficiency. The trabecular and cortical components of this bone site, however, were found to be different among the groups. The percentage of the total bone area that was trabecular bone was significantly greater and the percentage that was cortical bone was significantly reduced in the amenorrheic adolescent athletes compared to nonathletic controls. These findings were not observed when comparing the eumenorrheic adolescent athletes to nonathletic controls, indicating that amenorrhea likely contributes to alterations in bone remodeling and therefore bone structure.

As such, we surmise that amenorrheic exercising women will experience similar skeletal adaptations, resulting in changes to bone area as well as compositional makeup of the area (i.e. cortical versus trabecular contributions). Total bone area is hypothesized to be similar in amenorrheic and eumenorrheic exercising women at the tibia. However, due to the energy deficiency and resultant hormone adaptations that are thought to be the primary cause (i.e., alterations in metabolic hormones and the H-P-O axis) and the primary marker (i.e., suppressed estrogen) of amenorrhea among these women, it is likely that amenorrheic women will experience greater bone resorption and decreased bone formation on the endosteal surface of the bone. For this reason, it is expected that amenorrheic exercising women will demonstrate increased trabecular area and decreased cortical area of the bone compared to exercising. This will result in an increased endosteal circumference while preserving periosteal circumference. Consequently, cortical thickness will likely be decreased among amenorrheic exercising women.

At the radius, it is possible that changes in total bone area will not be observed in exercising women of differing menstrual status, unless the exercising women participate
in activities that expose the upper body to high impact loading (i.e. gymnastics, racquet sports). However, the compositional differences in the trabecular and cortical makeup of the bone may be similar to the results seen at the tibia since that is a process that is surmised to depend largely on hormonal factors and is not thought to differ to any great extent as the result of loading. That is, amenorrheic women may experience greater bone resorption and decreased bone formation on the endosteal surface of the radius, thereby increasing the ratio of trabecular area to cortical area compared to exercising eumenorrheic women while keeping total bone area constant.

BSI and SSI are estimates of bone strength based on geometric and densitometric properties of the bone. BSI is calculated at the distal aspect of the bone, which is comprised of more trabecular bone than more proximal sites, and takes into account the total bone area and the square of the total vBMD to estimate the bone’s strength against compressive forces. If total vBMD was less in amenorrheic exercising women and total area was comparable between amenorrheic and eumenorrheic exercising women in the radius and tibia, it would follow that BSI would be lower in amenorrheic exercising women at both extremities. If found to be true, these results would be consistent with the study conducted by Ducher et al. of retired gymnasts, in which the investigators reported that retired gymnasts with a history of amenorrhea and non-gymnast control subjects both presented with significantly lower BSI values at the distal radius than retired gymnasts without a history of amenorrhea (Ducher, Eser et al. 2009), thus indicating that the beneficial effects of loading on BSI are lost when loading is coupled with severe menstrual dysfunction, such as amenorrhea.
SSI, on the other hand, is calculated at the proximal aspect of the bone, which is comprised mainly of cortical bone. As such, it takes into account cortical density as well as section modulus, a property that depends largely on bone size. If both amenorrheic and eumenorrheic exercising women were found to have similar total bone area at the tibia, as hypothesized, as well as similar cortical density, it would follow that SSI would be similar between the groups at the tibia. At the radius, unless the exercising women participate in sports that load the upper extremities to a great extent, fewer changes in differences in area may be observed and therefore fewer differences in SSI at the radius may be encountered. Hence the supposition that SSI will likely be similar at the radius among groups.

The hypotheses presented are supported based on available literature featuring amenorrheic and eumenorrheic athletes. However, due to the age and activity differences between the subjects of previous studies and the subjects in the present study, it is possible that the conclusions drawn based on the results of the previous studies are not completely representative of what will be observed in exercising adult women. In the study of retired gymnasts, the subjects participated in a high training volume (at least 15 hours per week) during the childhood and adolescent years in a sport that is known for its exceptionally high impact loading. The osteogenic nature of gymnastics, particularly during key developmental years when the skeleton is exceptionally sensitive to the hormonal and loading environment, may contribute to results for bone that may not be similarly observed as a result of lower impact exercise, such as that typically engaged in by young, exercising women. In addition, the retired gymnasts had not been practicing their sport for at least 3 years, during which time they participated in no greater than two
hours of regular physical activity per week. It is possible that skeletal adaptations occurred during this time period, deeming the results of the study not completely representative of the effects of menstrual dysfunction on bone health in adolescent athletes. In the study by Ackerman et al., subjects were strictly adolescent athletes or athletes in very early adulthood participating in a variety of sports (Ackerman, Nazem et al. 2011). Since activity was occurring during the growth period, it is possible that the skeletal effects encountered at that time would differ from the effects of activity during adulthood.

To date, these parameters have not been examined in a population of solely young exercising women with menstrual disturbances. By examining the densitometric and geometric properties of bone in amenorrheic and eumenorrheic exercising, we will be able to draw further conclusions about the effects of energy and estrogen deficiency on vBMD, bone geometry and estimated bone strength.
References

Ackerman, K. E., T. Nazem, et al. (2011). "Bone microarchitecture is impaired in adolescent amenorrheic athletes compared with eumenorrheic athletes and nonathletic controls." J Clin Endocrinol Metab 96(10): 3123-3133.


1. Abstract

Low bone mineral density (BMD) is frequently observed among exercising women with functional hypothalamic amenorrhea as a consequence of an energy deficiency. Reports describing the impact of energy deficiency on bone health have primarily measured BMD using two-dimensional imaging techniques such as dual-energy x-ray absorptiometry (DXA). DXA is capable of measuring areal BMD but not bone geometry, thereby providing an incomplete picture of bone strength. Conversely, three-dimensional imaging techniques, such as peripheral quantitative computed tomography (pQCT), provide measurements of volumetric BMD (vBMD), bone geometry, and bone microarchitecture. Furthermore, pQCT is capable of separately assessing the cortical and trabecular compartments of bone and estimating bone strength, thereby serving as a useful tool for evaluating the underlying mechanisms of low bone mass in amenorrheic exercising women. This review examines the current literature regarding vBMD, bone geometry and microarchitecture, and estimated bone strength in amenorrheic athletes, female athletes with stress fractures, and anorexic girls and women in order to gain further understanding of 1) the impact of energy deficiency on bone health in amenorrheic athletes and 2) bone characteristics that may contribute to stress fracture risk among amenorrheic athletes. Amenorrheic athletes and anorexic individuals present with decreased trabecular vBMD and compromised trabecular microarchitecture, indicating
that the synergistic effects of an energy deficiency and estrogen deficiency impair bone quantity and quality, especially within trabecular regions. Likewise, exercising women with stress fractures display reduced trabecular vBMD, suggesting that amenorrheic athletes may experience increased fracture risk due to the compromised density of trabecular bone.

2. Introduction

Among exercising women, failure to consume the required energy to meet the demands of energy expenditure typically results in an energy deficiency that may contribute to serious health consequences due to unfavorable physiological adaptations. Such adaptations include a decrease in resting metabolic rate (De Souza, Lee et al. 2007) and altered concentrations of metabolic hormones, i.e., decreased triiodothyronine (De Souza, Lee et al. 2007), leptin (Christo, Cord et al. 2008), and insulin-like growth factor-1 (IGF-1) (Christo, Prabhakaran et al. 2008), and elevated ghrelin (De Souza, Leidy et al. 2004; De Souza, Lee et al. 2007; Christo, Cord et al. 2008) and peptide YY (Scheid, Williams et al. 2009), all of which influence bone metabolism or bone mineral density (BMD) (Hotta, Fukuda et al. 2000; Kaufman, Warren et al. 2002; Misra, Miller et al. 2005; Scheid, Toombs et al. 2011; Sienkiewicz, Magkos et al. 2011; Wojcicka, Bassett et al. 2013). A more overt symptom of an energy deficiency in exercising women and adolescent girls is menstrual dysfunction, such as functional hypothalamic amenorrhea [1], which is often accompanied by poor bone health that is characterized by changes in bone size and shape [3], decreased bone mineral density (BMD) [3-6], and increased
fracture risk [7]. To date, the relationship between amenorrhea and BMD in exercising women and adolescent girls with amenorrhea has been extensively investigated using two-dimensional (2-D) imaging techniques such as dual-photon absorptiometry (DPA) and dual energy x-ray absorptiometry (DXA), producing a conclusive understanding that areal BMD (aBMD) is often decreased in these women and adolescent girls compared to their eumenorrheic counterparts (Drinkwater, Nilson et al. 1984; Nelson, Fisher et al. 1986; Drinkwater, Bruemner et al. 1990; Rencken, Chesnut et al. 1996; Christo, Prabhakaran et al. 2008; West, Scheid et al. 2009; Scheid, Toombs et al. 2011).

However, three-dimensional (3-D) measurements of bone geometry and microarchitecture in amenorrheic exercising women and adolescent girls have not been documented as thoroughly in the literature.

Peripheral quantitative computed tomography (pQCT) is a 3-D imaging technique that produces images of both cortical and trabecular bone with a resolution better than that of DXA, allowing for descriptions of bone size and shape, collectively referred to as bone geometry, and volumetric BMD (vBMD), known as true BMD. These parameters allow for calculated estimates of bone strength, a surrogate indicator of fracture risk, at the radius and tibia (Schnackenburg, Macdonald et al. 2011). High resolution pQCT (HR-pQCT), flat panel volume CT, and finite element analysis (FEA) are another step beyond traditional pQCT, allowing for assessment of trabecular microarchitecture as well as bone stiffness and failure load. When used in combination with DXA, 3-D imaging techniques, such as pQCT, HR-pQCT, flat panel volume CT, and FEA can lead to a greater understanding of bone quantity (i.e., bone density) and bone quality (i.e., bone structure to include bone geometry and microarchitecture) (Rubin, Turner et al. 2002;
Adams 2013), which are key components of bone strength, in exercising amenorrheic women and adolescents.

To date, the literature available that describes bone geometry and microarchitecture obtained from pQCT in amenorrheic exercising women and adolescent girls is limited. However, the results from investigators who have explored bone health using pQCT in this population have been relatively consistent, potentially providing important information about the characteristics of bone that may be influenced by an environment of habitual loading and suppressed reproductive hormones. Therefore, we review the current literature regarding vBMD, bone geometry and microarchitecture, and estimated bone strength in amenorrheic athletes (AA) in an effort to gain further understanding of bone health in this population that is at risk for low bone mass and fractures. We also review vBMD, bone geometry and microarchitecture, and estimated bone strength in exercising women with stress fractures and in adolescent girls or women with anorexia nervosa (AN), a model of severe energy deficiency, as a means to inform us of the presumably less severe energy-deficient model of exercising amenorrheic women and girls. In doing so, the purpose is to 1) identify characteristics of bone strength and propensity to fracture in women along a spectrum of energy deficiency ranging from mild to severe using models of exercising women/girls and anorexic women/girls and 2) describe characteristics of bone within exercising women who incur stress fractures in order to gain better insights into factors that characterize bone that is susceptible to fracture, as these factors may be present in AA.
3. Search Strategy

To identify studies pertinent to this review, an electronic search of the PubMed database was performed using the following search terms: amenorrhea and pQCT; stress fracture and pQCT; anorexia nervosa and pQCT; anorexia nervosa and CT; exercise and amenorrhea and CT. The terms amenorrhea, stress fracture, anorexia, and anorexia nervosa were also searched with the term peripheral quantitative computed tomography. We only included articles that contained data in premenopausal, exercising women and girls or those with AN with bone health measurements as the primary outcome variables. In addition, among the stress fracture articles, we only included studies that assessed women with a diagnosed stress fracture. We excluded abstracts, case studies, and articles not published in English. Because the purpose of the paper was to review vBMD, bone geometry and microarchitecture, and estimated bone strength, we did not include studies that only reported vBMD at the lumbar spine using CT and did not include other parameters of bone strength. As such, the studies highlighted in this review emphasize estimates of bone strength and the variables measured to derive these estimates at the radius and tibia.

4.1 Volumetric Bone Density, Geometry, and Estimated Strength in Amenorrheic Athletes

Currently, five papers have been published that describe vBMD, bone geometry and estimated bone strength in AA (To, Wong et al. 2005; Ruffing, Nieves et al. 2007; Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012). The low aBMD observed among AA when using DPA and DXA (Drinkwater, Nilson et
al. 1984; Nelson, Fisher et al. 1986; Drinkwater, Bruemner et al. 1990; Rencken, Chesnut et al. 1996; Christo, Prabhakaran et al. 2008; West, Scheid et al. 2009; Scheid, Toombs et al. 2011) is consistent with the results of studies assessing vBMD, bone geometry, and estimated bone strength among a similar population of women and girls (To, Wong et al. 2005; Ruffing, Nieves et al. 2007; Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012). To date, studies using pQCT to assess bone health among exercising women and adolescents have been limited to cross-sectional studies comparing athletes with amenorrhea or with a history of amenorrhea to eumenorrheic athletes and non-athletic controls (To, Wong et al. 2005; Ruffing, Nieves et al. 2007; Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012). Results from these studies have revealed that athletes with amenorrhea or a history of amenorrhea have lower trabecular vBMD at the radius but not the tibia when compared to eumenorrheic athletes or non-exercising controls (Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012) (Table 1). Ducher et al. (Ducher, Eser et al. 2009) studied a sample of retired elite gymnasts between the ages of 17-36 years who were grouped according to self-reported history of amenorrhea and a control group of non-exercising women similar in age. Trabecular vBMD of the distal radius was 16% lower in retired gymnasts with a history of amenorrhea compared to the gymnasts without a history of amenorrhea; however, when compared to the control group, gymnasts with a history of amenorrhea did not display significantly lower trabecular vBMD (Ducher, Eser et al. 2009). At the proximal radius, cortical thickness (CoTh) was significantly lower (12%) in the gymnasts with a history of amenorrhea compared to that of non-athletic controls; however, there was no significant difference (3.2%) in CoTh
between gymnasts without a history of amenorrhea and control subjects (Ducher, Eser et al. 2009). Ackerman et al. (Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012) reported slightly different findings in adolescent AA, perhaps due to the differences in sport type between the osteogenic activity of gymnastics (Ducher, Eser et al. 2009) and the low-impact loading of running (Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012) as well as the age difference between populations (i.e., adolescent vs. adult); however, the compromised bone health remained evident in AA.

At the ultradistal radius, trabecular vBMD was 16% and 12% lower among AA compared to non-athletic controls and eumenorrheic athletes, respectively, after adjusting for height (Ackerman, Nazem et al. 2011). Unadjusted results demonstrated decreased total vBMD at the distal radius and cortical vBMD at the distal tibia by 15% and 3.5%, respectively, among AA compared to non-athletic controls (Ackerman, Nazem et al. 2011), which coincided with a lower cortical area/total area ratio, and a greater cortical porosity at the distal tibia (Ackerman, Putman et al. 2012). Moreover, AA had a significantly lower trabecular number (TbN) and significantly greater trabecular separation (TbSp) at the distal tibia when compared to both eumenorrheic adolescent athletes and non-athletic controls, indicating the deterioration of the trabecular microarchitecture (Ackerman, Nazem et al. 2011).

It is logical that trabecular vBMD was lower among athletes who were either currently amenorrheic or had been amenorrheic in the past when compared to their eumenorrheic counterparts or non-athletic controls. Trabecular bone is more sensitive to hormonal changes than cortical bone due to its greater rate of bone turnover (Biller, Saxe et al. 1989). Estrogen serves to inhibit osteoclast action; therefore, in an environment of
hypoestrogenism, low bone mass is often first observed at sites primarily composed of trabecular bone, such as the vertebrae, which explains the consistent reports of low aBMD of the lumbar spine in AA (Christo, Prabhakaran et al. 2008; West, Scheid et al. 2009) and the low trabecular vBMD observed at these peripheral distal sites (Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011). Hypoestrogenism in AA is confirmed by detailed reports from our lab of estrogen exposure (area under the curve) over a 28-day period; among AA, estrogen exposure is 48% of the estrogen exposure for a similar time period (one menstrual cycle versus one 28-day monitoring period) in ovulatory, regularly-menstruating athletes (Mallinson, Williams et al. 2013). Figure 1 depicts estrogen exposure in amenorrheic versus ovulatory athletes.

Interestingly, it has also been observed that adolescent AA and retired athletes with a history of amenorrhea have a greater total bone area at the tibia and radius compared to non-athletic controls (Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011), a positive finding worthy of further investigation due to the expected poor bone health in this population. In fact, in their sample of young retired female gymnasts, Ducher et al. (Ducher, Eser et al. 2009) reported that those with a history of amenorrhea had a 10% greater total area of the distal radius compared to retired gymnasts without a history of amenorrhea. These findings suggest that AA still acquire the benefit, in part, of the osteogenic stimulus secondary to weight-bearing exercise with respect to periosteal expansion of bone, but rather than having greater bone area coupled with increased CoTh, as is often observed in menstruating female athletes (Nikander, Sievanen et al. 2006; Smock, Hughes et al. 2009). Ducher et al. (Ducher, Eser et al. 2009) reported that previously-amenorrheic retired gymnasts present with thinner cortices. Reduced CoTh
was also observed by Ruffing et al. (Ruffing, Nieves et al. 2007) among oligoamenorrheic military recruits compared to their eumenorrheic counterparts. A possible explanation for these findings involves the poor estrogen exposure coupled with habitual exercise that is experienced by these athletes with amenorrhea or a history of amenorrhea. Chronic exercise training and loading of bone result in bone formation and periosteal expansion at the site of the stress (Forwood and Burr 1993; Judex, Gross et al. 1997; Burt, Naughton et al. 2011; Ducher, Bass et al. 2011). Thus, bones that are subjected to loading or muscle forces typically increase in size (Burt, Naughton et al. 2011; Ducher, Bass et al. 2011). A bone that has its mass distributed farther from the center of the bone is typically a stronger bone (Borer 2005); thus, habitual exercise is beneficial for bone health by causing an increase in the size of the bone and, therefore, the strength of bone (Burt, Naughton et al. 2011; Ducher, Bass et al. 2011). Estrogen also has an influence on the size of bone such that the increase in estrogen production that occurs during puberty causes periosteal apposition of bone to cease but encourages endosteal apposition to continue, thus preventing further increases in bone size but allowing for increases in CoTh (Schoenau, Neu et al. 2001; Seeman 2003). Therefore, athletes with amenorrhea or a history of amenorrhea during the critical adolescent and pubertal bone accretion years may have a larger area and thinner cortex than eumenorrheic athletes or non-athletic controls. This finding would presumably be due to poor estrogen exposure, which may inhibit the typical suppression and stimulation of periosteal and endosteal apposition, respectively (Frost 1999; Saxon and Turner 2005). On the other hand, estrogen is also extremely important for optimal bone health due to its role in inhibiting osteoclast action, particularly at trabecular sites that are characterized
by rapid turnover (Compston 2001). Thus, without adequate estrogen exposure, it is apparent that athletes with amenorrhea or a history of amenorrhea during the adolescent years may demonstrate the effects of partially uninhibited periosteal expansion and bone resorption as evidenced by a greater total bone area and reduced trabecular vBMD.

Assessment of the geometry and vBMD of bone via pQCT provides valuable information about the structural and densitometric properties of bone (Engelke, Adams et al. 2008). These factors can be used to calculate estimates of bone strength such as the bone strength index (BSI) and the strength strain index (SSI) (Ducher, Eser et al. 2009). The BSI, typically calculated at the distal radius and tibia, is the product of total bone area (ToA) and the square of total vBMD (ToD): $\text{BSI} = \text{ToA} \times \text{ToD}^2$ (Ducher, Eser et al. 2009) and estimates the strength of the bone against compressive forces. Ducher et al. (Ducher, Eser et al. 2009) reported that BSI was 17% lower at the distal radius in retired gymnasts with a history of amenorrhea compared to gymnasts without a history of amenorrhea. In a study of adolescent dancers, To et al. (To, Wong et al. 2005) observed a 13% and 15% greater BSI at the distal radius and tibia, respectively, in eumenorrheic dancers compared to non-exercising controls, a benefit that was lost when comparing amenorrheic dancers with control subjects. These findings suggest that the effects of loading on BSI are lost when loading is coupled with severe menstrual dysfunction such as amenorrhea.

Another estimate of bone strength, SSI, is most often calculated at the proximal radius and tibia and takes into account section modulus (Z) and cortical density (CoD) in order to estimate the bone’s resistance to bending and torsion: $\text{SSI} = Z \times \frac{\text{CoD}}{\text{CoD}_{\text{max}}}$ (Ducher, Eser et al. 2009). Ducher et al. (Ducher, Eser et al. 2009) reported that SSI
tended to be greater (p<0.09) in the tibia of retired gymnasts with a history of amenorrhea compared to those gymnasts without a history of amenorrhea and was significantly greater than the SSI of the non-athletic controls. These conflicting results of lower BSI but greater SSI in retired gymnasts with a history of amenorrhea may be explained by the fact that the two measurements assess strength at different bone regions. BSI is calculated at the distal epiphysis, which is comprised largely of trabecular bone; therefore, the low bone mass may be present more rapidly at distal sites rather than proximal sites, causing bone strength to be compromised to a greater extent at distal sites. SSI, on the other hand, is calculated at the proximal shaft, which is largely cortical bone and therefore may not be as susceptible to an early loss of bone mass or failure to accrue appropriate bone mass. On the contrary, the proximal region of these peripheral sites may be experiencing an increase in total area as observed by Ducher et al. (Ducher, Eser et al. 2009) and Ackerman et al. (Ackerman, Nazem et al. 2011), thus resulting in greater strength at the shaft.

It must be noted that the studies to date evaluating vBMD, bone geometry and microarchitecture, and estimated bone strength among AA represent a variety of ages, sport types, and life stages with respect to the onset of amenorrhea. Each of these factors influence the results obtained; therefore, the population assessed in each study must be taken into account prior to drawing conclusions about the effect of menstrual dysfunction on bone quantity and quality in athletes. For example, the athletes in the investigation conducted by Ducher et al. (Ducher, Eser et al. 2009) participated in gymnastics, a potent osteogenic stimulus due to both the magnitude and heterogeneity of the loading forces (Daly, Rich et al. 1999), during childhood and adolescence. The combination of low
estrogen and participation in gymnastics during the growth years when the skeleton is very sensitive to both the hormonal and loading environments may have contributed to the comparatively large bone size that was observed at all bone sites among the retired athletes with a history of amenorrhea. The athletes in the studies by Ackerman et al. (Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012), however, represented adolescents and young women that primarily engaged in low-impact loading such as running; therefore, the greater total and trabecular area at the weight-bearing tibia (but not at the non-weight bearing radius) and the reduced trabecular vBMD at the non-weight bearing radius (but not at the weight-bearing tibia) may be specific to athletes at this age and participating in this low-impact loading modality. As such, exercise-associated amenorrhea may have different effects on bone health depending on the developmental stage during which the activity is performed and the menstrual dysfunction occurs.

However, the bones of AA exhibit structural and densitometric properties that indicate a decreased ability to withstand increased loading without failure, particularly at areas of primarily trabecular bone. Investigators have demonstrated that low trabecular vBMD (Hung, Wu et al. 2005) and thin cortices (Chevalley, Bonjour et al. 2013) are risk factors for fracture. Further, athletes with menstrual disturbances have been found to be 2 to 4 times more likely to incur a stress fracture than those with normal menstrual function (Bennell, Matheson et al. 1999). The energy-deficient environment often observed among AA results in metabolic alterations such as suppressed IGF-1 and leptin and elevated peptide YY and ghrelin (Christo, Cord et al. 2008; Christo, Prabhakaran et al. 2008; Scheid, Williams et al. 2009) that may negatively affect bone formation and negate potential positive changes in bone geometry and density that occur with chronic
exercise training (Christo, Prabhakaran et al. 2008; Scheid, Toombs et al. 2011; Sienkiewicz, Magkos et al. 2011). In fact, a recent report among female military recruits undergoing basic training revealed that those who suffered a stress fracture during the 4-week intense training period displayed a 67.8% decrease in bioavailable IGF-1 concentrations, a change that was significantly different than the 19.3% increase that was observed among the recruits who did not fracture (Strohbach, Scofield et al. 2012). These results suggest that a decrease in IGF-1 as is observed in an energy-deficient state often characterized by amenorrhea (De Souza, Lee et al. 2007; Christo, Prabhakaran et al. 2008) may be associated with increased fracture risk. In addition, energy deficiency suppresses bone formation and increases bone resorption, thereby negatively influencing bone turnover and creating an environment within bone tissue that is more susceptible to fracture (Ihle and Loucks 2004; De Souza, West et al. 2008).

To date, there are currently no reports on the bone geometry and microarchitecture of AA who develop stress fractures; however, research conducted in athletes diagnosed with stress fractures, irrespective of menstrual status, may provide insight into the characteristics of bones that are at risk for stress fracture.

4.2 Volumetric Bone Density, Geometry, and Estimated Strength in Exercising Women with Stress Fractures

Few investigators have employed pQCT to assess vBMD, bone geometry, and estimated bone strength among exercising women with stress fractures (Popp, Hughes et al. 2009; Schnackenburg, Macdonald et al. 2011) (Table 2). In fact, to date, only one study has explored bone quality among exercising women with a current stress fracture (Schnackenburg, Macdonald et al. 2011), and in the case of this study, there was a large
range in time since diagnosis (1-47 weeks). Other investigators have assessed bone geometry and estimated bone strength among exercising women with a history of stress fracture (Popp, Hughes et al. 2009). Results demonstrate that female athletes with bones characterized by a small cortical area may be at increased risk for stress fracture. Schnackenburg et al. (Schnackenburg, Macdonald et al. 2011) compared bone geometry and estimated bone strength using HR-pQCT among exercising women with a lower limb stress fracture (SF group) to exercising women who reported no history of stress fracture (NSF group). The SF and NSF groups were matched for age, sport, and weekly training volume. HR-pQCT scans of the ultradistal and distal tibia were analyzed by anatomical regions that included the anterior, posterior, lateral, and medial sites. In the posterior quadrant of the tibia, a common site for stress fractures (Brukner, Bradshaw et al. 1996), cortical area was significantly lower at both the ultradistal (7%) and distal (5%) locations among athletes with a stress fracture compared to those who had never experienced a stress fracture (Schnackenburg, Macdonald et al. 2011). Additionally, in the SF group, the posterior region of the distal tibia displayed significantly lower trabecular vBMD (20%) and impaired trabecular microarchitecture as evidenced by a trend toward lower trabecular thickness (TbTh) (6%, p=0.09) and TbN (18%, p=0.08) compared to the NSF group. Furthermore, both total area (8%) and cortical area (2%) of the distal tibia in the SF group were reduced compared to the NSF group (Schnackenburg, Macdonald et al. 2011).

Likewise, Popp et al. (Popp, Hughes et al. 2009) reported a significantly reduced cortical area of the tibia at multiple proximal sites among female runners with a history of stress fracture (SFX group) compared to female runners without a history of stress
fracture (NSFX group). Runners who had experienced a stress fracture in the past 5 years but did not have a current stress fracture were compared to women who had never experienced a stress fracture. Cortical area of the tibia was 6.9%, 7.7%, and 9.9% lower among the SFX group compared to the NSFX group at the 45%, 50%, and 66% sites, respectively (Popp, Hughes et al. 2009). Estimated bone strength was impaired in the SFX group as evidenced by a significantly lower SSI at both the 50% and 66% sites (Popp, Hughes et al. 2009). Taken together, it appears that small bones and less cortical bone may increase the risk for stress fracture among female athletes.

As described above, the equations for estimating bone strength from pQCT use measurements that represent the size and/or shape of the bone as well as the density of the bone (Ducher, Eser et al. 2009), such that a bone that is larger, denser, and has its mass distributed farther away from the central axis is a stronger bone. Thus, it can be deduced that a bone that is more prone to fracture has a smaller area, particularly in the cortical compartment where the majority of the bone mass is located. Using computed tomography (CT), Franklyn et al. (Franklyn, Oakes et al. 2008) observed that section modulus, which indicates bone’s resistance to bending, was significantly lower among exercising women with stress fracture compared to exercising controls due to less favorable distribution of bone within the cross section, supporting the notion that both the size and shape of the bone may contribute to stress fractures among exercising women.

Lean mass and muscle forces also have a large impact on bone size and mass, and, therefore, bone strength (Burr 1997; Robling 2009; Mallinson, Williams et al. 2013). Results from a recent study conducted among non-elite female gymnasts demonstrated that total bone area of the radius, BSI, lean mass, and muscle cross-sectional area
(MCSA) were greater in high-training gymnasts compared to non-gymnasts (Burt, Naughton et al. 2012). These differences disappeared, however, after adjusting for MCSA, suggesting that the difference in bone area and estimated bone strength may be partially explained by differences in muscle size (Burt, Naughton et al. 2012). Similarly, differences in cortical area and SSI in exercising women with stress fracture compared to exercising controls were no longer significant after controlling for MCSA in the previously-described study of Popp et al. (Popp, Hughes et al. 2009). Taken together, these findings indicate that muscle size may also be a determinant factor for stress fracture risk (Popp, Hughes et al. 2009; Burt, Naughton et al. 2012). It appears that smaller bones and less cortical bone may increase the risk for stress fracture among female athletes and that muscle size may have an indirect effect on stress fracture risk via its influence on bone strength and its ability to dissipate the forces exerted on bone through loading (Popp, Hughes et al. 2009; Burr 2011; Schnackenburg, Macdonald et al. 2011). Thus, according to the findings of Popp et al. (Popp, Hughes et al. 2009), female athletes with a smaller muscle size may have smaller bones and less distribution of bone mass to areas of high stress simply due to less muscle force. In turn, these women may therefore be at a greater risk for stress fracture.

Although Schnackenburg et al. (Schnackenburg, Macdonald et al. 2011) did observe a decrease in trabecular vBMD and a trend toward reduced TbN in the distal tibia in accordance with the findings of trabecular vBMD and microarchitecture in AA (Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011), the primary outcomes of pQCT measurements of AA and athletes with a stress fracture (or athletes with a history of either) display stark contrasts. While adolescent AA displayed a greater total area of the
bone compared to non-athletic controls and a similar total bone area when compared to their eumenorrheic counterparts (Ackerman, Nazem et al. 2011), athletes with stress fractures displayed a smaller total area and cortical area compared to athletes without a stress fracture (Schnackenburg, Macdonald et al. 2011). Additionally, retired athletes with a history of amenorrhea displayed a trend (p<0.09) toward greater SSI than retired gymnasts without a history of amenorrhea (Ducher, Eser et al. 2009); whereas, athletes with a history of stress fracture demonstrated lower SSI results compared to athletes without a history of stress fracture (Popp, Hughes et al. 2009) (Figure 2). These findings may provide an explanation for the observation that not all AA experience stress fractures and indicate that other factors likely play a role in the occurrence of these fractures.

Interestingly, AA with a small muscle size may be at greatest risk due to the potential for a small bone size, unfavorable bone shape, and less energy dissipation from lean mass as well as decreased vBMD and deterioration of the trabecular microarchitecture. Thus, weight training may be an effective technique for stress fracture risk reduction among AA with a small frame.

Notably, the majority of women in the studies to date that explored bone health among those with a stress fracture were runners who typically have lean physiques, characterized by small body and bone size. As such, the suppositions about characteristics of bone size and shape that contribute to fracture risk may be biased, and it must be considered that other factors may play a role in fracture risk among athletes of other sport types.

The energy deficient environment of AA may also represent a factor that contributes to an increased risk for stress fracture due to the role that energy deficiency
has in the uncoupling of bone turnover and suppression of IGF-1 concentrations (Ihle and Loucks 2004; Christo, Prabhakaran et al. 2008; De Souza, West et al. 2008). To determine the influence of an energy deficiency on parameters of bone strength in AA, it may be insightful to examine bone geometry and structure among those with a severe energy deficiency independent of exercise status, i.e., women and girls with AN.

4.3 Volumetric Bone Density, Geometry, and Estimated Strength in Anorexia Nervosa

Bone mass among women and adolescents with AN, an eating disorder characterized by extreme dietary restriction is notably low, likely attributable to both severe energy deficiency associated with starvation and estrogen deficiency if amenorrhea is present (Hay, Hall et al. 1989; Hay, Delahunt et al. 1992; Grinspoon, Thomas et al. 2000; Milos, Spindler et al. 2005; Lawson, Miller et al. 2010; Olmos, Valero et al. 2010). In fact, Grinspoon et al. (Grinspoon, Thomas et al. 2000) observed that 92% and 38% of anorexic women were osteopenic and osteoporotic, respectively. Anorexic women may present with similar bone deficiencies as AA, but perhaps to a greater severity due to the additional factors of low body weight, starvation-related energy deficiency, long duration of amenorrhea, and the potential lack of osteogenic loading from habitual exercise. Both an energy and estrogen deficit have been shown to impact bone health exclusively and, when present in combination, can result in notably poor bone quality (Bredella, Misra et al. 2008; De Souza, West et al. 2008; Walsh, Phan et al. 2010; Ackerman, Nazem et al. 2011). To date, data from nine studies that have used flat panel volume CT, HR-pQCT, and traditional pQCT to assess bone health variables at the radius among adolescent and adult women with AN have illustrated
compromised bone vBMD, geometry, and microarchitecture (Resch, Newrkla et al. 2000; Fricke, Tutlewski et al. 2005; Milos, Spindler et al. 2005; Milos, Spindler et al. 2007; Bredella, Misra et al. 2008; Fricke, Beccard et al. 2010; Lawson, Miller et al. 2010; Walsh, Phan et al. 2010; Faje, Karim et al. 2013) (Table 3, Figure 3).

To date, information about bone geometry and microarchitecture in anorexic adolescents (Bredella, Misra et al. 2008; Faje, Karim et al. 2013) and women (Resch, Newrkla et al. 2000; Fricke, Tutlewski et al. 2005; Milos, Spindler et al. 2005; Milos, Spindler et al. 2007; Fricke, Beccard et al. 2010; Lawson, Miller et al. 2010; Walsh, Phan et al. 2010) is limited to the distal radius; however, these reports have demonstrated strong agreement that total, trabecular, and cortical bone quantity and/or quality are compromised among women and adolescent girls with AN (Resch, Newrkla et al. 2000; Fricke, Tutlewski et al. 2005; Milos, Spindler et al. 2005; Bredella, Misra et al. 2008; Lawson, Miller et al. 2010; Walsh, Phan et al. 2010; Faje, Karim et al. 2013) (Table 3).

For example, using traditional pQCT, Resch et al. (Resch, Newrkla et al. 2000) observed significantly lower total vBMD of 18% at the distal radius among anorexic women compared to age-matched controls. Similarly, investigators that used HR-pQCT, which provides better resolution than standard pQCT, reported that total and trabecular vBMD were significantly lower, on average, by 14% and 12%, respectively, among anorexic women and adolescents compared to healthy controls (Milos, Spindler et al. 2005; Faje, Karim et al. 2013). Trabecular microarchitecture was also compromised as evidenced by significantly lower TbN (5%), greater TbSp (7%), and a lower ratio of trabecular bone volume to total bone volume (BV/TV) (13%) compared to the control group (Milos, Spindler et al. 2005). These findings were confirmed by several investigators who used
flat panel volume CT (Bredella, Misra et al. 2008; Lawson, Miller et al. 2010; Walsh, Phan et al. 2010). Trabecular BV/TV, TbN, and TbTh were lower and TbSp was greater among the anorexic patients compared to controls, highlighting the consequences that severe energy deficiency has on bone quality, in particular the integrity of trabecular bone (Bredella, Misra et al. 2008; Lawson, Miller et al. 2010; Walsh, Phan et al. 2010).

An energy deficiency is characterized by a decline in circulating concentrations of certain metabolic hormones known to have anabolic effects on the skeleton, specifically IGF-1 and leptin (Munoz, Morande et al. 2002; Mika, Holtkamp et al. 2007; Misra, Miller et al. 2007), which in turn suppresses the hypothalamic-pituitary-ovarian axis, contributing to low concentrations of reproductive hormones. The effects of these factors on bone extend beyond the scope of this review and readers are directed to other publications (Merriman, La Tour et al. 1990; Soyka, Grinspoon et al. 1999; LeRoith 2000; Pasco, Henry et al. 2001; Thomas, Burguera et al. 2001; Audi, Vargas et al. 2002; Gordeladze, Drevon et al. 2002; Welt, Chan et al. 2004; De Souza, West et al. 2008; West, Scheid et al. 2009; Chou, Chamberland et al. 2011; Mantzoros, Magkos et al. 2011; Sienkiewicz, Magkos et al. 2011). In essence, the hormonal environment characteristic of AN results in an uncoupling of bone turnover, as defined by elevated bone resorption concomitant with suppressed bone formation, similar to that observed among amenorrheic exercising women and girls. Because trabecular bone is more metabolically active than cortical bone (Biller, Saxe et al. 1989; Borer 2005), it follows that an energetic environment that encourages unfavorable alterations in bone metabolism will be accompanied by profound and relatively consistent detriments in trabecular bone.
In addition to the detriments observed in trabecular bone (Resch, Newrkla et al. 2000; Milos, Spindler et al. 2005; Bredella, Misra et al. 2008; Lawson, Miller et al. 2010; Walsh, Phan et al. 2010; Faje, Karim et al. 2013), cortical bone has also been observed to be impacted by AN. Similar to the decreased CoTh observed in the distal radius of AA (Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012), the cortical shell of the ultradistal radius has been reported to be thinner in women and adolescents with AN compared to healthy controls (Milos, Spindler et al. 2005; Faje, Karim et al. 2013). These results give rise to the concern of increased fracture risk in anorexic women. As mentioned, studies of women who experience stress fractures demonstrated that those with stress fractures had significantly decreased cortical area, leading to the supposition that loss of cortical bone can lead to fractures (Popp, Hughes et al. 2009). In anorexic women, therefore, the decreased CoTh and resultant decreased cortical area could contribute to a greater risk for stress fractures and other fractures (Rigotti, Neer et al. 1991; LaBan, Wilkins et al. 1995; Lucas, Melton et al. 1999). It is important to note, however, that the cortical compartment of the tibia, a weight-bearing bone, was assessed among women with stress fractures (Franklyn, Oakes et al. 2008; Popp, Hughes et al. 2009; Schnackenburg, Macdonald et al. 2011); whereas, the radius, a non-weight bearing bone, was evaluated among anorexic women and girls (Resch, Newrkla et al. 2000; Fricke, Tutlewski et al. 2005; Milos, Spindler et al. 2005; Milos, Spindler et al. 2007; Bredella, Misra et al. 2008; Fricke, Beccard et al. 2010; Lawson, Miller et al. 2010; Walsh, Phan et al. 2010). Although it is possible that bone quality at the radius among the anorexic population is similar to bone quality observed at the tibia among women with stress fractures, such an assumption must be made with caution, and further studies
that explore bone quality at both the radius and tibia among exercising women with stress fractures and anorexic women are needed.

The impact of an energy deficit on bone health among anorexic women is influenced by the severity and duration of the energy-deficient, starvation environment (Hay, Delahunt et al. 1992). Milos et al. (Milos, Spindler et al. 2007) compared vBMD and bone structure between anorexic women who increased body mass index (BMI) during a two-year treatment and those whose BMI remained unchanged or decreased. Baseline measurements revealed that the group with the longer duration of illness and being underweight (“BMI unchanged or decreased group”) had significantly lower total vBMD, trabecular vBMD, TbN, and CoTh than the group that increased BMI and demonstrated a shorter duration of illness (Milos, Spindler et al. 2007). These findings suggest that the longer the exposure to an energy-deficient environment and the more severe the energy deficit, the greater the negative consequences on bone density and structure. It must also be noted that during the course of the treatment greater declines in total vBMD, trabecular vBMD, and CoTh were observed in the “BMI increased” group, demonstrating that despite weight recovery in severely malnourished individuals, bone health may remain compromised within the short term (Milos, Spindler et al. 2007). Likewise, results from a two-year prospective study that assessed changes in aBMD after inpatient refeeding and weight rehabilitation among adolescent girls with AN demonstrated that low bone mass persists even two years after weight rehabilitation (Mika, Holtkamp et al. 2007). A longer follow-up of greater than 3 years revealed similar results; no significant differences in trabecular and cortical vBMD, cortical and total bone area, and perosteal and endosteal circumference at the distal radius were
observed between those who presented with a persistent eating disorder and those who were recovered (Fricke, Beccard et al. 2010). Therefore, the impact of an energy deficit on bone health among anorexic women may also persist for years following recovery despite the increase in body weight that accompanies an improved nutritional environment (Mika, Holtkamp et al. 2007).

Mechanical properties of bone, which serve as indicators of bone strength (Macneil and Boyd 2008) have also been reported to be affected by the energy-deficient and estrogen-deficient environment characteristic of AN (Walsh, Phan et al. 2010). Walsh et al. (Walsh, Phan et al. 2010) reported that stiffness and failure load as assessed by FEA of flat panel CT images was significantly lower (-27% and -29%, respectively) in anorexic women compared to healthy controls. Therefore, not only does an energy deficit appear to negatively affect bone mass and architecture, it also contributes to an impaired ability of bone to withstand loading, presumably increasing the risk for fracture (Walsh, Phan et al. 2010).

5. Lessons from Stress Fractures and Anorexia Nervosa

Bone strength among AA is notably compromised as a result of the synergistic actions of an energy and estrogen deficiency, thereby placing these women and girls at increased risk for fracture (Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012). On the other hand, the loading achieved through chronic exercise training may help to preserve some components of bone strength among this population as evidenced by greater total bone area compared to non-athletic controls (Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011). However, among athletes with
amenorrhea or a history of amenorrhea, the energy-deficient environment coupled with an estrogen deficiency leads to decreases in trabecular vBMD, impaired trabecular microarchitecture, and decreased cortical thickness and area (when considered as a percent of total area) (Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012). This deterioration of bone density and structure is strikingly similar to what is observed among anorexic adolescent girls and women (Resch, Newrkla et al. 2000; Milos, Spindler et al. 2005; Bredella, Misra et al. 2008; Lawson, Miller et al. 2010; Walsh, Phan et al. 2010; Faje, Karim et al. 2013); adolescent girls and women with AN were observed to have significantly lower total and trabecular vBMD, thinner cortical shells, and degradation of the trabecular microarchitecture as evidenced by a decrease in TbN and an increase in TbSp compared to healthy girls and women (Resch, Newrkla et al. 2000; Milos, Spindler et al. 2005; Bredella, Misra et al. 2008; Lawson, Miller et al. 2010; Walsh, Phan et al. 2010; Faje, Karim et al. 2013) (Figure 4). The similarity of these findings within the trabecular bone compartment of AA and anorexic girls and women indicates that energy and estrogen deficiencies are likely the primary contributors to the deterioration of trabecular bone.

Notably, athletes with stress fractures demonstrated significantly lower trabecular vBMD and a trend toward reduced TbN compared to athletes without a history of stress fracture (Popp, Hughes et al. 2009; Schnackenburg, Macdonald et al. 2011), which are characteristics that also described the vBMD and microarchitecture of AA (Figure 2). Thus, these similarities suggest that AA are at increased risk for fracture, possibly due to the poor quality of trabecular bone. Stress fractures occur when microdamage within bone accumulates and is not adequately repaired with remodeling (Burr 2011). The
presence of microdamage within bone is a normal and even healthy characteristic of bone given that it dissipates energy from loading thereby delaying the occurrence of a complete fracture and, physiologically, stimulates the remodeling process to renew the bone matrix (Burr 2011). In the case of a stress fracture, however, microdamage accumulates to a critical limit due to overuse without adequate repair, most likely as a result of the suppression of remodeling (Burr 2011). Therefore, the uncoupling of bone turnover that is observed among AA may be a primary contributor to the increased risk of stress fracture among these girls and women through both direct mechanisms (inadequate repair of microdamage) and indirect mechanisms (low vBMD and impaired bone structure).

6. Conclusions

Among AA, bone strength as estimated by 3-D imaging techniques is compromised. Striking similarities are observed when comparing the bone characteristics of AA with those of athletes with stress fractures and adolescent girls and women with AN. The data from athletes with stress fractures inform us that bones with a small total and cortical area as well as low trabecular vBMD may be at increased risk for fracture; whereas, the data from the anorexic population inform us that an energy deficiency typically combined with hypoestrogenism primarily may contribute to deterioration of trabecular bone, which is characterized by low trabecular vBMD and degradation of the trabecular microarchitecture. As such, these findings provide further support that the impaired bone health in AA 1) is due to an energy and estrogen deficiency and 2) causes the AA to be at increased risk for stress fracture. Among AA,
both bone quantity and structure are compromised, thereby suggesting reduced bone strength among these athletes.

Education and awareness of the consequences of amenorrhea on bone strength and fracture risk are important among girls and women who are engaged in habitual exercise training. In addition, due to the persistent nature of the deficits in both aBMD and vBMD even after reversal of an energy deficit (Mika, Holtkamp et al. 2007; Milos, Spindler et al. 2007), regular monitoring of bone health among these athletes as well as encouragement to maintain a healthy body weight, adequate energy intake, and regular menstrual function is essential. Participation in resistance training may also help to improve the bone health of AA and decrease the risk for fracture by potentially increasing the size of the bone and improving muscle strength that will help dissipate energy from loading (Burr 2011). As such, targeting both adequate energy intake and increased lean mass may lead to improved bone quantity and quality with the hope of reducing fracture risk.

7. Acknowledgements

We thank Dan Schiferl of Bone Diagnostics, Inc. for reviewing the manuscript.
References

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Chapter 3
Methods

Experimental Design

This study is a cross-sectional analysis in exercising women aged 18-35 years who were categorized according to menstrual status (i.e. amenorrheic (AM), n=18 or eumenorrheic (EU), n=9). Subjects were considered exercising if they participated in at least 2 hours of purposeful exercise per week. Menstrual status was determined by self-reported menses within the past year. Body composition and areal BMD (aBMD) were assessed using dual energy x-ray absorptiometry (DXA). vBMD, measurements of bone geometry, and measurements of estimated bone strength (BSI and SSI) were evaluated using peripheral quantitative computed tomography (pQCT).

The current investigation merges data from three studies conducted in young, exercising women, including 1) baseline data from a randomized controlled trial assessing the effects of a 12-month intervention of increased caloric intake on indices of bone health and menstrual status in premenopausal exercising women with severe menstrual disturbances, 2) a cross-sectional study assessing bone strength in exercising and sedentary women of varying menstrual status, and 3) an observational study of female collegiate cross-country runners. The studies were approved by the Institutional Review Board at Penn State University, and all participants signed an approved informed consent form.
Recruitment and Screening

Subjects were recruited through campus and community flyers, classroom announcements and handouts, listserv emails, and through research volunteer website postings at Penn State University, University Park, PA. Eligibility criteria for participation in these studies included 1) 18-35 years of age, 2) generally good health, 4) BMI of 16-29.9 kg/m\(^2\), 6) ≥2 hrs·wk\(^{-1}\) of purposeful exercise, 7) reporting at least 10 cycles in the past 12 months, if regularly menstruating, 8) no menses in the past 3 months, if amenorrheic, 9) non-smoker, 10) no form of hormonal therapy for the past 6 months, 11) not pregnant or lactating, and 12) no additional contraindications that would prevent study participation. After consent was obtained, height and weight were measured and participants completed questionnaires to assess demographics, medical history, exercise history, menstrual history, eating behaviors, and bone health.

Classification of Menstrual Status

Classification of menstrual status was based on self-reported history of menses within the past 12 months. Participants completed a questionnaire that asked them to recall the number of menses that they had experienced in the past 3, 6, 9, and 12 months. Amenorrhea was defined as the absence of menses for the past 3 months (De Souza, Toombs et al. 2010). Eumenorrhea was defined as having at least 10 menses in the past 12 months which corresponds to a cycle length ≤35 days (Drinkwater, Bruemner et al. 1990; De Souza, Toombs et al. 2010).

Anthropometrics and Body Composition

Body weight and height were measured without shoes in the laboratory using a digital scale and stadiometer, respectively. Body mass index (BMI) was calculated as the
ratio of weight to height (kg/m$^2$). DXA scans of the total body, posteroanterior lumbar spine, and dual hip were performed to assess body composition and aBMD. Participants were scanned on a GE Lunar iDXA scanner (GE Lunar Corporation, Madison, WI, enCORE 2008 software version 12.10.113). Scans were performed by two certified technicians using guidelines consistent with the International Society of Clinical Densitometry. Quality control and phantom scans were performed prior to each scan according to manufacturer guidelines.

**Measurement of vBMD, Bone Geometry, and Estimated Bone Strength**

vBMD, bone geometry, and estimated bone strength were assessed using pQCT. Scans of the proximal (66%) and distal (4%) non-dominant radius and opposite tibia were performed on a Stratec XCT 3000 (software version 6.00B, Stratec Medical, Pforzheim, Germany). If a participant had a history of fracture in the radius or tibia, the opposite limb was scanned. The 4% analysis was performed using contour mode 31 and a threshold of 169 mg/cm$^3$ to find total bone. Next, peel mode 4 with a threshold of 650 mg/cm$^3$ was used to separate out the trabecular region, with an additional 10% concentric peel from the endosteum to exclude any cortical bone. This analysis also provides a measure of cortical bone in the metaphysis using the cort_sub results. The 66% density and area analysis was performed using cortical mode 2 and a threshold of 710 mg/cm$^3$. For the 66% SSI analysis, cortical mode 2 at a threshold of 480 mg/cm$^3$ was performed. From these measurements, estimates of bone strength were calculated. BSI was calculated at the distal site using the equation: BSI=total area*(total density$^2$) (Ducher, Eser et al. 2009). SSI was calculated at the proximal site using the equation: SSI=section modulus*(cortical density/max cortical density) (Ducher, Eser et al. 2009). Muscle area
at the proximal site only was also measured. For muscle area image filtering was first applied using a combination kernel filter C03C05C05 supplied with the pQCT software. The filter smoothed edges and intra muscle adipose tissue to better separate muscle area from fat and bone. Separation thresholds of 40mg/cm³, and 710mg/cm³ were used to separate fat and bone from the muscle area respectively. To determine if muscle size corresponded with bone size, the ratio of total bone area to total muscle area (total bone area/total muscle area) for the radius and tibia at the 66% site was calculated. A pictorial representation of the variables can be found in Figure 1a (radius) and 1b (tibia).

vBMD, bone geometry, and estimated bone strength were also analyzed with respect to lean body mass, as this is a factor that may contribute to differences in these variables regardless of energy or menstrual status. It has been previously reported that lean body mass is positively correlated with BMD and hip strength analysis in exercising women (Douchi, Matsuo et al. 2003; Petit, Beck et al. 2004; Ackerman, Pierce et al. 2013; Mallinson, Williams et al. 2013), indicating a likely effect of lean body mass on bone that should be recognized. Hence, the variables examined in this study were additionally analyzed to control for differences in lean body mass.

Prior to scanning, ulna and tibia length were measured with a measuring tape. The ulna was measured from the ulnar styloid process to the olecranon process. The tibia was measured from the tibial plateau to the medial malleolus. pQCT scans were performed at 4% and 66% of the ulnar and tibial length, proximal to the radius and tibia endplates. A scout view scan was performed prior to each scan to position the reference line in the endplates. One tibia scan of women in the AM group was excluded from analysis due to movement artifacts.
Statistics and Data Analysis

Data were screened for outliers, normality, and homogeneity of variance within each group. Normality and homogeneity of variance were assessed using the Shapiro-Wilk test and Levene’s test, respectively. For variables that were normally distributed and displayed homogeneity of variance, independent t-tests were performed to determine unadjusted group differences. Non-parametric Mann Whitney-U tests were performed to determine unadjusted group differences for variables that were not normally distributed and/or did not display homogeneity of variance. To control for the known effects of lean mass on bone health, group differences in the pQCT measurements were also assessed using analysis of covariance (ANCOVA) with lean body mass as a covariate. Each model was screened for homogeneity of variance and homogeneity of regression slopes, which are two important ANCOVA assumptions, and results for the models that violated these assumptions were not reported.

A p-value of p <0.10 was considered statistically significant. SPSS software (version 19.0; Chicago, IL) was used to perform all analyses, and data were reported as mean ± standard error mean (SEM).
References


Abstract

Exercise is known to be an osteogenic stimulus, improving bone mineral density (BMD) and reducing the long-term risk of fracture. However, through metabolic and hormonal mechanisms, exercising women with menstrual cycle disturbances could be at risk for decreased BMD and negative adaptations in bone geometry that compromise bone strength secondary to insufficient caloric intake to compensate for energy expenditure. The purpose of this study was to compare volumetric BMD (vBMD), bone geometry, and estimated bone strength (bone strength index (BSI) and strength strain index (SSI)) between eumenorrheic (EU, n=9) versus amenorrheic (AM, n=18) exercising women. Bone variables were assessed at the tibia and radius using peripheral quantitative computed tomography. EU and AM women were similar in age (p>0.05) (20.7±0.5yrs), weight (57.2±1.5kg), BMI (20.6±0.4kg/m²), and body composition. Volumetric BMD, bone geometry, and estimated bone strength at the distal and proximal tibia and at the distal radius were not different (p>0.05) between EU and AM women. At the proximal radius, total vBMD, cortical vBMD, cortical thickness, endosteal circumference, muscle area, and the ratio of bone area to muscle area were also statistically similar (p>0.05) between the groups. However, EU women demonstrated a larger total area (p=0.045), cortical area (p=0.064), periosteal circumference (p=0.045), and SSI (p=0.057) at the proximal radius compared with AM women. These findings
were no longer significant after controlling for lean body mass. The results suggest that the alteration in reproductive hormones that typically results from an energy deficiency may negatively impact bone geometry and, consequently, bone strength. Because these findings were observed only at the radius, it may be that the osteogenic effect of exercise on the weight-bearing limbs is protective against the skeletal consequences of an energy deficiency in young, exercising women. However, this study may be underpowered and require a larger sample size to adequately address the research question.

Introduction

Bone growth and development are notably influenced by the mechanical stimuli the bone is exposed to, and particularly those exposures during purposeful, weight-bearing exercise (Frost 1987; Goodship 1992). It is well-established that high-impact exercise has an osteogenic effect on bone and is beneficial for improving bone mineral density (BMD) and preventing degenerative bone diseases, such as osteoporosis (Heinonen, Sievanen et al. 1996; Vainionpaa, Korpelainen et al. 2005). High-impact exercise has also been shown to cause geometric changes to bone, such as increased total bone area, which may further contribute to increased bone strength (Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012).

In spite of the beneficial bone-building influence of weight-bearing exercise, one variable that may negatively impact bone health among exercising women is the presence of an energy deficiency, which occurs when caloric intake is insufficient to support energy expenditure (De Souza and Williams 2005). An energy deficit results in physiological adaptations to conserve fuel, which suppress the hypothalamic-pituitary-
ovarian (HPO) axis (Stafford 2005; Scheid and De Souza 2010). An overt symptom of this suppression is menstrual dysfunction, specifically amenorrhea, which is the absence of menses for at least 3 months and is characterized by chronically low estrogen concentrations (De Souza and Williams 2004). A less conspicuous consequence of an energy deficiency is its detrimental effects on bone health. Low energy availability causes a variety of metabolic adaptations that ultimately result in decreased bone formation (Ihle and Loucks 2004). Indeed, amenorrheic exercising women (Christo, Prabhakaran et al. 2008) and anorexic women (Brick, Gerweck et al. 2010) (Soyka, Grinspoon et al. 1999; Misra, Aggarwal et al. 2004) have been demonstrated to have both suppressed concentrations of both IGF-1 and markers of bone formation. Estrogen, on the other hand, has a potent impact on the suppression of bone resorption due to its role of inhibiting the action of osteoclasts (Compston 2001). Hypoestrogenism, therefore, results in a removal of osteoclast inhibition and a subsequent increase in bone resorption (De Souza, West et al. 2008). When present in combination, hypoestrogenism and an energy deficiency create an uncoupling of bone turnover, characterized by an increase in bone resorption and a decrease in bone formation, which negatively impacts bone mass (De Souza, West et al. 2008).

While there has been thorough documentation in the literature regarding the effect of exercise on areal BMD (aBMD) assessed via dual energy x-ray absorptiometry (DXA) (De Souza, West et al. 2008; West, Scheid et al. 2009; Scheid, Toombs et al. 2011; Callreus, McGuigan et al. 2012), aBMD is just one of the many factors that influences bone strength. Volumetric BMD (vBMD) is a three-dimensional measurement of BMD that provides a “truer” indication of BMD. In addition, geometric factors are
known to influence bone strength (Ducher, Hill et al. 2009). Bone geometry refers to the size and the shape of the bone, which are desirable parameters for the evaluation of bone strength since a larger bone tends to be a stronger bone (Kanis, Melton et al. 1994). Furthermore, a bone that has its mass distributed farther from the center, a measurement known as cross-sectional moment of inertia (CSMI), tends to be a stronger bone (Bouxsein 2007).

Peripheral quantitative computed tomography (pQCT) is an imaging technique capable of providing measurements of vBMD and geometry not only for the total bone of interest, but also for the individual cortical and trabecular compartments (Adams 2013). Together, these densitometric and geometric variables are used in various combinations to calculate bone strength index (BSI) and strength strain index (SSI), which are estimates of bone strength against compressive and torsional/bending forces, respectively (Ducher, Eser et al. 2009). In fact, BSI was shown to be highly correlated with actual fracture load in a study of 103 excised rat femurs (Ferretti, Capozza et al. 1996), while SSI was shown to have a high prediction rate for fracture load in the forearm in a study using cadaveric human radii (Wilhelm, Felsenber et al. 2000). Therefore, these densitometric and geometric variables provide a comprehensive illustration of the structure of bone, which plays a large role in bone strength, and thus may provide valuable information about properties of bone that are indicative of an increased risk of fracture.

Exercising women with menstrual disturbances provide a unique model in which to investigate bone health due to the coupling of the skeletal consequences of an energy deficiency and the typical osteogenic effects of exercise. Up to this point, vBMD, bone
geometry, and estimated bone strength have been evaluated in adolescent female athletes, a limited number of young, active women (Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012) and in retired gymnasts (Ducher, Eser et al. 2009). Further research to evaluate these parameters in a broader population of physically active adult women is warranted in order to further elucidate the effects of exercise on bone when accompanied by the reproductive hormone alterations indicative of an energy deficiency. Therefore, the purpose of this study was to compare vBMD and bone area of total, trabecular, and cortical bone, cortical thickness, periosteal and endosteal circumference, and estimates of bone strength (BSI and SSI) in the proximal and distal radius and tibia of amenorrheic exercising women versus eumenorrheic exercising women. We hypothesized that amenorrheic exercising women would have significantly lower total and trabecular vBMD than eumenorrheic exercising women at the radius and tibia but cortical vBMD would be similar between amenorrheic and eumenorrheic exercising women. Concerning bone geometry, we hypothesized that total bone area at the tibia and radius would not be different between amenorrheic and eumenorrheic exercising women; however, at the tibia, amenorrheic women would have a decreased cortical area and increased trabecular area compared to their eumenorrheic counterparts. Accordingly, amenorrheic exercising women would have a decreased cortical thickness at the tibia and thereby an increased endosteal circumference versus eumenorrheic exercising women; however, periosteal circumference at the tibia would be similar between the groups. Finally, with regard to estimated bone strength, we hypothesized that, at the tibia and radius, amenorrheic exercising women would have significantly lower BSI but similar SSI at the radius and tibia when compared with eumenorrheic exercising women.
Methods

Experimental Design

This study is a cross-sectional analysis in exercising women aged 18-35 years who were categorized according to menstrual status (i.e. amenorrheic (AM), n=18 or eumenorrheic (EU), n=9). Subjects were considered exercising if they participated in at least 2 hours of purposeful exercise per week. Menstrual status was determined by self-reported menses within the past year. Body composition and areal BMD (aBMD) were assessed using dual energy x-ray absorptiometry (DXA). vBMD, measurements of bone geometry, and measurements of estimated bone strength (BSI and SSI) were evaluated using peripheral quantitative computed tomography (pQCT).

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Body weight and height were measured without shoes in the laboratory using a digital scale and stadiometer, respectively. Body mass index (BMI) was calculated as the ratio of weight to height (kg/m²). DXA scans of the total body, posteroanterior lumbar spine, and dual hip were performed to assess body composition and aBMD. Participants were scanned on a GE Lunar iDXA scanner (GE Lunar Corporation, Madison, WI, enCORE 2008 software version 12.10.113). Scans were performed by two certified
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\[ \text{vBMD, bone geometry, and estimated bone strength were also analyzed with respect to lean body mass, as this is a factor that may contribute to differences in these variables regardless of energy or menstrual status. It has been previously reported that lean body mass is positively correlated with BMD and hip strength analysis in exercising women (Douchi, Matsuo et al. 2003; Petit, Beck et al. 2004; Ackerman, Pierce et al. 2013; Mallinson, Williams et al. 2013), indicating a likely effect of lean body mass on bone that should be recognized. Hence, the variables examined in this study were additionally analyzed to control for differences in lean body mass.} \]

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A p-value of p<0.10 was considered statistically significant. SPSS software (version 19.0; Chicago, IL) was used to perform all analyses, and data were reported as mean ± standard error mean (SEM).

Results

Demographic Characteristics, Body Composition, and Areal Bone Mineral Density

Demographic and body composition characteristics for EU (n=9) and AM (n=18) subjects are provided in Table 1. All subjects were between 18 and 26 years of age. The groups did not differ (p>0.05) in age, age of menarche, history of exercise minutes per week in the past six months, body weight, height, body mass index, body fat percentage, fat mass, or lean body mass. Areal BMD at the total body, lumbar spine (L1-L4), femoral neck, and total hip were also similar (p>0.05) between the groups, as shown in Table 2.
pQCT results for the distal and proximal tibia and radius are shown in Tables 3 and 4, respectively. At the distal and proximal tibia and the distal radius, EU and AM women did not differ in any pQCT measurements, including vBMD, bone geometry, or estimated bone strength. At the distal and proximal tibia, one EU scan was unable to be analyzed due to movement that occurred during the scan. Likewise, at the proximal radius, EU and AM women presented with similar total vBMD, cortical vBMD, cortical thickness, endosteal circumference, muscle area, and the ratio of bone area to muscle area. However, EU women had a (p<0.10) larger total area (p=0.045), cortical area (p=0.064), periosteal circumference (p=0.045), and SSI (p=0.057) than AM women at the proximal radius. When controlling for lean body mass, these findings were no longer significant.

Discussion

Results of this investigation revealed that EU women exhibited larger bone size and greater estimated bone strength at the proximal radius compared with AM women. However, no significant differences were observed at the tibia. The results were in contrast to our hypotheses, which predicted differences in vBMD at the radius and tibia, differences in bone area at the tibia but not the radius, differences in BSI at the radius and tibia, and no differences in SSI at the radius or tibia. Interestingly, the differences that we observed in bone size and estimated strength between EU and AM women occurred in a non-weight-bearing bone but not a weight-bearing bone, suggesting that in exercising women, hypoestrogenism, which may be occurring synchronously with an energy deficit
(De Souza, Lee et al. 2007) may contribute to greater skeletal decrements at non-weight-bearing sites. This is the first published study to examine these variables in a population that consists solely of young exercising women with and without severe menstrual disturbances. As such, the results offer novel insight as to how exercise during young adulthood can affect bone when it is accompanied by the hormonal and metabolic consequences associated with menstrual disturbances (De Souza, Lee et al. 2007).

At the radius, our results were not consistent with previous studies that examined similar variables to those presented herein but in populations that differed from our population (Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011; Ackerman, Putman et al. 2012). Ackerman et al. (2011) used high resolution pQCT to assess vBMD, bone geometry and structure, and trabecular microarchitecture in adolescent and young adult athletes with and without amenorrhea. Although measurements at the proximal radius were not obtained, eumenorrheic athletes exhibited significantly greater trabecular vBMD at the ultradistal radius compared with amenorrheic athletes when controlling for bone age, bone age and BMI, bone age and lean mass, and bone age and height (Ackerman, Nazem et al. 2011). Furthermore, in a cross-sectional analysis that explored vBMD, bone structure, and estimated bone strength in retired gymnasts grouped according to menstrual history, it was reported that retired gymnasts without a history of amenorrhea experienced greater cortical thickness and decreased medullary area at the proximal radius compared to retired gymnasts with a history of amenorrhea (Ducher, Eser et al. 2009).

At the tibia, our results also differed from previous findings. Ackerman et al. (2011) reported that eumenorrheic adolescent and young adult athletes had higher total
vBMD at the distal tibia than amenorrheic athletes when controlling for bone age (Ackerman, Nazem et al. 2011). Moreover, Ducher et al. (2009) observed that retired gymnasts without a history of amenorrhea had greater trabecular vBMD at the distal tibia but smaller SSI at the proximal tibia when compared with retired gymnasts with a history of amenorrhea (Ducher, Eser et al. 2009).

Important differences between the population investigated herein and that of the aforementioned studies (Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011) may explain the discrepancies between the findings of these previous studies and the results reported in our investigation. Notably, the study by Ackerman et al. (2011) included girls and women aged 15-21 years, while our sample included women aged 18-26 years. Since the greatest increases in bone mass occur during puberty (Theintz, Buchs et al. 1992; Soyka, Fairfield et al. 2000; Gilsanz, Chalfant et al. 2011), exercise during adolescence has a profound impact on improving BMD and enhancing the attainment of peak bone mass (Burrows 2007). The effects of this augmented bone mineral accrual due to physical activity during adolescence persists into young adulthood (Baxter-Jones, Kontulainen et al. 2008). Since such large increases in bone mass occur during adolescence, estrogen exposure during adolescence is perhaps even more crucial than during young adulthood when the rate of change in bone mass is much slower (Theintz, Buchs et al. 1992). Amenorrhea during adolescence may lead to even larger discrepancies in BMD between amenorrheic and eumenorrheic athletes than amenorrhea experienced during young adulthood. Therefore, the inclusion of adolescents in the investigation conducted by Ackerman et al. (2011) may have greatly impacted the findings. Since our present study did not include athletes under the age of 18 and, additionally, we did not
control for activity levels during adolescence, duration of amenorrhea, or history of amenorrhea during adolescence, we are unable to determine if these are confounding variables.

There are additional reasons to take caution when attempting to extrapolate the findings of Ducher et al. (2009) to female athletes of all ages who are participating in various types of sports. The women in the Ducher et al. (2009) investigation participated in competitive gymnastics at a high level for at least 4 years during childhood and adolescence and had been retired from the sport for at least 3 years. Furthermore, since retirement from the sport, the women had not participated in more than 2 hours of physical activity per week (Ducher, Eser et al. 2009). Due to the high-impact loading experienced with gymnastics training, large differences in BMD between gymnasts or retired gymnasts and other types of athletes have been consistently reported and are, in fact, expected (Nichols, Sanborn et al. 1994; Kirchner, Lewis et al. 1996; Taaffe, Robinson et al. 1997; Bass, Pearce et al. 1998; Zanker, Osborne et al. 2004; Pollock, Laing et al. 2006; Maimoun, Coste et al. 2013). Since the retired gymnasts in the Ducher investigation participated in a highly osteogenic sport during childhood and adolescence, the combined influence of physical activity and estrogen exposure (or lack thereof) on bone density and structure in this population may differ when compared with the influence of physical activity and estrogen status on bone health in a population of young adults.

Additionally, for our study in particular, sport type was heterogeneous among the women, unlike the women studied in the investigation by Ducher et al. (Ducher, Eser et al. 2009). In the present study, 78% of the EU women participated in repetitive low
impact sports, including running (n=6) and general cardio training (n=1). The other 22% of the EU women participate in non-weight-bearing sports, namely swimming (n=1) and cycling (n=1). Sport type among the AM women was more varied, with 6%, 11%, and 6% of women participating in odd impact, high magnitude, and non-weight-bearing sports, respectively. Odd impact training included tennis (n=1), high magnitude sports included strength/weight training and/or pilates (n=2), and the non-weight-bearing athlete was a swimmer. However, the majority of women in the AM group (78%), participated in repetitive low impact sports, including running (n=9) and general cardio training (n=4). The effects of exercise on bone may differ considerably depending on the activity, so noting these variations in sport type may explain why our findings were not consistent with previous studies that investigated more homogenous samples.

The physiological mechanism underlying the results presented herein is currently unclear; however, there are several factors that may have contributed to our findings. Estimated bone strength was lower in AM compared with EU women at the proximal radius but not at the distal tibia, suggesting that hypoestrogenism may contribute to reductions in bone strength at non-weight-bearing sites. This theory is supported by an additional investigation by Ackerman et al. (2012) which reported lower estimates of stiffness and failure load at the distal radius in amenorrheic adolescent and young adult athletes compared to non-athletic control subjects. This same finding was not observed at the distal tibia; rather, amenorrheic athletes demonstrated similar stiffness and failure load when compared with non-athletic controls (Ackerman, Putman et al. 2012).

Moreover, menstrual disturbances in female athletes are often caused by an underlying energy deficit (De Souza, Lee et al. 2007); therefore, the skeletal influence of
metabolic hormones must be considered. Peptide YY (PYY) is an anorexigenic peptide hormone that is elevated in amenorrheic athletes with an energy deficiency (Scheid, Williams et al. 2009) and also negatively associated with BMD (Scheid, Toombs et al. 2011). Further, circulating concentrations of leptin, an adipocytokine, which is frequently lower in amenorrheic exercising women compared to ovulatory exercising women (Christo, Cord et al. 2008; Corr, De Souza et al. 2011; Mallinson, Williams et al. 2013) is a significant predictor of bone geometry, specifically in the femoral neck (Mallinson, Williams et al. 2013). In addition, insulin-like growth factor-1 (IGF-1) is an anabolic hormone that may also be decreased in amenorrheic athletes (Christo, Prabhakaran et al. 2008). IGF-1 predicts lumbar spine BMD and markers of bone formation in amenorrheic athletes ranging from adolescence to young adulthood (Zanker and Swaine 1998; Christo, Prabhakaran et al. 2008), highlighting its contribution to bone health. The alterations in these metabolic hormones that occur with an energy deficit, in combination with the suppression of estrogen that is characteristic of exercise-associated amenorrhea, likely impact bone turnover (Compston 2001; Russell and Misra 2010; Mantzoros, Magkos et al. 2011). In female runners with chronic amenorrhea, the suppression of bone formation and elevation of bone resorption resulting in a negative bone remodeling balance has been reported (Zanker and Swaine 1998). This is in opposition to the positive bone remodeling balance observed in their eumenorrheic counterparts (Zanker and Swaine 1998). In addition, correlations between decreased estradiol concentrations and decreased concentrations of markers of bone formation, specifically osteocalcin and bone-specific alkaline phosphatase (BSAP), in amenorrheic distance runners has been observed, indicating that amenorrhea and the associated
estrogen deficiency are linked to suppression of bone formation (Zanker and Swaine 1998). Considering these findings, it is possible that the hormonal differences between AM and EU women is enough to cause changes in bone turnover, particularly at non-weight-bearing sites that do not receive any skeletal protection from the mechanical stimuli associated with exercise. Hence, the underlying reason for a larger bone size and greater estimated bone strength of non-weight-bearing bones in amenorrheic compared with eumenorrheic exercising women may be due to the changes in bone metabolism, particularly a decrease in bone formation, that may accompany amenorrhea resulting from an energy deficiency.

Elaborating on the effect of hypoestrogenism on non-weight-bearing bones, it has been consistently reported that amenorrheic athletes have lower BMD at the lumbar spine, a non-weight-bearing site (Drinkwater, Nilson et al. 1984; Nelson, Fisher et al. 1986; Rencken, Chesnut et al. 1996; Christo, Prabhakaran et al. 2008; West, Scheid et al. 2009; Ackerman, Nazem et al. 2011; Duckham, Peirce et al. 2013; Mallinson, Williams et al. 2013). However, reports comparing BMD at weight-bearing sites such as the femoral neck and hip between female athletes of differing menstrual status are inconsistent; some investigators have reported no differences (West, Scheid et al. 2009; Duckham, Peirce et al. 2013; Mallinson, Williams et al. 2013) while other investigators have reported differences between amenorrheic and eumenorrheic athletes (Rencken, Chesnut et al. 1996; Christo, Prabhakaran et al. 2008; Ackerman, Nazem et al. 2011; Duckham, Peirce et al. 2013). These findings support the supposition that hypoestrogenism associated with exercise-associated amenorrhea, and the potential underlying energy deficit, causes detriments to the non-weight-bearing bones while the
weight-bearing bones, though experiencing an identical hormonal environment, are at least moderately protected by the osteogenic effects of regular weight-bearing physical activity.

There are several limitations to this study. Due to the small and largely unbalanced sample size, additional data collection is necessary to improve the power of the study, balance the sample sizes, and confirm that the findings are sound. Doing so may also reveal further significant findings. The heterogeneity of the sample is a further limiting factor. Subjects represent a wide variety of sport types, all of which may cause distinct effects on bone due to differences in the magnitude of loading, frequency of loading, and varying angles of impact. In regard to menstrual categorization, subjects were assigned as amenorrheic or eumenorrheic according to self-reported history of menses in the past 12 months. Confirmation of menstrual status via collection of daily urinary samples for one menstrual cycle or 28-day monitoring period to assess urinary metabolites of the reproductive hormones estrogen, progesterone, and luteinizing hormone would strengthen the analysis, allowing for identification of classic hormone profiles that indicate amenorrhea or normal, ovulatory menstrual cycles. Furthermore, we did not consider the duration of amenorrhea or whether subjects were amenorrheic during adolescence, both of which may influence the impact that amenorrhea has on bone. For instance, since it takes several months to years to observe clinically significant changes in bone, women who have been amenorrheic for an extended period of time have had more of an opportunity to experience changes in bone than women who have only recently become amenorrheic. Our analyses could be strengthened by further controlling for these factors.
Future research should compare the variables measured in the current study with vBMD, bone geometry, and estimated bone strength in sedentary young adult women. Using a sedentary, eumenorrheic control group, it may be possible to further distinguish the effects of exercise on bone with and without the presence of menstrual disturbances.

Conclusion

In conclusion, the purpose of this study was to determine if differences existed between AM and EU exercising women with respect to vBMD, bone geometry, and estimated bone strength. Our results showed larger bone size and estimated bone strength at the radius, but not at the tibia, in eumenorrheic compared with amenorrheic exercising women. This supports the notion that menstrual disturbances in exercising women, which is typically due to an underlying energy deficit, can negatively impact bone geometry and therefore bone strength. In turn, this decrease in bone strength may contribute to an increased risk of fracture throughout life (Melton, Christen et al. 2010). Our results highlight the importance of promoting sufficient caloric intake to support energy expenditure among female athletes, thereby ensuring adequate energy status and optimal reproductive status to maintain and optimize bone health.
References

Ackerman, K. E., T. Nazem, et al. (2011). "Bone microarchitecture is impaired in adolescent amenorrheic athletes compared with eumenorrheic athletes and nonathletic controls." J Clin Endocrinol Metab 96(10): 3123-3133.


Chapter 5  
Conclusion and Future Directions

The purpose of this study was to determine if there are differences in volumetric (vBMD), bone geometry, and estimated bone strength at the radius and tibia between amenorrheic (AM) and eumenorrheic (EU) exercising women. It has been previously reported that amenorrhea in exercising women is frequently the result of an energy deficiency that is caused by insufficient caloric intake to support energy expenditure (De Souza and Williams 2005). The hormonal alterations that accompany an energy deficit and menstrual dysfunction have potential negative effects on bone, such as increased bone resorption, decreased bone formation, and ultimately decreased bone mineral density (BMD) (De Souza, West et al. 2008). Based on published results from previous studies in female athletes (Ducher, Eser et al. 2009; Ackerman, Nazem et al. 2011), we hypothesized that, in general, EU exercising women would have denser, larger, and stronger bones than AM exercising women at the tibia, but predicted only a select few of these variables, such as vBMD, would be different at the radius since it is non-weight-bearing. However, contrary to our hypothesis, we observed no differences in vBMD or bone structure between EU and AM exercising women at the distal and proximal tibia and at the distal radius. Unexpectedly, at the proximal radius, AM women had decreased total area, cortical area, periosteal circumference, and strength strain index (SSI) compared to EU women. In summary, a non-weight-bearing bone of AM exercising women appears to be smaller and weaker than that of their EU counterparts; however, vBMD, bone geometry, and estimated bone strength of the weight-bearing tibia appears
to be less influenced by menstrual dysfunction than the radius in exercising women. It must be noted, however, that these findings at the proximal radius were no longer significant when controlling for lean body mass, indicating a potential effect of lean body mass on bone size and strength regardless of menstrual status.

The non-weight-bearing nature of the radius is worth noting. It is well-established that weight-bearing physical activity has a beneficial, osteogenic effect on bone such that the mechanical stimuli of habitual exercise contributes to increases in BMD and strength-promoting changes in bone geometry (Heinonen, Sievanen et al. 1996; Nikander, Sievanen et al. 2005; Vainionpaa, Korpelainen et al. 2005). However, the hormonal alterations associated with amenorrhea have an opposite effect on bone. Specifically, hypoestrogenism associated with exercise-induced amenorrhea causes increased bone resorption and a disruption of bone turnover, resulting in a net negative bone balance (De Souza, West et al. 2008). Furthermore, AM exercising women frequently present with decreased areal BMD (aBMD) compared to their EU counterparts (Drinkwater, Nilson et al. 1984; Nelson, Fisher et al. 1986; Rencken, Chesnut et al. 1996; Christo, Prabhakaran et al. 2008; West, Scheid et al. 2009; Ackerman, Nazem et al. 2011; Mallinson, Williams et al. 2013). Notably, in the present investigation, vBMD did not appear to be affected by menstrual dysfunction at either the weight-bearing tibia or non-weight-bearing radius in a similar sample of women. Rather, the bone that is not largely influenced by the mechanical stimulus of exercise was different in regard to size and strength between EU and AM exercising women, reflecting the potentially potent impact of hormonal factors on bone geometry. Furthermore, since similar detriments were not observed in the weight-bearing tibia, it may be that exercise has a protective effect on bone, particularly
in young adults whose change in bone mass is generally less than the change occurring during adolescence (Theintz, Buchs et al. 1992).

This investigation contributes valuable information to the literature regarding the effect of exercise on bone when it is coupled with menstrual dysfunction. Previous studies have used DXA to examine aBMD in exercising women categorized according to menstrual status (De Souza, West et al. 2008; West, Scheid et al. 2009; Scheid, Toombs et al. 2011; Callreus, McGuigan et al. 2012); however, aBMD is a 2-dimensional measurement of a 3-dimensional property, BMD. Although aBMD from DXA certainly has clinical value, aBMD is only an estimate of true BMD. Peripheral quantitative computed tomography (pQCT), the imaging technique used for our analyses, provides a volumetric, 3-dimensional measurement of BMD, thereby providing a truer value when compared to DXA. pQCT is also able to separate cortical and trabecular measurements (Adams 2013) in a way that has not been previously reported in the population studied herein. In addition to allowing for estimates of bone strength, the measurements obtained from pQCT provide a more accurate picture of the bone as a whole. By revealing characteristics of bone that have been negatively impacted by menstrual dysfunction, which is most likely caused by an energy deficit (De Souza, Lee et al. 2007), it is evident that sufficient energy intake likely plays a vital role in maintaining bone size and strength in exercising women and perhaps preventing fractures. In fact, SSI was shown to have a high prediction rate for fracture load in the forearm in a study using cadaveric human radii (Wilhelm, Felsenber et al. 2000), such that a greater SSI was protective against fractures. As such, the decreased SSI that was observed in the proximal radius of AM exercising women may indicate an increased fracture risk.
Several limitations of this study may impact the validity and reproducibility of our findings. First of all, small, unbalanced sample sizes are particularly limiting for outcomes related to bone health. By increasing the total sample size and achieving more balanced groups, the power of the study will be improved, potentially leading to the identification of additional and greater differences in vBMD and bone structure between the AM and EU women. Moreover, a potential source of error may have resulted from solely using self-reported menstrual history rather than confirming menstrual status with measurement of reproductive hormones. Additionally, duration of amenorrhea may also have been a confounding variable, considering that it takes several months for bone to experience observable changes in response to a change in hormonal environment. Consequently, women who have been amenorrheic for an extended period of time would have had more opportunity to experience detriments to bone than women who have only recently become amenorrheic. Furthermore, we did not take into account whether subjects were amenorrheic during adolescence, during which time the hormonal impact on bone may be even greater than during adulthood (Theintz, Buchs et al. 1992). Furthermore, without distinguishing sport type, we are unable to account for the differences in magnitude and frequency of loading on bone between and among the groups. These factors may all have contributed to the discrepancies between our hypotheses and our findings.

In addition to collecting additional data in the population studied herein to strengthen the present investigation, it would also be prudent to add a sedentary control group. Assessment of vBMD, bone geometry, and estimated bone strength in sedentary eumenorrheic women would allow for further elucidation of the impact of exercise on
bone when it is accompanied by menstrual disturbances. Specifically, addition of a sedentary control group would allow us to determine if AM exercising women reap no, partial, or full skeletal benefits from regular physical activity.

In conclusion, the present study delved into a largely uninvestigated area of research in exercise physiology and, more specifically, in the health of physically-active women. Investigations that provide further insight into the combined and independent effects of an energy deficiency and estrogen deficiency on vBMD and bone structure are necessary in order to effectively prevent, manage, and treat female athletes with exercise-associated menstrual disturbances.
References

Ackerman, K. E., T. Nazem, et al. (2011). "Bone microarchitecture is impaired in adolescent amenorrheic athletes compared with eumenorrheic athletes and nonathletic controls." J Clin Endocrinol Metab 96(10): 3123-3133.


Theintz, G., B. Buchs, et al. (1992). "Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of


Appendix A

Tables and Figures for Literature Review
Table 1. Summary of pQCT Results from Studies Assessing Bone Strength in Amenorrheic Athletes

<table>
<thead>
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<th>Study</th>
<th>Participants</th>
<th>Tool</th>
<th>Sites</th>
<th>Significant Results</th>
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*p<0.05 AA vs. NAC; *p<0.05 AA vs. EA; *p<0.05 EA vs. NAC; a p<0.05 AO vs. EA
NS: not significant; nr: not reported; na: not applicable; AA: amenorrheic athletes; EA: eumenorrheic athletes; NAC: non-athletic controls; AO: amenorrheic/oligomenorrheic; ToD: total volumetric bone mineral density (vBMD); ToA: total bone area; TbD: trabecular vBMD; TbA: trabecular area; CoD: cortical vBMD; CoA: cortical area; CoTh: cortical thickness; TbN: trabecular number; TbTh: trabecular thickness; TbSp: trabecular spacing; BSI: bone strength index; SSI: strength strain index; pQCT: peripheral quantitative computed tomography; HR: high resolution
*Analysis adjusted for bone age and height; **Analysis adjusted for height
Table 2. Summary of pQCT Results from Studies Assessing Bone Strength in Women with Stress Fractures

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</tbody>
</table>

ns: not significant; nr: not reported; na: not applicable; SF: athletes with history of/current stress fracture; NSF: athletes without history of/current stress fracture; ToD: total volumetric bone mineral density (vBMD); ToA: total bone area; TbD: trabecular vBMD; CoD: cortical vBMD; CoA: cortical area; CoTh: cortical thickness; TbN: trabecular number; TbTh: trabecular thickness; TbSp: trabecular spacing; BSI: bone strength index; SSI: strength strain index; MCSA: muscle cross-sectional area; pQCT: peripheral quantitative computed tomography; HR: high resolution

*Analysis adjusted for age and tibial length
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Tool</th>
<th>Sites</th>
<th>Significant Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample</td>
<td>Age (yr)</td>
<td></td>
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<td>Cross-Sectional Studies</td>
<td></td>
<td></td>
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<tr>
<td>Bredella et al., 2008 [61]</td>
<td>10 AN 10 HC</td>
<td>15.9±1.6 15.9±2.1</td>
<td>Flat-panel volume CT</td>
<td>Ultradistal Radius</td>
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<tr>
<td>Walsh et al., 2010 [60]</td>
<td>8 AN 6 HC</td>
<td>26.6±7.1 26.3±6.9</td>
<td>Flat-panel volume CT</td>
<td>Ultradistal Radius</td>
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<tr>
<td>Lawson et al., 2009 [56]</td>
<td>12 AN 11 HC</td>
<td>28.8±1.7 27.0±1.8</td>
<td>Flat-panel volume CT</td>
<td>Ultradistal Radius</td>
</tr>
<tr>
<td>Faje et al., 2013 [66]</td>
<td>21 AN 23 HC</td>
<td>19.1±0.2 19.3±0.5</td>
<td>HR-pQCT</td>
<td>Ultradistal Radius</td>
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<tr>
<td>Milos et al., 2005 [57]&lt;sup&gt;*&lt;/sup&gt;</td>
<td>36 AN 30 HC</td>
<td>23.4±3.7 22.8±2.1</td>
<td>HR-pQCT</td>
<td>Ultradistal Radius</td>
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<tr>
<td>Resch et al., 2000 [62]</td>
<td>20 AN 20 HC</td>
<td>23 23</td>
<td>pQCT Stratec XCT 1400</td>
<td>Distal Radius</td>
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<td>Longitudinal or Follow-Up Studies</td>
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<tr>
<td>Milos et al., 2007 [63]&lt;sup&gt;**&lt;/sup&gt;</td>
<td>15 AN-R 9 AN-NR</td>
<td>27.3±6.7 24.7±6.6</td>
<td>HR-pQCT</td>
<td>Ultradistal Radius</td>
</tr>
<tr>
<td>Fricke et al., 2005 [64]</td>
<td>13 AN-R 12 AN-NR</td>
<td>22.2±2.5 21.6±2.1</td>
<td>pQCT Stratec XCT 900</td>
<td>Distal Radius</td>
</tr>
<tr>
<td>Fricke et al., 2010 [65]</td>
<td>12 AN-R 9 AN-NR</td>
<td>22.2±2.5 21.6±2.1</td>
<td>pQCT Stratec XCT 900</td>
<td>Distal Radius</td>
</tr>
</tbody>
</table>
*p<0.05 AN vs. HC; **p<0.05 AN-R vs. AN-NR for annual change
NS: not significant; nr: not reported; AN: anorexic women/girls; HC: healthy controls; AN-NR: anorexic-not recovered; AN-R: anorexic-recovered; ToD: total volumetric bone mineral density (vBMD); ToA: total bone area; TbD: trabecular vBMD; TbA: trabecular area; CoD: cortical vBMD; CoA: cortical area; CoTh: cortical thickness; TbN: trabecular number; TbTh: trabecular thickness; TbSp: trabecular spacing; BV/TV (%): apparent trabecular bone volume fraction; BSI: bone strength index; SSI: strength strain index; CT: computed tomography; pQCT: peripheral quantitative computed tomography; HR: high resolution
*adjusted for age, **reflects annual change
**Figure 1**

a) Cycles of Ov Women (n=37)

![Graph showing follicular and luteal phases with E1G and PdG levels](image)

b) Cycles of Amen Women (n=45)

![Graph showing minimal changes in E1G and PdG levels over the cycle](image)
Figure 2

Amenorrheic Athletes ↔ Stress-Fractured Athletes

 ↔ ToD
 ↓ CoD
 ↑ ToA
 ↑ CoA
 ↑ TbSp
 ↓ BSI
 ↑ SSI

 ↔ ToD
 ↔ CoD
 ↓ ToA
 ↓ CoA
 ↔ TbSp
 ↔ BSI
 ↓ SSI
Figure 4

Amenorrheic Athletes ↔ Anorexic Girls and Women

↔ ToD
↓ CoD
↑ ToA
↑ CoA
↔ TbTh
↓ BSI

↓ TbD
↓ CoTh
↓ TbN
↑ TbSp
↓ TbD
↔ CoD
↔ ToA
↓ CoA
↓ TbTh
↓ Stiffness
↓ Failure Load
Figure Captions

Fig. 1 Composite menstrual graphs of reproductive hormones. A.) Composite menstrual graph featuring the reproductive hormone profile of menstrual cycles of ovulatory (Ov) women. The estrone-1-glucuronide (E1G) peak and pregnanediol glucuronide (PdG) peak in the follicular and luteal phases, respectively, are classic characteristics of an ovulatory cycle. The line represents the day of the luteinizing hormone surge and ovulation. B.) Composite menstrual graphs demonstrating the reproductive hormone profile of a 28-day monitoring period in amenorrheic (Amen) women. The chronic suppression of estrone-1-glucuronide (E1G) and pregnanediol glucuronide (PdG) are classic characteristics of the hormonal status in amenorrhea. Used with permission from Mallinson RJ, Williams NI, Hill BR, De Souza MJ. Body composition and reproductive function exert unique influences on indices of bone health in exercising women. Bone. 2013 May 20;56(1):91-100.

Fig 2 Similarities and differences in pQCT variables between amenorrheic and stress-fractured athletes. Directional changes for amenorrheic athletes are compared to eumenorrheic athletes or non-athletic controls, and directional changes for stress-fractured athletes are compared to athletes without a current stress fracture or history of stress fracture. Amenorrheic and stress-fractured athletes both display a decrease in trabecular density and trabecular number. Results after adjustment for bone age and height were used from Ackerman et al. [21]. ToD: total volumetric bone mineral density; CoD: cortical volumetric bone mineral density; TbD: trabecular volumetric bone mineral density; ToA: total bone area; CoA: cortical bone area; TbSp:
trabecular spacing; TbN: trabecular number; BSI: bone strength index; SSI: strength strain index. *indicates trend toward lower TbN (p=0.08) in stress-fractured athletes.

**Fig 3** Bone structure and microarchitecture in anorexia nervosa. Flat-panel volume CT images of distal radius were obtained in a.) control subject and b) woman with anorexia nervosa. Corresponding three-dimensional models for c.) control subject and d) woman with anorexia nervosa were created using MIMCS software. Deterioration of the trabecular microarchitecture and thinning of the cortical shell can be seen in the distal radius of the anorexic woman. Used with permission from Walsh CJ, Phan CM, Misra M, Bredella MA, Miller KK, Fazeli PK, et al. Women with anorexia nervosa: finite element and trabecular structure analysis by using flat-panel volume CT. Radiology. 2010 Oct;257(1):167-74.

**Fig 4** Similarities and differences in pQCT variables between amenorrheic athletes and anorexic girls and women. Directional changes for amenorrheic athletes are compared to eumenorrheic athletes or non-athletic controls, and directional changes for anorexic women or adolescents are compared to healthy controls. Amenorrheic athletes and anorexic girls and women both present with decreased total and trabecular density, decreased cortical thickness and trabecular number, and increased trabecular spacing. Results after adjustment for bone age and height were used from Ackerman et al. [21]. ToD: total volumetric bone mineral density; CoD: cortical volumetric bone mineral density; TbD: trabecular volumetric bone mineral density; ToA: total bone area; CoA: cortical bone area; TbSp: trabecular spacing; TbN: trabecular number; TbTh: trabecular thickness; BSI: bone strength index.
Appendix B

Tables and Figures for Manuscript
Table 1- Demographic and body composition characteristics for eumenorrheic and amenorrheic women

<table>
<thead>
<tr>
<th></th>
<th>EU (n=9)</th>
<th>AM (n=18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>20.7 ± 0.9</td>
<td>20.8 ± 0.6</td>
<td>0.734</td>
</tr>
<tr>
<td>Age of Menarche (yr)</td>
<td>13.4 ± 0.6</td>
<td>13.3 ± 0.3</td>
<td>0.783</td>
</tr>
<tr>
<td>Gynecological Age (yr)</td>
<td>7.2 ± 1.2</td>
<td>7.5 ± 0.7</td>
<td>0.917</td>
</tr>
<tr>
<td>History of Past Exercise</td>
<td>513.1 ± 104.8</td>
<td>502.3 ± 127.3</td>
<td>0.246</td>
</tr>
<tr>
<td>History of Past Exercise (min/wk)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>60.3 ± 2.6</td>
<td>55.6 ± 1.8</td>
<td>0.217</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.7 ± 1.7</td>
<td>165.1 ± 1.3</td>
<td>0.120</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>21.1 ± 0.6</td>
<td>20.4 ± 0.6</td>
<td>0.424</td>
</tr>
<tr>
<td>Body Fat Percentage (%)</td>
<td>25.6 ± 1.3</td>
<td>24.2 ± 1.2</td>
<td>0.227</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>15.4 ± 1.2</td>
<td>13.6 ± 1.0</td>
<td>0.150</td>
</tr>
<tr>
<td>Lean Body Mass (kg)</td>
<td>42.0 ± 1.6</td>
<td>39.7 ± 1.1</td>
<td>0.244</td>
</tr>
</tbody>
</table>
Table 2- DXA results for eumenorrheic and amenorrheic women

<table>
<thead>
<tr>
<th></th>
<th>EU (n=9)</th>
<th>AM (n=18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Body z-score</td>
<td>0.8 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>0.319</td>
</tr>
<tr>
<td>Total Body BMD (g/cm²)</td>
<td>1.144 ± 0.033</td>
<td>1.104 ± 0.021</td>
<td>0.302</td>
</tr>
<tr>
<td>Lumbar Spine z-score</td>
<td>-0.2 ± 0.2</td>
<td>-0.6 ± 0.2</td>
<td>0.239</td>
</tr>
<tr>
<td>Lumbar Spine BMD (g/cm²)</td>
<td>1.145 ± 0.028</td>
<td>1.086 ± 0.028</td>
<td>0.194</td>
</tr>
<tr>
<td>Femoral Neck z-score</td>
<td>0.6 ± 0.5</td>
<td>0.2 ± 0.3</td>
<td>0.398</td>
</tr>
<tr>
<td>Femoral Neck BMD (g/cm²)</td>
<td>1.065 ± 0.042</td>
<td>1.017 ± 0.032</td>
<td>0.384</td>
</tr>
<tr>
<td>Total Hip z-score</td>
<td>0.6 ± 0.5</td>
<td>0.1 ± 0.3</td>
<td>0.333</td>
</tr>
<tr>
<td>Total Hip BMD (g/cm²)</td>
<td>1.069 ± 0.047</td>
<td>1.013 ± 0.030</td>
<td>0.304</td>
</tr>
</tbody>
</table>

*z-score: n=6 EU and n=12 AM; reference values not available to calculate z-score for populations under the age of 20
Table 3- Measurements of BMD, bone geometry, and estimated bone strength at the proximal and distal tibia

<table>
<thead>
<tr>
<th></th>
<th>EU (n=8)</th>
<th>AM (n=18)</th>
<th>P value</th>
<th>P* value</th>
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</thead>
<tbody>
<tr>
<td><strong>DISTAL TIBIA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>310.7 ± 14.6</td>
<td>325.4 ± 11.0</td>
<td>0.453</td>
<td>0.358</td>
</tr>
<tr>
<td>Total Area (mm²)</td>
<td>1001.8 ± 39.1</td>
<td>959.8 ± 26.5</td>
<td>0.387</td>
<td>0.599</td>
</tr>
<tr>
<td>Trabecular vBMD (mg/cm³)</td>
<td>254.6 ± 13.3</td>
<td>271.2 ± 8.0</td>
<td>0.277</td>
<td>0.196</td>
</tr>
<tr>
<td>Trabecular Area (mm²)</td>
<td>805.0 ± 36.3</td>
<td>767.0 ± 24.8</td>
<td>0.399</td>
<td>0.582</td>
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<tr>
<td>Cortical vBMD (mg/cm³)</td>
<td>533.5 ± 21.7</td>
<td>533.4 ± 18.0</td>
<td>0.996</td>
<td>0.885</td>
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<tr>
<td>Cortical Area (mm²)</td>
<td>196.7 ± 5.5</td>
<td>192.8 ± 4.8</td>
<td>0.640</td>
<td>0.990</td>
</tr>
<tr>
<td>Bone Strength Index (mg²/mm⁴)</td>
<td>98.0 ± 9.7</td>
<td>102.1 ± 6.0</td>
<td>0.720</td>
<td>0.470</td>
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<tr>
<td><strong>PROXIMAL TIBIA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>668.7 ± 31.2</td>
<td>691.4 ± 19.1</td>
<td>0.528</td>
<td>0.532</td>
</tr>
<tr>
<td>Total Area (mm²)</td>
<td>553.3 ± 19.6</td>
<td>519.9 ± 17.1</td>
<td>0.260</td>
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<tr>
<td>Cortical vBMD (mg/cm³)</td>
<td>1120.8 ± 7.4</td>
<td>1127.6 ± 4.2</td>
<td>0.405</td>
<td>0.801</td>
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<td>Cortical Area (mm²)</td>
<td>296.0 ± 15.0</td>
<td>284.2 ± 7.6</td>
<td>0.444</td>
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<tr>
<td>Cortical Thickness (mm)</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.1</td>
<td>0.974</td>
<td>0.753</td>
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<tr>
<td>Periosteal Circumference (mm)</td>
<td>83.3 ± 1.5</td>
<td>80.6 ± 1.3</td>
<td>0.239</td>
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<tr>
<td>Endosteal Circumference (mm)</td>
<td>56.5 ± 2.4</td>
<td>53.9 ± 1.8</td>
<td>0.423</td>
<td>0.650</td>
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<tr>
<td>Strength Strain Index (mm³)</td>
<td>2373.7 ± 105.6</td>
<td>2176.5 ± 81.3</td>
<td>0.175</td>
<td>0.526</td>
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<tr>
<td>Muscle Area (mm²)</td>
<td>6681.0 ± 225.8</td>
<td>6430.2 ± 176.3</td>
<td>0.149</td>
<td>-</td>
</tr>
<tr>
<td>Bone Area to Muscle Area Ratio</td>
<td>0.083 ± 0.003</td>
<td>0.081 ± 0.003</td>
<td>0.677</td>
<td>0.743</td>
</tr>
</tbody>
</table>

Data reported as mean ± SEM
*p value when adjusted for lean body mass
vBMD = Volumetric Bone Mineral Density
Table 4 - Measurements of vBMD, bone geometry, and estimated bone strength at the proximal and distal radius

<table>
<thead>
<tr>
<th></th>
<th>EU (n=9)</th>
<th>AM (n=18)</th>
<th>P value</th>
<th>P* value</th>
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<tbody>
<tr>
<td><strong>DISTAL RADIUS</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Total vBMD (mg/cm(^3))</td>
<td>350.7 ± 27.9</td>
<td>327.9 ± 10.6</td>
<td>0.363</td>
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<tr>
<td>Total Area (mm(^2))</td>
<td>298.1 ± 13.8</td>
<td>303.9 ± 10.4</td>
<td>0.746</td>
<td>0.760</td>
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<tr>
<td>Trabecular vBMD (mg/cm(^3))</td>
<td>205.5 ± 9.6</td>
<td>218.4 ± 5.7</td>
<td>0.234</td>
<td>0.218</td>
</tr>
<tr>
<td>Trabecular Area (mm(^2))</td>
<td>202.6 ± 15.1</td>
<td>215.7 ± 9.5</td>
<td>0.451</td>
<td>0.573</td>
</tr>
<tr>
<td>Cortical vBMD (mg/cm(^3))</td>
<td>624.9 ± 39.0</td>
<td>582.1 ± 17.6</td>
<td>0.257</td>
<td></td>
</tr>
<tr>
<td>Cortical Area (mm(^2))</td>
<td>95.6 ± 3.9</td>
<td>88.2 ± 3.1</td>
<td>0.162</td>
<td>0.353</td>
</tr>
<tr>
<td>Bone Strength Index (mg(^2)/mm(^4))</td>
<td>36.4 ± 4.2</td>
<td>32.7 ± 1.9</td>
<td>0.355</td>
<td>0.675</td>
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<tr>
<td><strong>PROXIMAL RADIUS</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total vBMD (mg/cm(^3))</td>
<td>789.2 ± 18.8</td>
<td>799.5 ± 19.6</td>
<td>0.741</td>
<td>0.394</td>
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<tr>
<td>Total Area (mm(^2))</td>
<td>117.1 ± 4.6</td>
<td>107.3 ± 3.1</td>
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<td>0.145</td>
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<tr>
<td>Cortical vBMD (mg/cm(^3))</td>
<td>1138.7 ± 7.3</td>
<td>1138.3 ± 3.5</td>
<td>0.952</td>
<td>0.601</td>
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<tr>
<td>Cortical Area (mm(^2))</td>
<td>76.3 ± 3.6</td>
<td>70.1 ± 1.9</td>
<td><strong>0.064</strong></td>
<td>0.252</td>
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<tr>
<td>Cortical Thickness (mm)</td>
<td>2.5 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>0.560</td>
<td>0.994</td>
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<tr>
<td>Periosteal Circumference (mm)</td>
<td>38.3 ± 0.7</td>
<td>36.7 ± 0.5</td>
<td><strong>0.045</strong></td>
<td>0.137</td>
</tr>
<tr>
<td>Endosteal Circumference (mm)</td>
<td>22.6 ± 0.7</td>
<td>21.4 ± 0.8</td>
<td>0.330</td>
<td>0.294</td>
</tr>
<tr>
<td>Strength Strain Index (mm(^3))</td>
<td>288.3 ± 17.8</td>
<td>249.6 ± 11.4</td>
<td><strong>0.057</strong></td>
<td>0.154</td>
</tr>
<tr>
<td>Muscle Area (mm(^2))</td>
<td>2585.8 ± 110.0</td>
<td>2512.8 ± 86.8</td>
<td>0.554</td>
<td>-</td>
</tr>
<tr>
<td>Bone Area to Muscle Area Ratio</td>
<td>0.046 ± 0.002</td>
<td>0.043 ± 0.002</td>
<td>0.370</td>
<td>0.108</td>
</tr>
</tbody>
</table>

Data reported as mean ± SEM

*P value when adjusted for lean body mass

vBMD = Volumetric Bone Mineral Density
Figure 1a- Diagram of pQCT variables measured in the radius
Figure 1b- Diagram of pQCT variables measured in the tibia


Objective
To obtain a position to further my graduate education

Education
The Pennsylvania State University
University Park, PA
- Expected Graduation: May 2014
- B.S. in Kinesiology – Movement Science Option
- Scholar in the Schreyer Honors College

Related Experience
Research Lab Assistant 2011 – Present
The Pennsylvania State University
Women’s Health and Exercise Lab
Department: Kinesiology – Exercise Physiology
- Competent in processing blood and urine samples
- Competent in performing the following assessments
  - VO2 Max Testing
  - Tests of Resting Energy Expenditure
- Have shadowing experience in the following areas
  - Bone Density Scans
    - DXA
    - pQCT
  - Underwater Weighing
- Completed NIH and PSU IRB Training Courses
- ISCD Certified Bone Densitometry Technologist  In progress

Teaching Assistant 2012
The Pennsylvania State University
KINES 202- Functional Human Anatomy
- Undergraduate Teaching Assistant

Conferences
Attended the American College of Sports Medicine Annual Meeting 2012
### Awards and Scholarships

- **Ruth Stevens Tewksbury Honors Scholarship** 2010-2012
- **Marie Underhill Noll Endowment for Undergraduate Research in Exercise and Sport Science** 2012, 2013
- **Francis A. and Ruth Coates Wodock Scholarship** 2012-2014
- **Member of The Pennsylvania State University chapter of The Honor Society of Phi Kappa Phi** 2013
- **Mary Boyle Weaver and Rebecca Boyle Sutherland Scholarship** 2013-2014
- **Honor’s Thesis Research Grant from the Schreyer Honors College** 2013-2014

### Activities

#### Schreyer Honors College Student Council 2010-2011
- Attended weekly meetings to help plan various events
- Volunteered at fundraisers
- Served as a student ambassador for Schreyer Honors College recruitment events

#### Penn State Gymnastics Club 2010 – Present
- Secretary (2012-2014)
- Member of the competitive team
- Regularly attend practice, meetings, and fundraisers
- Travel to several competitions yearly
- National Association of Intercollegiate Gymnastics Clubs national competition
  - 4th Place Team: 2013
  - 2nd Place Team: 2012
  - 4th Place Team: 2011
- Have developed teamwork, communication, and leadership skills

#### Penn State Panhellenic Dance Marathon (THON) 2010 – Present
- THON Chair for Penn State Gymnastics Club (2011-2012)
- Organized and assisted in fundraising efforts for the Four Diamonds Fund at Hershey Medical Center
- Dancer for THON 2014 representing Penn State Gymnastics Club

#### Study Abroad 2011
- Studied in Palma de Mallorca, Spain
- Obtained credit for a Mediterranean Marine Environment class
- Learned about the culture of Spain through homestay and peers
- Improved my Spanish language skills

#### SHO Time Mentor 2011
- Student mentor for the Schreyer Honors College Orientation (SHO Time)
- Served a peer leader for incoming scholars to the Schreyer Honors College
Kinesiology Peer Mentor Program 2013-2014
• Assist in mentoring underclassmen, transfer students, and incoming freshmen in Kinesiology

Work Experience

Beach Bounders Gymnastics and Athletics 2008-2010
Fruitland, MD
• Recreational gymnastics instructor for ages 18 months through 13 years
• Helped young children and athletes develop basic motor and gymnastics skills

Fenwick Island State Park 2011
Fenwick Island, DE
• Worked in the Beach Accessory Rental Facility
• Developed customer service skills

Computer Skills
Competency in Microsoft Office Suite

References
Available upon request